METHODS FOR THE STUDY OF MARINE BENTHOS

Fourth Edition

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Fourth Edition

Edited by

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*Professor Carlo Heip of the Royal Netherlands Institute for Sea Research, co-author of Chapter 8, sadly passed away just as this volume of the Handbook was in production. His contribution to marine science provided a great deal to our understanding of the marine environment. He will be greatly missed.

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Dedication

This edition of the handbook is dedicated to the memory of Alasdair McIntyre, co-editor and contributor from its inception. A marine scientist whose work achieved worldwide recognition, Alasdair McIntyre left an enduring legacy to benthic research, for which we are and will remain grateful.

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Preface to the Fourth Edition

Bearing in mind that the present edition of this handbook has a long and honourable history, a short account of its provenance may prove useful for the first-time reader. The International Biological Programme's global plan of coordinated research into ecosystem ecology (1964–1974) led to the publication of a series of handbooks designed to meet the needs of workers in a wide range of thematic areas and disciplines, one of which was the study of marine benthos. The first edition of the handbook targeted not only newcomers to the field but also the isolated worker who had to have ready access to the existing literature, as well as others in related disciplines who needed to carry out seabed sampling and those who initiated benthic studies.

It is now more than 40 years since the publication of that edition of the handbook (Holme & McIntyre, 1971), which was followed by two further editions (Holme & McIntyre, 1984; Eleftheriou & McIntyre, 2005). These later editions came about because major new approaches had emerged in response to ever-increasing demands for detailed information on bottom-living communities, as well as to the rapid advances of twenty-first-century technologies. The present edition has retained the same overall layout of previous editions, though there have been substantial updating and revisions as an outcome of the many changes that have occurred. This includes the rearrangement of some chapters that focus on the latest advances in marine technology and the reinstatement of a chapter on phytobenthos. The chapters on benthic deep-sea sampling, diving, imaging analysis, acoustic techniques used for the determination of the seabed characteristics and seabed sediment studies have been substantially rewritten. However, where a specific sampler or technique is essential to the work to be carried out, the information has remained much the same. During the rewriting of the handbook, care has been taken to keep to a minimum any significant overlapping in the descriptions of gear equipment and methods to be used for different purposes or different biota, by cross-referencing or by means of brief descriptions of commonly used gears or methods. Nevertheless, it was felt that such overlapping was sometimes inevitable, as for instance, workers searching for information concerning deep-sea sampling gears would not expect to find the relevant information available merely as a cross-reference to an entirely different chapter.

There have been unavoidable changes in authorship, as over its 40 years' continuum, new authors and co-authors have replaced those who have retired or who are

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no longer with us. Sadly, since the third edition, John Gage, expert on the deep-sea macrobenthos, senior author of Chapter 7, and Alasdair McIntyre, co-editor of the handbook since its first edition, have both passed on.

During the last 20 years, it has been recognised that the underlying mechanisms and interactions in ecosystem functioning are complex and can be understood only through multidisciplinary research networks. It is now accepted that the only way to understand the functioning of marine ecosystems is to formulate conceptual models based upon information concerning exchanges and interactions of the ecosystem components resulting from the collaboration between the subdisciplines of benthic ecology and other disciplines. These ecosystems have been shown to be a sensitive index of alterations and changes and, as such, demand long-term and effective monitoring.

The editor has throughout been fully aware of the important priorities concerning the identification and the linking of benthic patterns and processes and the development of suitable techniques for such linkages. While it is true that important inroads have been made in several sectors of the marine sciences, such as biogeochemical fluxes in the benthic boundary layer, the role of bacteria and meiobenthos in the benthic environment, the dynamics of recruitment, the linking of biodiversity to the ecosystem and carbon flow and its role in ecological stability, these are only a few of the priority areas in ecological research that require concomitant technological development.

Over the past 30 years, though there has been a steady evolution in methods and techniques in benthic investigations, which have seen significant advances in acoustic techniques, deep-sea exploration, diving and imaging techniques, methods and techniques in the study of macrobenthos have remained relatively static. This is an indication of a slowdown in technological progress in this field, or perhaps of a scarcity of ideas in technical and methodological issues or a result of the noticeable decline in large-scale and intensive benthic investigations, which were one of the features of the previous 30 years.

It cannot be concluded that the existing methodology concerning sampling an extremely complex environment, which we cannot always understand, has reached a stage of perfection. It should be stressed, therefore, that in an attempt to study marine benthos as comprehensively as possible, the many tools and methods described in the present edition of the handbook are complementary and should be used in parallel when and where appropriate.

Anastasios Eleftheriou, *Heraklion, May 2012*

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Acknowledgements

For this fourth edition of the handbook, I remain grateful to Norman Holme and Alasdair McIntyre, the two previous editors who were in the first instance instrumental in pulling together all available knowledge on the subject, for the benefit of so many researchers who have subsequently worked on marine benthos.

I express my deep gratitude to all the authors who have contributed their know-how and expertise to this volume; working with them has been both a valuable and memorable experience.

I owe a great debt of gratitude to my wife, Margaret, without whose encouragement, support and forbearance this edition would not have appeared.

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Chapter 1

Design and Analysis in Benthic Surveys in Environmental Sampling

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Abstract

Measuring environmental impacts affecting benthic habitats requires detection of specific patterns of statistical interactions in data sampled before and after a potential impact, in the potentially impacted place and in control or reference locations. This is complex because ecological assemblages and populations vary at many spatial and temporal scales. Here, we introduce methods to ensure appropriate, independent replication of sampling at hierarchical scales in space and time. For statistical analysis, the logic of sampling design is critical. Determining precision of estimates and maximising power to detect impacts require care in the design, analysis and interpretation of the relevant data.

Keywords benthic variance, environmental impact, environmental sampling, independence, precautionary principle, precision, replication, scale, sampling design, statistical interaction

1.1 Introduction

Quantified sampling, particularly to test applied and logically structured hypotheses about patterns and processes in marine habitats, is of increasing importance in underpinning the understanding of natural processes and to predict changes in response to environmental influences. There have been numerous advances in soul-searching (Peters, 1991), methods of analysis (Clarke, 1993; Anderson, 2001), commentaries on logic (Underwood, 1990; Resetarits & Bernardo, 1998; Lawton, 1999) and the need for better understanding of environmental impacts (Schmitt & Osenberg, 1996; Sparks, 2000). As a result, it is not possible in a general summary to review comprehensively even the new material, let alone everything relevant to

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the topic of improved benthic sampling. Suffice it to say that it is also essential to help with management and conservation of diversity, natural resources, systems and functions. Taking care in the acquisition of quantitative information should, therefore, be of paramount importance to all marine biologists and ecologists.

This chapter, therefore, presents a general overview of the issues concerned. It is not, nor could it be, a 'cookbook' of procedures that might work. Rather, it is an attempt to consider fundamental issues of replication, in space and time, the nature of variables examined, issues about designing comparative sampling programmes and so forth.

These topics are considered against a general background of the logical structure underpinning sampling methodology. The issue is a simple one – unless the aims and objectives of any study are clearly identified at the outset, the least damaging outcome will be wastage of time, money and resources. The worst outcome would be a complete lack of valid information on which to build understanding, predictive capacity and managerial/conservatory decision-making. Where aims are vague, designs of sampling are usually (if not always) inadequate, data do not match necessary assumptions, analyses are invalid and conclusions suspect.

In contrast, where aims and purposes are logical, coherent and explicit, it is usually possible to design a robust, effective, efficient and satisfactory sampling programme, which will allow aims to be achieved with minimal uncertainty. This seems such common sense that it does not need to be stated – but common sense indicates that the world is flat and that the sun rotates round the earth. Common sense is not enough.

For example, Hurlbert (1984) published a devastating critique of the failure of many published studies to demonstrate a valid basis for reaching conclusions, because the samples analysed were inappropriately or not at all replicated. His study was confined to the *published* studies, in refereed journals, subject to independent scrutiny. The overall situation, taking into account rejected papers, less intensively scrutinised journals and the flood of unreviewed grey literature, was clearly much worse. It is clear, from practical inspection of more recent literature and through reviewing manuscripts and applications for grants, that the situation (though now better) has not improved substantially (Hurlbert, 2004).

The starting point for studies needing quantitative sampling is that objectives should be clear, the variables to be measured should be defined and the sorts of patterns anticipated in the data should be clearly identified (as testable hypotheses). Wherever possible, as much information as is available should have been collected (and understood) about the operational processes operating, their spatial and temporal scales and about the biological interactions in assemblages and responses to environmental variables. Ideally, the constraints of money, time and equipment should also have all been taken into account. In other words, the professional components of scientific work should all be in place. Under these circumstances, it should be possible to design sampling to achieve minimal probabilities of error in analyses.

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As a result, the focus in this chapter is on general issues and procedures to provide help and guidance with setting objectives, formulating hypotheses and designing sampling, particularly for measuring ecological impacts. This will serve as an aide-memoire for contemplating issues of logic when dealing with spatial and temporal variability in biological systems. It will also provide an introduction into the broader literature where many advances have been made in methodologies dealing with the problems of biological complexity in the real world.

1.2 Variability in benthic populations

Surveys must always be designed to take into account the fact that benthic animals and plants are extremely patchy in distribution and abundance. Patchiness is caused by processes external to the assemblage, particularly disturbances and recruitment, in addition to processes operating within the existing assemblage. Although anthropogenic disturbances are often very severe (e.g. large-scale trawling or dredging can cause extreme changes in benthic assemblages; Hall & Harding, 1997; Lindegarth *et al*., 2000), natural disturbances are common and can be important contributors to spatial variability of populations. These vary from small-scale disturbances, e.g. being overturned by waves affects the assemblage on a boulder (Sousa, 1979) and potentially the assemblage in the sediment below it, to large-scale processes, such as the erosion of nearshore sediments (Shanks & Wright, 1986) and the destruction of assemblages in response to large storms (Underwood, 1998).

However, the most important contribution to patchiness is probably due to unpredictable and variable patterns of recruitment (Underwood & Denley, 1984). Both settlement itself and post-settlement mortality typically vary at a hierarchy of spatial scales (Caffey, 1985; Gaines & Bertness, 1992). Patterns at larger scales tend to be more predictable because most species are confined to particular habitats within a biogeographic range (Brown & Gibson, 1983). However, at small scales within patches of habitat, there is considerable variability in recruitment (Keough, 1998), caused by local environmental variation (e.g. topographic features of habitat or localised water currents) or by the existing assemblage itself (e.g. gregarious settlement in response to conspecific adults or consumption of larvae by large numbers of sessile species). The numbers of larvae competent to settle that arrive in any site are themselves influenced by a multitude of external processes, many of which act in the water column well away from the site of settlement. The localised processes, both physical and biological, that influence recruitment are interactive, so recruitment is extremely variable in space and time.

In addition, numerous interactions within the assemblages themselves continually alter patterns of abundances and these effects, too, occur at a range of spatial scales. For example, though predation may decrease abundances at the scale of a shore or habitat, within that habitat predation may eliminate species from certain patches, but leave other patches alone. Feeding by eagle rays or shore birds can

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create extreme small-scale patchiness in abundances of their prey, although these effects are complicated by environmental factors, such as currents and movement of sediment (reviewed by Thrush, 1999). Even in areas with heavy predation, prey may settle in particular microhabitats, where they can grow large enough to escape predation (Dayton, 1971).

Competition, either for space among sessile animals or plants or for food among mobile animals, also contributes greatly to patchiness of assemblages. Therefore, over-growth or dislodgment of one species by another (Keough, 1984) causes very patchy assemblages of sponges, ascidians and other colonial animals in subtidal habitats and of barnacles and various types of algae in intertidal habitats. Although species that are superior competitors for space or food may eliminate inferior species, the relative strength of interspecific competition may be balanced with that of intraspecific competition. This ensures that neither species is eliminated, but that both persist in very variable and patchy numbers.

Processes such as these are better understood for benthic assemblages living on hard surfaces because, first, the patterns are often readily visible and, second, the processes are relatively easily investigated experimentally. They are, however, also important for assemblages in soft sediments, where recruitment may be equally variable (Skilleter, 1992; Whitlatch *et al*., 1998) and local disturbances, competition and predation alter local abundances, causing very patchy distributions at a hierarchy of spatial and temporal scales (Morrisey *et al*., 1992a, 1992b; Ysebaert & Herman, 2002; Fig. 1.1). To estimate abundances of benthic animals and plants accurately, measures must be made at the range of spatial scales relevant for the species, assemblage or process under consideration.

1.3 Appropriate replication

Appropriate spatial replication

Whatever the hypothesis being tested and the ultimate use of the data from sampling, spatial replication is a mandatory component of any benthic study. The large variability in numbers and varieties of benthic species from place to place at many spatial scales (see Section 1.2) creates fundamental problems for determining at what scales replication is necessary.

Consider a relatively simple problem of determining the influence of type of sediment on the numbers and types of benthic species. To keep the example very simple, suppose that a particular species of polychaete is generally more abundant where sediments are coarse than where fine sediments form the major proportion. In any ecological study of these worms in some new area, a relevant hypothesis to be tested is, therefore, that abundance will, on average, be greater in an area of coarse sediment than in a corresponding area of fine sediment. Furthermore, suppose that patches of the different types of sediment are about 800 m in diameter. Finally, as is virtually inevitable, imagine that numbers of worms per m^2 of habitat may vary

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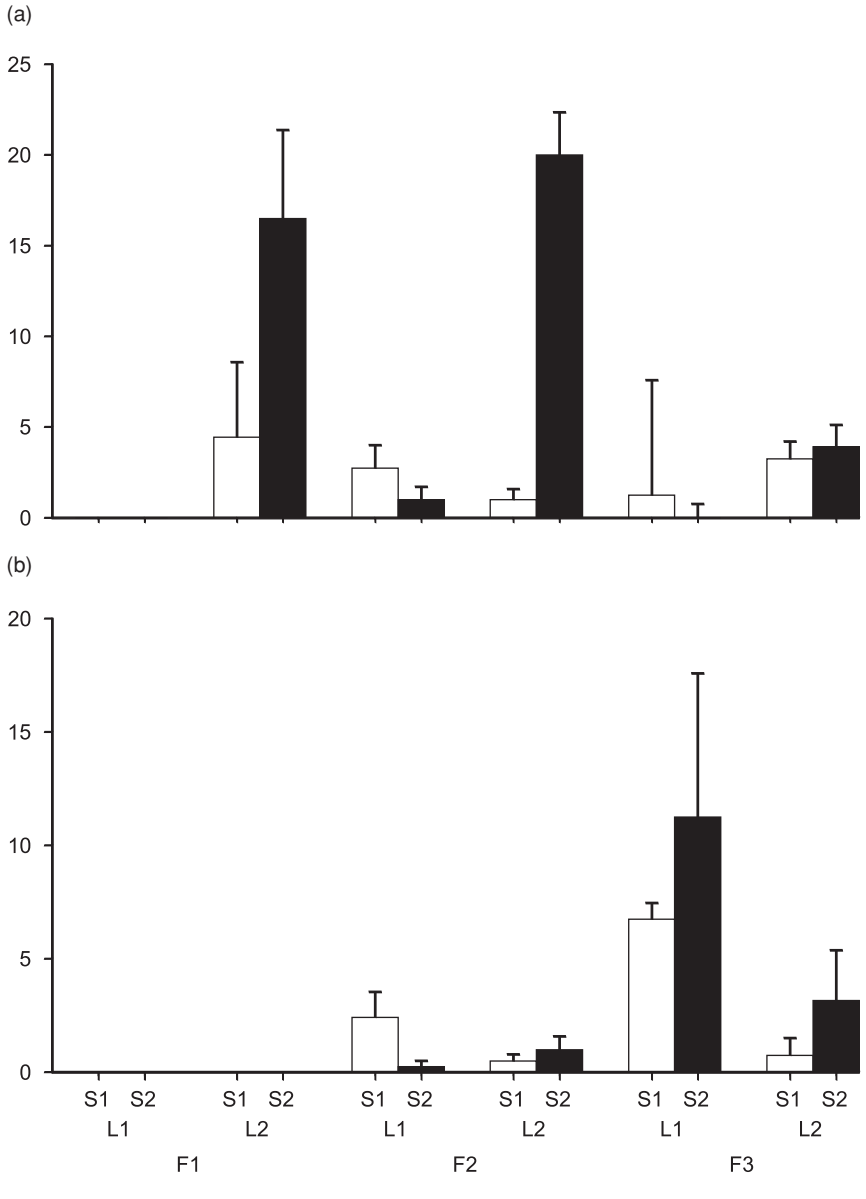

Fig. 1.1 Mean (Standard Error; SE) abundance of two species of amphipods (a) and (b) between sites (S1, S2; tens of metres apart) in each of two locations (L1, L2; 100 m apart) in each of three mangrove forests (F1, F2, F3; kilometres apart). Note that each species shows significant variation at each spatial scale, but these differ between the two species. Patterns of variation at the scale of sites and locations also vary from one mangrove forest to another.

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substantially at scales of tens of metres and at scales of hundreds of metres, even in the same type of sediment. Consider a sampling scheme in which 10 box cores are sampled in one patch of coarse sediment and 10 cores are taken from a patch of finer sediment some 2 km away. The mean number of worms is greater in the cores from the coarser sediment, which is consistent with the hypothesis. Nevertheless, this does *not* allow a valid interpretation that the difference is associated with the difference in sediment (as was predicted). Such an interpretation or influence is confounded by the alternative that numbers are different simply because the two areas are 2 km apart. In other words, if samples are taken in two patches of fine or two patches of coarse sediment separated by 2 km, numbers of worms may differ as much as is observed in the two samples shown here. The alternative models explaining why numbers of worms vary are that the sediments differ or that natural variation from place to place, regardless of sediment, caused the numbers to be different. The only way to separate these two models is to predict (from the first model) that differences from one type of sediment to the other are greater than expected from natural variation in either type of sediment.

The study, therefore, requires replication of sites of each type of sediment to estimate natural variation that is not associated with type of sediment. It is sometimes argued that such a study as described is replicated because there were several cores in each patch. This is an example of what Hurlbert (1984) called 'pseudoreplication' – the replicate units in each sample are at the wrong scale and are estimating variability at tens of metres, not at the hundreds of metres at which there are differences among patches of the same type. A better-constructed design would solve the problem by sampling several patches of coarse sediment spaced, say, 2 km apart and several patches of finer sediment at similar spacing, about 2 km from the nearest area of coarse sediment sampled. The two types of patch should be *interspersed*, i.e. chosen to be higgledy-piggledy on a map, so that no systematic trend or gradient makes them different for reasons other than the type of sediment (see Underwood (2000) for examples and illustrations of this issue).

Using such a design, the variation of scales of tens of metres within a patch and at hundreds of metres from patch to patch of the same type of sediment can be estimated. A hierarchical or spatially nested analysis of the data can then be used to test the hypothesis that the difference, on average, between the two types of sediment is greater than the natural spatial variation from patch to patch of the same type. Examples of such analysis for benthic infauna are in Green and Hobson (1970) and Morrisey *et al.* (1992a, 1992b), and the form and structure of analyses are described in detail in Underwood (1997a).

The lengthy description of a very simple case is made necessary by the large number of unreplicated studies, with logically invalid conclusions, that still keep appearing in (or are submitted to and rejected from) ecological journals. It should be noted that better sampling is no substitute for good biological knowledge. So, if the variation in numbers of polychaetes from site to site with finer sediments is known from previous studies to be of the order of tens to hundreds of worms

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per m^2 and there are 1500 more worms per m^3 in the single coarser site than in the single finer site sampled, it can be argued that this size of difference is much greater than natural variation among sites with finer sediments. In such a case, the conclusion that more worms are found in coarser sediments would be valid.

This argument will, however, only be correct if there has been adequate previous study to demonstrate the general validity (from area to area of the world and from time to time) of the notion that numbers of worms vary naturally by tens to hundreds per metre. It is not usually the case that sufficient information of this type is available. It is, in fact, often the case that previous studies were not replicated at appropriate spatial scales to justify reaching such general conclusions. If such an argument is to be used, it is, therefore, essential to provide the details of the previous studies that might justify it. Often, it is more efficient (and always more valid scientifically, because it does not depend on inductive inferences) to use appropriate replication in any new study.

One further example of problems of logical conclusions from sampling at only one or two spatial scales will be considered. Suppose that dredging is being done at several places in an estuary, to keep channels open for shipping. The sediments in shipping channels are contaminated by heavy metals. Sampling is required in order to detect any impact on benthic infauna in areas around the sites being dredged (not in the sites being dredged – they are not just impacted, but the habitat is actually removed). The concern is that fine sediments with associated heavy metals may wash from the dredged sites to areas up to 100 m away. A replicated study can be designed (as in Fig. 1.2), with several dredged areas and several controls being sampled. In each area, replicated patches of sediment are sampled. Natural spatial variation from patch to patch and area to area is estimated and analysis can reveal any systematic difference between sites near dredging and control sites.

Suppose, however, that the movement of contaminated sediment accidentally released from a dredged area is actually much larger than anticipated. Contaminated fines may now be dispersed over the whole estuary, thus deleteriously affecting all of the control sites, in addition to the sites immediately adjacent to dredging. Changes in benthic fauna due to heavy metals will now occur over all the sites sampled, but there will be no apparent impact because the control sites and the sites next to dredging will not show any differences, when the data are analysed. It would seem that, during the course of the study, there has been an estuary-wide change in fauna not associated specifically with dredging. This sort of situation requires sampling at much larger scales, best of all in other estuaries where dredging is not occurring.

Designs of this type and procedures for analysing the data to detect impacts in such situations were discussed in detail by Green (1979) and Underwood (1992, 1994). The moral of this example is clear – when in doubt about the relevant spatial scale, use a design that can detect changes or differences at one or more of several of the possible scales.

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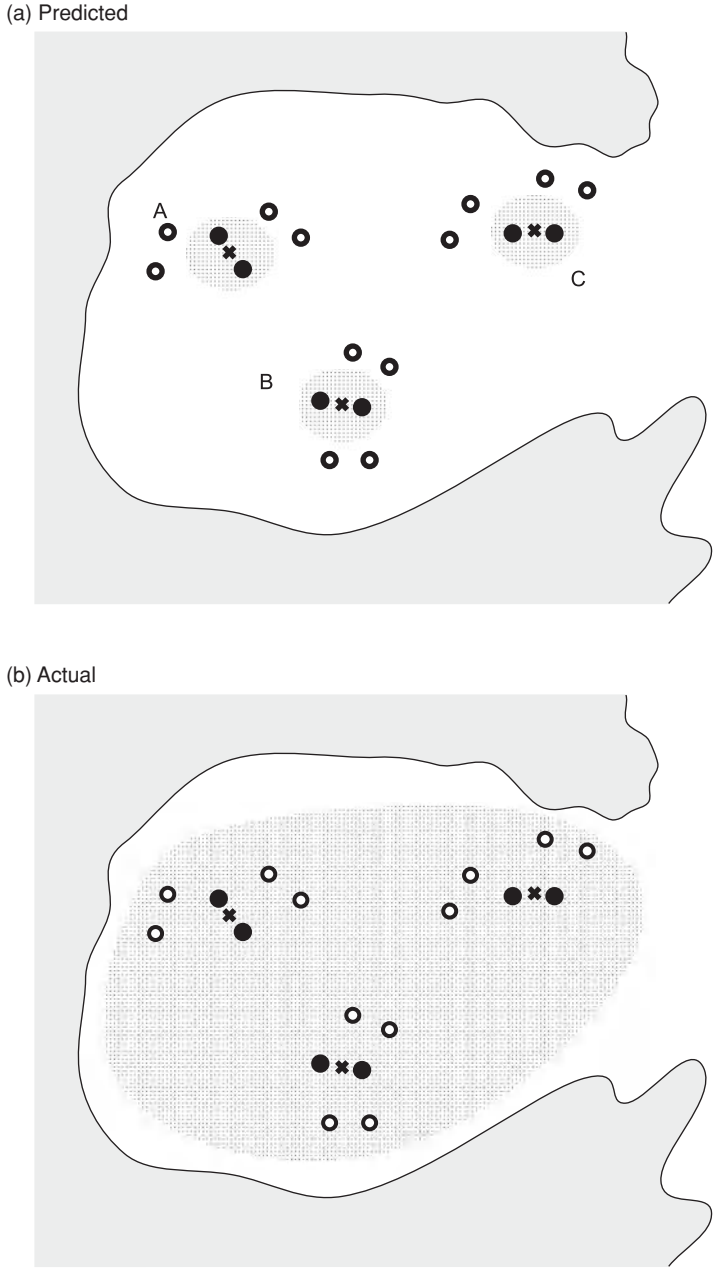

Fig. 1.2 Sampling to detect an impact due to escape of fine sediments from dredging at × in areas A, B and C in an estuary. In each area, there are sampling sites (●) within the distance predicted that sediment would disperse if accidentally released (i.e. over the stippled areas in (a)). Control sites (○) are outside the predicted area of impact and at similar distances apart as the potentially impacted sites. In (b) is the actual, much larger (stippled) area impacted; no impact would be detected because all the controls are also affected.

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Appropriate temporal replication

Many hypotheses concern temporal changes, e.g. seasonal patterns of variation or potential changes in populations due to disturbances. A major problem associated with many such studies is the confounding of temporal and spatial variability (Underwood, 1993; Stewart-Oaten & Bence, 2001). For example, to measure seasonal patterns of abundance, a common procedure is to collect a sample, using a set number of replicates, each month (or each season) in one or more places. Tests of temporal patterns then typically compare the abundances from one month (or season) to another to the variability among replicates in the different seasons (using procedures such as analyses of variance). The problem with such comparisons is that variation among seasons is indeed a measure of temporal variation, but variation within a sample is calculated from measures of spatial variation, because the replicates taken at any particular time are all taken at the one time, even though spatially scattered. In such a design, seasonal (or other temporal) patterns are not contrasted against temporal variation within each season, but against spatial variation.

To test for seasonal variation (or other *a priori* selected scales of temporal variation), temporal variation among the factors of interest must be compared to temporal variation *within* each factor of interest. In other words, temporal variation among seasons must be compared to the magnitudes of variation that occur in each season. To measure such variability, it is essential to collect samples several times within each season. With two or more scales of temporal sampling, seasonal or other long-term trends can be identified against background noise. Where there is no measure of shorter-term temporal variation and such variation is large, quite spurious seasonal (or other temporal) patterns will be seen in the data (Fig. 1.3).

Different scales of temporal sampling are extremely important for identifying environmental impacts. Disturbances to the environment may either be short-lived (pulse disturbances) or persist for long periods of time (press disturbances) (Bender *et al*., 1984). The responses of organisms to either type of disturbance may be relatively short-term (i.e. a pulse response); for example, abundances may rapidly increase, but soon drop to normal levels, irrespective of whether the disturbance persists or ceases. Alternatively, populations may show long-term responses (i.e. press responses) to continuing disturbances (because the disturbance continues to exert an effect) or to pulse disturbances (because the disturbance, although it ended long ago, caused a long-term change to another environmental or biological variable). The experimental designs needed to distinguish among pulse and press responses to pulse or press disturbances have been thoroughly described by Underwood (1991) and Glasby and Underwood (1996). All require a sampling design that can measure temporal variation at different temporal scales, measured at the spatial scales relevant to the pulse and press responses.

For examining most environmental impacts and many other ecological hypotheses, the temporal scales of change are not known and can seldom be predicted. The

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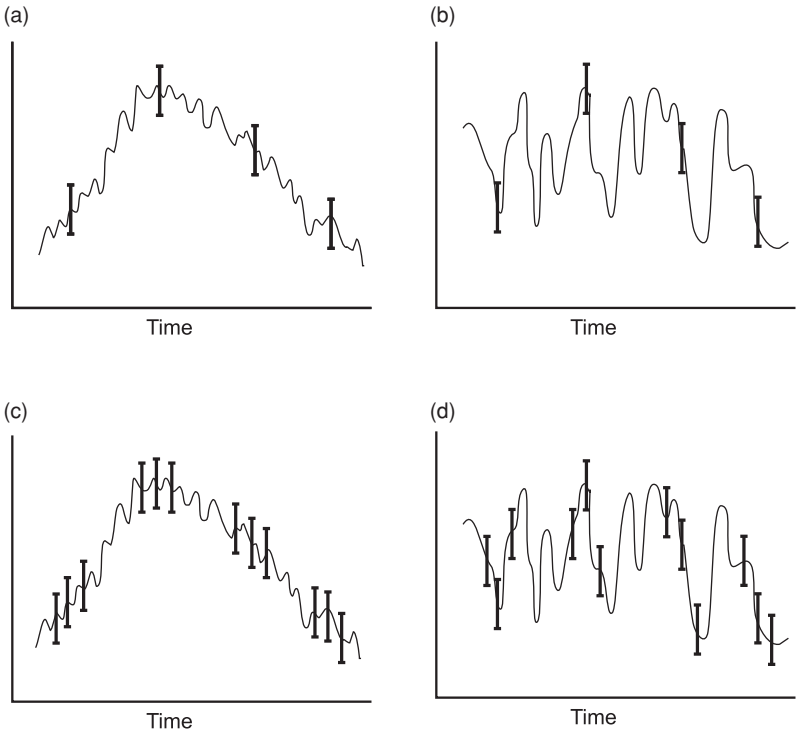

Fig. 1.3 With only a single sample at a series of time intervals a long time apart (e.g. each season), an apparent seasonal pattern could be identified in the variable being measured whether (a) there is, indeed, a long-term seasonal trend or (b) there is considerable short-term variability, but no long-term trend. Short-term temporal sampling is needed within each season. This provides the correct form of within-season replication to measure seasonal changes and identifies (c) long-term trends from (d) background 'noise'.

only way to identify important temporal change is to use a hierarchical temporal sampling scheme. Such designs (Underwood, 1991) not only identify whether any change indicates a pulse or press response but also quantify temporal variances at a number of scales. Impacts that change variances may be more common than and as important as those that change means (Underwood, 1991). If an impact causes much greater fluctuations in abundance (e.g. by altering water currents so that recruitment is more unpredictable), the average abundance of a species may not change over long periods of time; nonetheless the species may be very rare or very common at different times, depending on recent recruitment (Fig. 1.4). If a subsequent impact occurs during a period when abundance is small, such populations may be far more vulnerable to cumulative, but relatively minor impacts, which can then cause local extinctions. Sampling designs that measure changes in mean abundance at different temporal scales, in addition to changes in temporal variances of abundance, are most likely to be successful at identifying environmental impacts and natural ecological variation.

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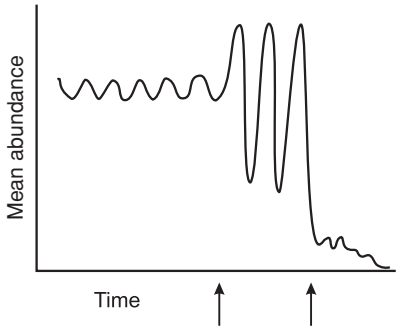

Fig. 1.4 Environmental impacts (at the times indicated by arrows) that affect the variability in abundances of a species, rather than the mean abundance, can cause increased vulnerability to further disturbance. If a subsequent impact occurs during a phase when abundances are very small, the species may easily be driven to local extinction.

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1.4 Size of sampling unit

Because abundances typically vary at a range of spatial scales, the size of the sampling unit selected to sample populations is very important in identifying patterns of abundance. For example, consider a population of polychaetes or other small benthic animals that typically aggregate in clusters, about 10 cm in diameter, with the clusters spaced about 20–30 cm apart (Fig. 1.5a). Therefore, some patches of sediment have very large numbers of animals, whereas other patches have very few or no animals at all. Sampling with very large cores or quadrats, for example 50 cm × 50 cm, will give the impression that the animals are very regularly spaced throughout the site. Each quadrat will probably sample one cluster, with perhaps a few individuals from adjacent clusters, giving very similar measures of abundance in each replicate (Fig. 1.5b). This is due to the fact that this size of sampling unit

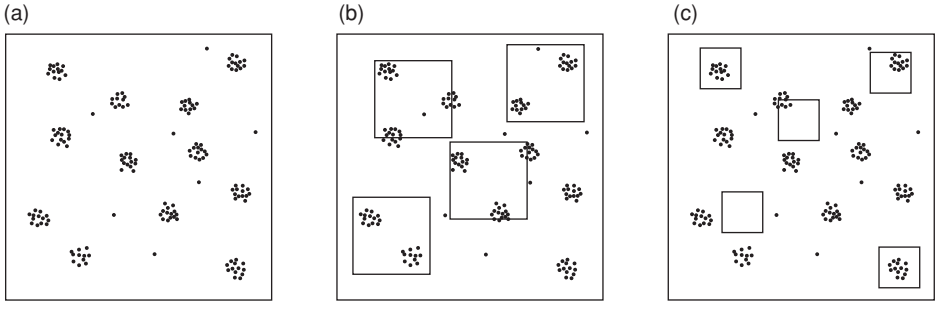

Fig. 1.5 (a) Small benthic animals frequently aggregate into clusters separated from one another. (b) Sampling with units that are much larger than the clusters tends to produce data that suggest a very regular distribution because each unit samples a similar number of individuals. (c) Sampling with smaller units shows the very clustered spatial pattern, with some units sampling clusters and others sampling the bare space among the clusters.

is too large to measure the spatial pattern because the ecological processes causing these patterns are operating at scales of 30 cm or smaller.

Sampling with a quadrat smaller than 30 cm, e.g. 15 cm (Fig. 1.5c), provides a more accurate picture of the spatial variability of these organisms. Some of these units will land near or around clusters, which will thus give very large numbers of the animals. Others will land in spaces among clusters, which will thus give very small numbers of the animals. The average of the replicates should be the same as when large quadrats are used (when scaled to the same area), as long as each set of quadrats representatively samples the population. The variance will, however, be much larger because it will more accurately represent the true pattern of dispersion, thus identifying the scale at which the ecological processes causing these patterns are operating.

In many cases, particularly for organisms in sediments or for very small or cryptic organisms, one cannot observe the patterns of dispersion prior to sampling, so it is not always easy to determine the appropriate size of a sampling unit. In these cases, a pilot study in one site at the start of the experiment, in which a range of sizes of sampling units are used, may identify such patterns and save subsequent, time-consuming efforts. Alternatively, good knowledge of the natural history of the species under investigation, or of other, similar species, or a critical evaluation of the sizes of sampling units others have used, with the reasons why and the patterns identified, may help.

When patterns of abundance of several species occupying the same habitat are being quantified, different-sized sampling units can be used for the different species simultaneously. Therefore, for intertidal and subtidal rocky shores in Australia, a 50 cm × 50 cm quadrat has been shown to provide independent samples that accurately quantify patterns of dispersion and abundance for several of the larger gastropods and barnacles (Underwood, 1981). There are many smaller species that show most variability at the scale of a few centimetres, e.g. littorinid snails (Underwood, 1981). To count these in a 50 cm × 50 cm quadrat is not only time-consuming (because there may be many thousands in a quadrat of this size), but would also not measure variation at the scale that is most important for these small animals. It is more useful, therefore, to count these and similar small species in replicated, smaller subquadrats, scattered representatively over the area of the large quadrat. Similarly, there may be large animals, such as sea urchins or large whelks that may be seldom sampled in a 50 cm × 50 cm quadrat, unless a very large number of quadrats is used. Therefore, these animals may be sampled in large units, e.g. 3 m × 3 m, within which one may sample some 50 cm × 50 cm quadrats, within each of which some 5 cm × 5 cm subquadrats (Fig. 1.6) may be sampled. Each of these scales is relevant to the species being measured and no single scale is appropriate for all species.

Another problem that can arise from the use of inappropriate or a single size of sampling unit may occur when attempting to measure patterns of association between abundances of two species. Consider two species of amphipods living

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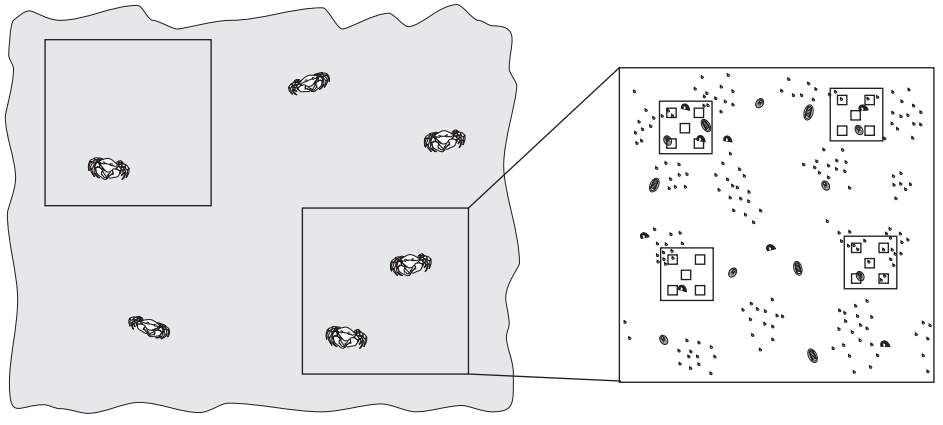

Fig. 1.6 When sampling a suite of species of different sizes and abundances, the most time-effective design is to sample large animals in large quadrats (or other sampling units) scattered over the site, smaller animals in a number of subquadrats, nested inside each of the large quadrats and very small or numerous animals in a set of even smaller areas scattered inside each subquadrat. Such sampling measures variation of the small animals over the same extent of the site as the large animals and allows the variation of the small animals to be partitioned among the different spatial scales.

in shallow, subtidal sediments. If sampled with large cores, say 25 cm in diameter, abundances of the two species may be very strongly positively correlated (Figs. 1.7a, b), i.e. they increase in abundance together. If sampled with small cores, say 10 cm diameter, abundances may be very strongly negatively correlated (Figs. 1.7c, d), i.e. where there are many of Species A, there are few of Species B and vice versa. These two different patterns could be caused because the two species are responding similarly to certain environmental variables, which cause their numbers to increase or to decrease similarly. For example, water depth may have a large-scale gradient across the site and both species are more common in shallower than in deeper water. At the same time, the two species may respond differently to other environmental variables, so that where numbers of one species are large, those of the other species are small and vice versa. For example, there may be small ripples in the sediment across the study site, with one species living up-current and the other down-current of each ripple. This would separate the animals at a scale smaller than 25 cm, thus revealing the negative correlation picked up with the smaller sampling unit (Fig. 1.7e).

Use of only one of the units would lead to the conclusion that the two species were either positively *or* negatively correlated. In fact, they are negatively *and* positively correlated. The different-sized units also direct attention to the scale of the important ecological processes. Therefore, it is necessary to consider factors that cause positive correlation at the scale of the entire site, perhaps over tens of metres, in addition to factors that cause negative correlation at the scale of cm within the site. The opposite pattern could equally be found. For example, species may be positively correlated at a small spatial scale, if they use the same microhabitat. At a large

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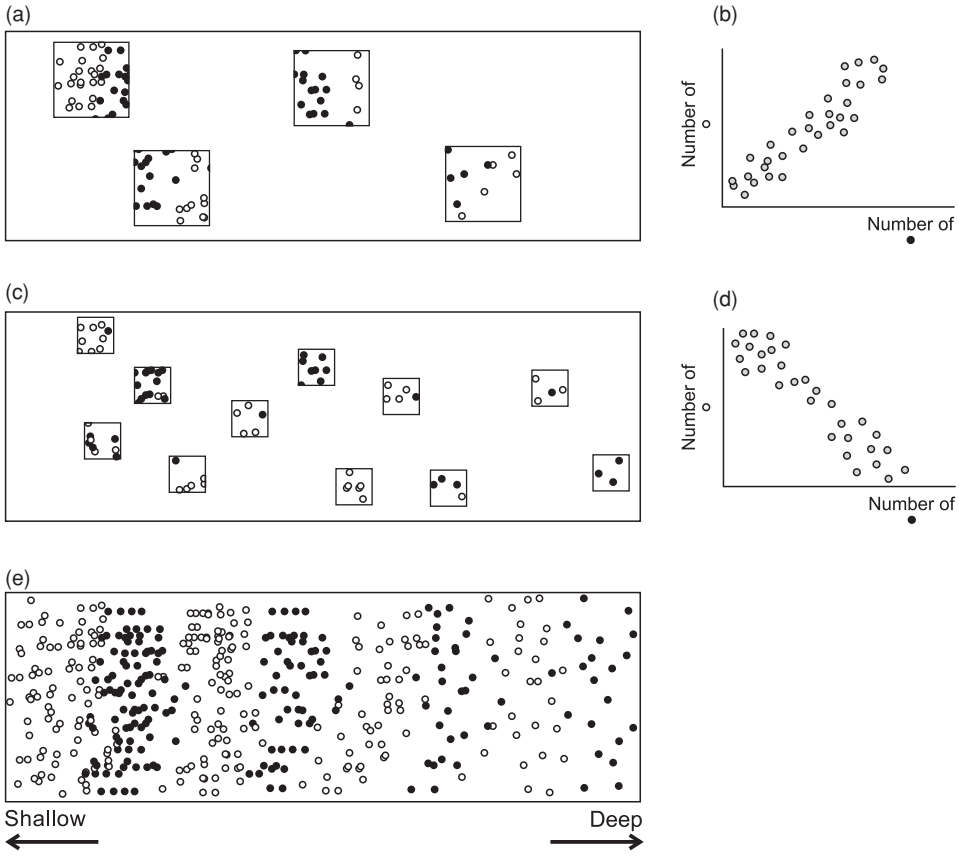

Fig. 1.7 Sampling two species using a large sampling unit (a) shows a positive relationship between the species (b), whereas a smaller unit (c) shows a negative relationship (d). (e) This occurs because the two species are responding to small-scale environmental variables that tend to separate them, while each of them increases in the same way across a broad environmental gradient. Note that the actual pattern in (e) is not visible or known when sampling most types of benthic organisms, so correlations at one scale may not be a good, nor complete, description of relationships between species.

spatial scale, e.g. across a depth gradient, they may be negatively correlated, with one species more common in shallow water and one more common in deeper water.

It is often very difficult to identify individuals of many sessile animals and plants. Therefore, they are usually sampled *in situ* by estimating the percentage of space that they occupy. This can be done by photographing patches of habitat and determining cover from the photographs using a grid of dots or planimetry, or directly in the field. Cover can be estimated from a grid of points, with the species under each point being scored (Underwood, 1981), or by dividing a quadrat into a series of smaller grids and estimating cover in each of these on a 0–4 scale (Benedetti-Cecchi *et al*., 1996). Comparing different methods of sampling cover of sessile organisms in the field, Foster *et al*. (1991) and Benedetti-Cecchi *et al*. (1996) have

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shown that estimates of cover vary according to the method used and a pilot study may be needed to determine which method gives greater accuracy and precision.

It is clear that the size of the sampling unit used will have a major influence on the spatial patterns that are identified and, hence, understanding of ecological processes. In their extensive review on this topic, Andrew and Mapstone (1987) strongly emphasised the need to consider carefully the sizes (and numbers) of sampling units used in a study to optimise any sampling design, rather than automatically using methods that are reported in the literature, without any idea of their suitability.

1.5 Independence in sampling

Many analytical procedures require that data be independently measured from place to place, time to time and replicate to replicate. This is a particularly important assumption for many univariate procedures (regressions, analysis of variance, chi-squared tests). The issues are not so stark for some multivariate analyses and for many tests where data are permuted to generate distributions of test statistics (Clarke, 1993). Nevertheless, even where the consequences of non-independence may not be clearly understood, they should not be ignored.

Correlations in space occur among data where, for example, density in one area influences what happens in surrounding areas. For example, for the numbers of the two species illustrated earlier (see Section 1.4), there would be positive correlations between data points taken on the peaks of ripples (where numbers of one species are relatively large). There would be negative correlation between any pair of sample units where one is in a trough (where numbers are small) and one is on a peak (where numbers are large).

Many biological processes cause correlations in spatial data. For example, if some species are scattered at random across an area of habitat, there will be no pattern of correlation between the numbers in pairs of sample units, wherever they are placed. If, in contrast, numbers are non-random because predatory fish eat the animals and the activity of fish is concentrated in a few sites, perhaps where there are rocks, there are now patches relatively devoid of the prey animals. Two or more sampling units (cores, grabs, quadrats) landing in one of the areas where predators are active will have small numbers of prey and there will thus be some positive correlation in numbers found in sample units where numbers are small.

Non-independence through time (more commonly called serial correlation) can be a more serious problem. Analysing differences in numbers of animals (numbers of species, or whatever other variable) is fraught with difficulties because the numbers in any given area tend to be correlated with future numbers. Even after trends due to seasonal cycles or mortality through time have been identified in analyses, there is often a tendency for the data at one time to be related to those at the next time, or to those at subsequent times of sampling.

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There are two general issues about serial correlation. The first is to determine whether it is present in any given set of data and the second is to determine what can be done about it. A general test for serial correlation is the Durbin–Watson test (Durbin & Watson, 1951), which uses residuals from whatever trend or time-course has been fitted to the data. If e_i represents the residual at time i and there are t times of sampling,

$$d = \frac{\sum_{i=1}^{t} (e_i - e_{i-1})^2}{\sum_{i=1}^{t} e_i^2}$$

is compared with tabulated values when there is no serial correlation. If positive correlation is present, differences between adjacent times of sampling are smaller than expected by chance and d will be smaller than in uncorrelated series of data.

If serial correlation is present and many times of sampling (or distances for spatial analyses) are available, time-series analytical procedures will help. Procedures are described in detail in Box and Jenkins (1976), Diggle (1990) and Cliff and Ord (1973). One possibility is to repeat the Durbin–Watson test at different temporal (or spatial) lags. Thus, compare e_i with e_{i-2}, e_{i-3}, etc., to identify an interval at which there is no longer any noticeable correlation. Data from such distances or times apart would then be sufficiently independent and could be analysed by traditional procedures. This must, however, result in a loss of data and a considerable waste of effort collecting data that are subsequently not useable.

What this means in practice for sampling biota is that, usually, it is not possible to have data for several temporal or spatial samples. Therefore, it is crucial to think in advance about the biological and environmental processes operating that will cause positive or negative correlations in the data. Then, it is often possible to ensure that sampling is done at large enough distances or times to ensure that the correlations do not turn up in the sampled data. Procedures such as hierarchical sampling schemes will also help to allow relevant analysis and some forms of fractal analysis may offer alternative approaches (Leduc *et al*., 1994).

1.6 Multivariate measures of assemblages

Examination of many types of models about natural ecological processes, such as predation or competition, or about changes in response to environmental disturbance or management, requires measures of assemblages of species. Differences in assemblages from place to place, or changes through time, are complex because several variables can change simultaneously. These include the species or taxa found in each sample, their relative abundances and their distribution among replicates within each sample, i.e. their spatial variation. Each of these is equally

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important in understanding natural ecological processes or changes in response to disturbances. Therefore, analytical tools that can identify changes in assemblages need to take all of these factors into consideration.

Several procedures attempt to reduce such multivariate data into univariate measures that summarise aspects of the entire data set, e.g. species richness, evenness, dominance, etc. None of these measures, however, deals simultaneously with the entire variability of the data. For example, showing that the number of species does not vary from place to place allows no ecological interpretation if it is not known that the identities of the species are different in different places. More useful procedures examine components of a multivariate set of data simultaneously, thus measuring the magnitude of differences between samples in composition of species, abundances and spatial pattern. These procedures are well described elsewhere (Clarke, 1993) and will be introduced only briefly here.

These procedures generally work on the same principle (illustrated in Fig. 1.8). Abundances (biomasses or equivalent measures) are measured in a suite of taxa (Fig. 1.8a) in each of a set of samples, each with a number of replicates. A similarity (or dissimilarity) matrix is then calculated for all pairs of replicates across all of the samples in the matrix (Fig. 1.8b). A number of measures of similarity can be used, but the Bray–Curtis coefficient of similarity,

$$S_{jk} = 100\left[1 - \frac{\sum_{i=1}^{p} |y_{ij} - y_{ik}|}{\sum_{i=1}^{p} (y_{ij} + y_{ik})}\right]$$

where p is the number of species and y_{ij} and y_{ik} represent the number of species i in any two sample units (units j and k), is generally considered most suitable for ecological data that tend to have many zero values and where abundances tend to be over-dispersed among replicates (Clarke *et al*., 2006a). The Bray–Curtis measure is also not affected by species that are absent from both of the replicates being compared. When two replicates are identical, the similarity measure is 100% (dissimilarity 0%) and when they have no species in common, similarity is 0% (and dissimilarity 100%).

These measures of (dis)similarity can then be compared within and among samples, assuming replication in each sample, using analyses such as ANOSIM (Clarke, 1993) or PERMANOVA (Anderson, 2001). These test the null hypothesis that the average magnitude in these measures between samples is not greater than it is within samples. These tests have been extended to consider many complex designs, but extreme care should be exercised in interpreting any significant result because, as described above, differences between samples are affected by the species present, their abundances and their occurrences across replicates. These

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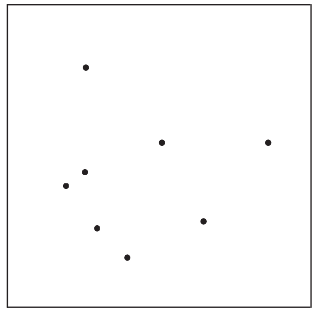
(a)

Data								
	s1	**s2**	**s3**	**s4**	**s5**	**s6**	**s7**	**s8**
Species 1	6	12	0	21	0	0	19	11
Species 2	1	0	0	0	2	0	0	0
Species 3	15	3	6	0	0	0	0	0
Species 4	2	1	1	0	1	0	0	0
Species 5	10	20	3	3	3	53	0	0
Species 6	54	55	23	31	79	87	99	56
Species 7	0	12	10	11	3	24	2	0
Species 8	0	13	23	0	0	15	0	0
Species 9	0	1	0	3	0	0	0	3
Species 10	0	5	0	0	0	5	1	0
Species 11	1	0	1	1	0	0	1	0
Species 12	0	0	1	0	3	0	0	0
Species 13	0	0	2	0	0	0	0	0
Species 14	0	0	0	3	0	4	0	0
Species 15	3	6	5	9	0	0	5	0
Species 16	6	0	1	0	0	0	0	0
Species 17	0	0	0	0	0	0	0	3
Species 18	18	1	10	1	3	2	0	3
Species 19	47	34	34	47	64	33	38	44
Species 20	2	1	1	0	0	0	0	0

(b)

Similarity matrix							
	s1	**s2**	**s3**	**s4**	**s5**	**s6**	**s7**
s2	68.7						
s3	58.0	66.0					
s4	62.4	67.3	61.4				
s5	67.5	60.2	48.7	59.0			
s6	51.0	71.8	50.0	46.5	63.0		
s7	61.8	66.3	45.5	65.1	73.7	63.4	
s8	75.1	71.8	49.8	72.0	74.1	53.1	73.7

(c)



(d)

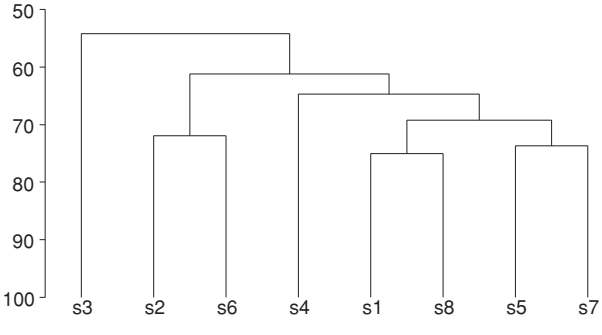


Fig. 1.8 Many useful multivariate analytical procedures convert (a) a matrix of samples, in this case the columns are abundances of a suite of taxa (the rows in the table), or (b) other variables into a matrix of similarities or dissimilarities. This summarises differences across all variables between each pair of samples into a single distance, or measure of difference between the pair. This information can be presented graphically in many ways. (c) Non-metric MultiDimensional Scaling (nMDS) plots illustrate similarity among samples in two- or three-dimensional space, using the ranked distances. Points closer together on the plot are more similar and vice versa. (d) Clustering attempts to identify 'natural' groups of samples by identifying which are more similar to each other.

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tests do not allow identification of the contribution that each of these types of variation is making to any overall patterns of differences.

Tests of complex designs have other potential statistical problems. For example, differences in dispersion (multivariate variance) within different samples cause difficulties in the interpretation of outcomes of tests for difference among samples. Unlike many univariate procedures, where methods for overcoming such problems have been examined in detail, there has not yet been such development of multivariate procedures.

As with univariate analyses, one must also consider transforming the data prior to analysis. It is common to see the data transformed to $X^{0.5}$ or $X^{0.25}$ in an attempt to minimise the effects of very common species and to allow rare species to contribute more to any patterns of difference. Transformations of different severity (e.g. $X^{0.5}$ is not as severe a transform as $\ln(X)$) affect the patterns found (Olsgard *et al*., 1997) and, thus, the interpretation of any analysis. Another consideration is the level of taxonomic resolution to use. Frequently, very similar results are obtained when organisms are sorted to species, or to genera, or families, or larger groups (Gray *et al*., 1988; Clarke, 1993). This will cause particular difficulties for interpretation when different species in an assemblage are affected differentially by the transformation. For example, species with relatively large abundances are affected more by square root transformation than are species with smaller abundances. Clarke *et al*. (2006b) described procedures to deal with assemblages in which species have different spatial variances among replicate sample units. At the start of any project, spending time to examine the effects of different transformations or levels of taxonomic resolution on the results obtained may well save considerable effort later. It is, however, very important to consider these issues carefully, especially with respect to the model and hypothesis being tested.

Measures of (dis)similarity can also be presented graphically, e.g. in Non-metric MultiDimensional Scaling (nMDS) plots. Such plots attempt to place all the replicates into a two- or three-dimensional diagram, maintaining the relative distances (or measures of dissimilarity) among the replicates (Fig. 1.8c). Replicates that plot closely together contain similar assemblages and replicates that plot further apart contain more dissimilar assemblages. Such plots are particularly useful for illustrating differences among samples and are usually used in conjunction with analyses, such as PERMANOVA.

In addition, samples can be subjected to cluster analyses, of which there are a range of different methods (Clifford & Stephenson, 1975). These attempt to find natural groups of samples, rather than to examine differences between predetermined groups. These groupings are often displayed in a dendrogram (Fig. 1.8d) against a scale of (dis)similarity (e.g. Bray–Curtis similarity), which identifies the degree of similarity that separates samples into different groups.

There are ongoing developments of relevant methods, some of which are very useful in analyses of environmental impacts. A recent summary of relevant techniques is given in Clarke and Gorley (2006).

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1.7 Transformations and scales of measurement

There are several reasons why data may need to be transformed prior to analysis. Some parametric and non-parametric analytical procedures (analysis of variance, Kruskall–Wallis tests of ranks) have underlying assumptions, for example, that the data are normally distributed, or that variances among samples are not heterogeneous (i.e. are of the same magnitude). If these assumptions are not met, there is increased possibility of Type I errors (i.e. increased chance of mistakenly finding apparent differences among samples, when they are, in fact, similar; Table 1.4). Although different analyses have different levels of sensitivity to making Type I errors, depending on the particular assumption being violated (Underwood, 1997a), it may be necessary to transform data prior to analysis to meet the assumptions of the analysis (Winer *et al*., 1991).

Three types of ecological processes are responsible for variances being of different sizes among samples. Counts of randomly dispersed animals or plants in quadrats, cores or other sampling units will be distributed approximately as Poisson distributions. This sort of pattern occurs where there are no ecological processes operating to cause the individuals to aggregate or to spread out, so that they are not scattered at random. In a Poisson distribution, the variance equals the mean. Therefore, when comparing samples in which means differ, it is also inevitable that variances will differ (Fig. 1.9a). Comparing samples with very large differences among means is likely to give significant values for heterogeneity of variances (e.g. using Cochran's test). In such situations, where the variance: mean plot is roughly linear, the data can usually be transformed using $\sqrt{X+1}$ to eliminate these problems.

Many ecological processes cause variances to increase exponentially with increases in means so that the data are log-normally distributed. For example, sizes of organisms caused by differing rates of growth, distances moved during dispersal or concentrations of enzymes in animals of different sizes are often log-normally distributed. It is common for most members of a population to grow at relatively similar rates or to disperse relatively similar distances, although a few grow extremely fast or disperse very long distances. These will cause log-normal distributions in the data. Similarly, while small animals may have large concentrations of enzymes, large animals may have small concentrations. If enzyme concentrations are scaled to the size of the animals, measures will be very large for small animals. In cases such as these, the Standard Deviation (SD), not the variance, is approximately linearly related to the mean (Fig. 1.9b). Such data will be more likely to meet assumptions of normality and homogeneity of variances if they are log-transformed prior to analysis (to whichever base seems most appropriate). Unfortunately, if there are zeros in the data, a value (e.g. 0.1 or 1, depending on the range of the data themselves) needs to be added to each datum prior to transformation. Adding these numbers needs careful consideration because this procedure can alter the relative

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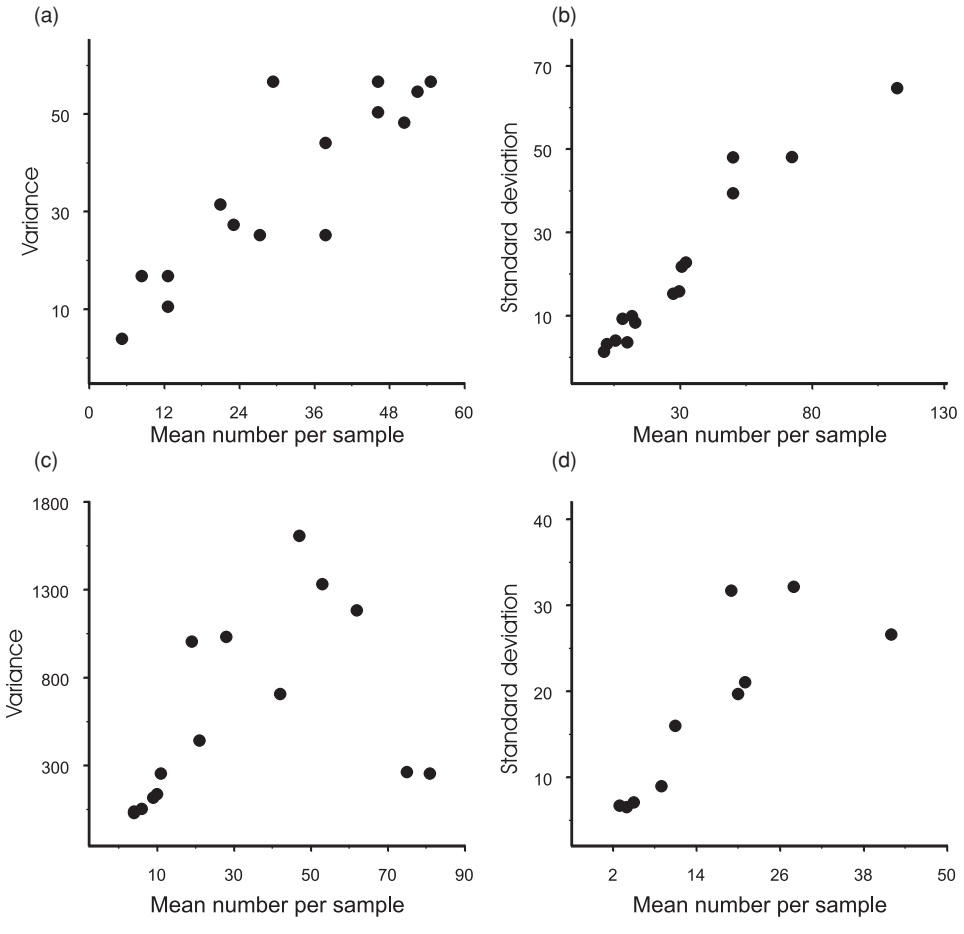

Fig. 1.9 (a) Linear relationship between means and variances in randomly distributed species; (b) linear relationship between Standard Deviations (SDs) and means in log-normally distributed data; (c) relationship between variances and means of proportional (or percentage) data; (d) relationship between SDs and means between constrained sets of proportional (or percentage) data. Parts (a) and (b) are numbers of microgastropods per cobble; (c) and (d) are percentage covers of unoccupied space underneath intertidal boulders.

relationships among means, especially in cases where the data are very variable, e.g. there are many zeros or very small numbers in some samples.

Finally, if data are percentages (or proportions), they may be distributed as binomial distributions. In such cases, variances may be small for samples where means are near the limits of the distribution (e.g. 0% or 100%), but relatively large when samples have means towards the middle of the distribution (e.g. 50%). In such cases, an arc-sine transformation may remove heterogeneity of variances (Fig. 1.9c). When the data do not span most of the range of 0–100%, they will often

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appear to be distributed like a log-normal distribution, so that a transformation to logarithms may be more appropriate (Fig. 1.9d).

In some cases, even if variances are not heterogeneous and, therefore, transformation is not necessary to meet the assumptions of some statistical test, it may still be appropriate to transform data prior to analysis. Therefore, if the ecological processes influencing the patterns being measured are multiplicative, rather than simply additive, it is appropriate to transform all data to logarithms when testing for differences among mean values. This would ensure that doubling the mean from 10 to 20 from one site to another would give the same measure of difference as doubling from a mean of 100–200. This may be very important, for example, for testing predictions about the effects of pollutants on populations of animals across a number of sites, where the natural density of animals differs greatly from site to site. If the pollutant is hypothesised to decrease densities similarly across a range of natural environmental conditions (and therefore across a range of natural densities), transforming the densities to logarithms will allow for such a test because the test will not be confounded by variation in natural densities. More insights into use of transformations in ecological sampling can be found in Elliott (1977).

In any case, whatever transformation is used for whatever reason, note that transforming data must change the scale over which the data are distributed and, therefore, must alter the relationships between means and variances. Transformations, therefore, also change the meaning or interpretation of any test of a hypothesis. This must be considered very carefully, particularly in multivariate analyses if the same transform is applied to the numbers of a set of species that have very different densities. It is essential that the relationships between the hypothesis being tested, the analytical procedures being used and the scale in which the data are analysed should be clearly maintained to ensure that results of any statistical tests are interpreted logically in terms of ecological processes.

1.8 Data-checking and quality control

Rigorous control of the quality of data is an essential requirement for any research project. This does not only mean collecting relevant, independent, representative data in the field or laboratory for appropriate tests of hypotheses of interest. Very careful translation of the data into the format necessary for analysis is also essential. Nowadays, most data are stored in databases or electronic spreadsheets. It is essential that as much quality control goes into checking that the data in the spreadsheet match those collected in the laboratory or field, as went into collecting the data in the first place. One infallible but time-consuming way to ensure that the data match is to make sure that, after each set of data is entered into the computer, it is also checked by two people, at least one of whom was not involved in the collection of the data. This helps to eliminate many errors that may arise from

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Table 1.1 Calculations of Standard Deviations (SDs) from counts of small snails in 15 samples. Note the very large SD for Sample 5 in Column 2, due to a value of 233 being entered instead of 23. When this was corrected (Column 3), Sample 5 still had a large SD, but this was correct. It was due to naturally large abundances and patchiness in this sample.

Sample	SD – not checked	SD – checked
1	0.00	0.00
2	0.45	0.45
3	4.92	4.92
4	0.00	0.00
5	101.04	15.96
6	8.26	8.26
7	7.46	7.46
8	7.60	7.60
9	2.59	2.59
10	7.14	7.14
11	5.45	5.45
12	5.02	5.02
13	4.04	4.04
14	3.42	3.42
15	3.65	3.65

illegible handwriting, fatigue or the sheer boredom associated with entering large amounts of data into databases or spreadsheets.

If it is not possible to develop a foolproof procedure, there are possible short-cuts. For example, if one has a large set of univariate data, such as the sizes of urchins, the diversity of amphipods or the numbers of polychaetes, from a number of sites, it is often useful simply to calculate the SD for each site. If data have been omitted, or very aberrant values have been incorrectly entered, they will cause a very small or a very large measure of SD in a site, relative to other sites (Table 1.1). Therefore, a very unusual SD may indicate an error either in collection of the data in the first place, or an error in transcribing data into the computer. This procedure is quick and easy to use as a form of data-checking, but it will not pick up relatively small errors, only those that cause substantially different estimates of SDs in samples.

When data from a number of species (or variables) have been collected, another form of quality control is to calculate distance measures among the different replicates (e.g. Bray–Curtis dissimilarities for species, or Euclidean distances for abiotic variables). If these are plotted as an nMDS (Non-parametric Multi Dimensional Scaling) plot in two- or three-dimensions, the plot will quickly show outliers, i.e. replicates that have abnormal values for one or more species/variables (Fig. 1.10a). Available procedures to identify which species/variables contribute most to measures of dissimilarity (Clarke, 1993) may help to focus on the particular data that should be checked.

Of course, not all samples with excessively large or small SDs or which form outliers in nMDS plots are necessarily the result of an error. They may simply reflect large natural variability. Similar procedures can also be used to determine whether the sampling strategy is appropriate or not. For example, if replicates

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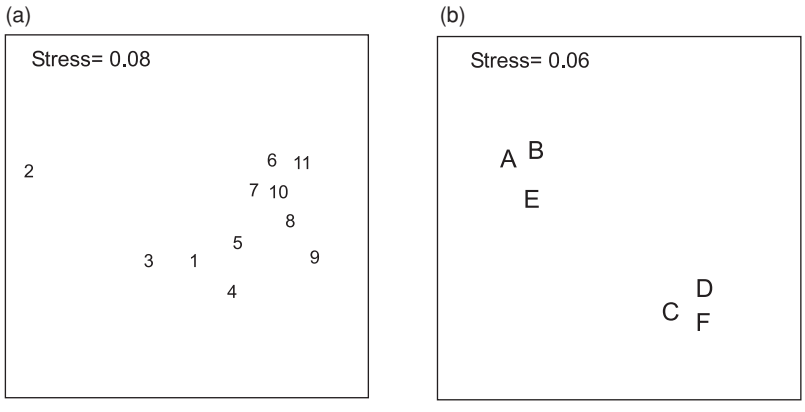

Fig. 1.10 (a) Replicate two in this sample of macrobenthos from a mangrove forest forms an outlier in the Non-metric MultiDimensional Scaling (nMDS) plot because the data for 3 of the 37 taxa in the set of data were omitted from this sample (and were, therefore, erroneously given a value of 0). When this was corrected, the replicates grouped into a single cluster. (b) If the points from a number of replicates in a single sample cluster into two or more distinct groups, it often suggests that the replicates are coming from two distinct strata and the sampling design should be stratified.

cluster into two distinct groups (Fig. 1.10b), it suggests that one might be dealing with two habitats or sets of environmental conditions, each with a different set of taxa. A stratified sampling strategy would probably be better in such a case, with, for example, A, B and E treated as replicates within one stratum and C, D and F as replicates in another (Fig. 1.10b; see Chapman & Underwood, 1999). Similarly, if a species were very aggregated in some sites, but more randomly distributed in others, the SDs would tend to fall into two distinct groups. This too would suggest that sampling should not be done across all sites as if they were equal. Instead, sites should be stratified into two groups (see Section 1.11).

Visual examination of patterns of means, variances, correlation and multivariate data is as important as the statistical tools used to analyse the data. It can provide important information about errors in data and about patterns of variability within and among samples. It should, therefore, be considered as an important precursor to any analysis, rather than looking at the data after statistical differences have been identified.

1.9 Detecting environmental impacts as statistical interactions

Much benthic sampling is done to detect or measure the size of environmental impacts. It is, therefore, worth considering the sorts of sampling designs that are best able to do this. Of course, many situations exist in which optimal designs cannot be done because of lack of time before a disturbance causing an impact,

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or lack of resources sufficient to achieve adequate spatial replication. Wherever a sampling design cannot be optimal, designing the sampling as carefully as possible will ensure the greatest chance of reliable and unambiguous detection of impacts. By understanding what is needed, greater appreciation can be achieved concerning what is not possible and the consequences for interpretation of data.

The main features of sampling designs necessary to detect impacts have been described in detail by Green (1979) and Underwood (1994, 2000). In an ideal design, there should be data from before to after a disturbance that might cause impacts, so that, if an impact does take place, it can be demonstrated that it appeared after the disturbance purported to have caused it. There must be proper temporal replication before and after the disturbance to provide reliable estimates of average conditions (Bernstein & Zalinski, 1983; Stewart-Oaten *et al*., 1986) and to estimate temporal variance, which might itself be altered by an environmental disturbance (Underwood, 1991).

There must be replicated, undisturbed controls to demonstrate that an impact, if it occurs, is associated with the disturbed area and is not a general phenomenon happening in that habitat, which is not due to that disturbance (Green, 1979). 'Undisturbed' in this context means subject to any other influence or process except the particular disturbance under investigation (Underwood, 2000). Control sites should be replicated to prevent confounding of interpretations (Underwood, 1992; see the section entitled 'Appropriate spatial replication').

Finally, as convincingly demonstrated by Green (1979), an environmental impact should always be considered as an ecological interaction. An impact is a change from before to after a disturbance (planned or accidental), which is not the same in the disturbed area as in an undisturbed control area. Therefore, there is a lack of consistency in the temporal pattern of change of the variables being measured in the disturbed area and the patterns of change in the controls (Fig. 1.11). Accordingly, in order to analyse impacts, it is necessary to design sampling that will provide data that can be analysed to detect and interpret statistical interactions.

One type of ideal design is illustrated in Fig. 1.11 and the analysis is shown in Tables 1.2 and 1.3. The situation concerns the construction of sewage outfalls along a coastline. They are to be situated on rocky headlands with fast currents, deep water and coarse offshore sediments. Two outfalls were examined, with two corresponding control areas with similar features of habitat (rocky headlands with fast currents, deep water and coarse sediments). Any coast-wide change in benthic fauna that is not due to discharge of sewage will affect the control areas and the outfall locations. An impact will cause a different type of change where there are outfalls from where there are no outfalls (hence an interaction in the spatial difference between outfall and control locations from before to after the outfalls are commissioned).

Data are collected three times (essentially chosen at random) over two years before the outfalls begin to discharge sewage and then three times over a period of two years, starting two years after the outfalls are commissioned (Fig. 1.11). At

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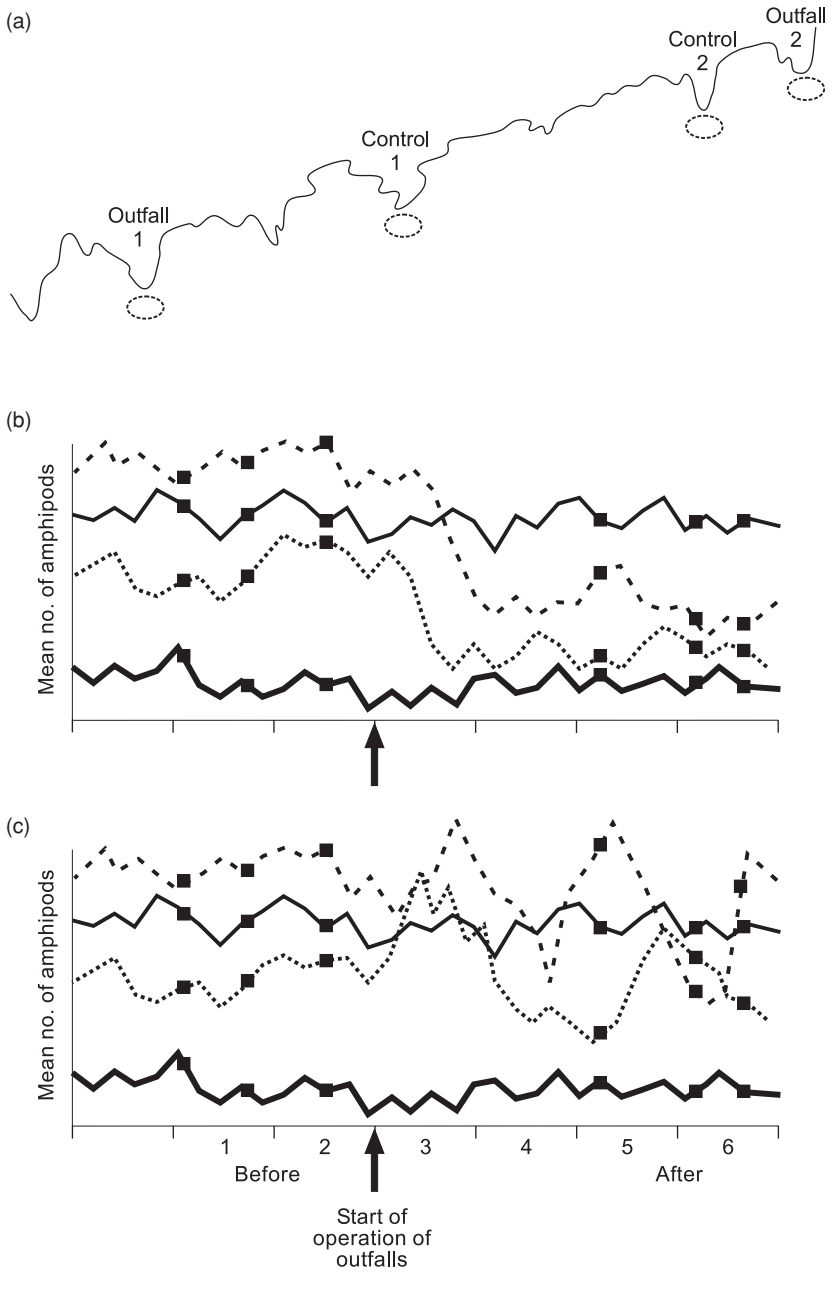

Fig. 1.11 (a) Sampling to detect impacts from construction of coastal sewage outfalls. Two outfalls (—, . . .) and two similar control sites (—, —) are sampled 3 times (■) before and again after the outfalls begin to discharge. (b) Data that would indicate a large-scale, consistent 'press' impact. (c) Data that would identify shorter-term fluctuating impacts.

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Table 1.2 Framework for analysis of hypothetical sampling to detect impacts due to construction of sewage outfalls (illustrated in 1.11). *O* indicates the comparison of mean numbers of an amphipod between outfall and control locations and is a fixed factor (see Winer *et al.*, 1991; Underwood, 1997a). There are two (randomly chosen) locations of each type, indicated by $L(O)$. Sampling before and after the outfalls were built represents a fixed factor; there are 3 randomly chosen times of sampling before and again after the outfalls start operation. $n = 5$ replicate cores are sampled at each time in each location.

Source of variation		Degrees of freedom	Test
Outfalls versus controls	$= O$	1	Irrelevant
Before versus after	$= B$	1	Irrelevant
$O \times B$		1	Indicates large-scale, long-term impact
Locations (outfall or control)	$= L(O)$	2	Irrelevant
$B \times L(O)$		2	Indicates different impacts at the two outfalls
Times of sampling (before or after)	$= T(B)$	4	Irrelevant
$O \times T(B)$		4	Indicates possible fluctuating impacts
$L(O) \times T(B)$		8	Indicates possible fluctuating impacts that differ between outfalls
Residual		96	
Total		119	

Table 1.3 Framework for an asymmetrical (beyond Before-After/Control-Impact (BACI)) analysis of sampling to detect impacts due to construction of a single sewage outfall. There is now no replication of outfall locations, but all other details are as in Table 1.2.

Source of variation		Degrees of freedom	Test
Outfalls versus controls	$= O$	1	Irrelevant
Before versus after	$= B$	1	Irrelevant
$O \times B$		1	Indicates large-scale, long-term impact
Locations (control)	$= L(C)$	1	Irrelevant
$B \times L(C)$		2	Indicates different impacts at the two outfalls
Times of sampling (before or after)	$= T(B)$	4	Irrelevant
$O \times T(B)$		4	Indicates possible fluctuating impacts
$L(C) \times T(B)$		4	Indicates possible fluctuating impacts that differ between outfalls
Residual		72	
Total		89	

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each time of sampling, a certain number, say five, replicate areas of sediment would be taken in each location and the animals of some species of interest (perhaps an amphipod) collected from each area.

From such data, the analysis in Table 1.2 can be completed. A large and consistent press impact (Bender *et al.*, 1984; Underwood, 1989, 1991) would cause an interaction identified as $O \times B$ in Table 1.2. A shorter-term, more fluctuating response (perhaps a pulse response; Bender *et al.*, 1984) would appear as an interaction $O \times T(B)$ in Table 1.2. Where an impact has a different effect on fauna in the two locations with outfalls, there will be an interaction $B \times L(O)$ or $L(O) \times T(B)$ depending on the rates of change caused by the sewage. Impacts causing changes in temporal variance rather than changes in mean abundance of animals (Fig. 1.11b) can also be analysed (Underwood, 1991, 1994).

Of course, in most cases, there is only one planned disturbance (in the present example, only one outfall is planned). Consequently, there is a loss of replication of the disturbed ('outfall') location and correspondingly less certainty about any impact. There must, however, still be replication of control locations, ensuring that extreme forms of confounding do not happen. Such asymmetrical designs are still analysable and interpretable (Table 1.3) and have been called 'beyond BACI' designs (Underwood, 1992) to distinguish them from earlier, spatially unreplicated and confounded BACI designs (Stewart-Oaten *et al.*, 1986). The acronym BACI is for 'Before-After/Control-Impact' sampling, where there is one (and only one) disturbed (called 'Impact') location and only one control area. The disadvantages and logical problems with BACI designs are well known (Underwood, 1992; Smith *et al.*, 1993).

In many situations, there can be no sampling before a disturbance, either because it is accidental (such as an oil spill) or because the impacts were unforeseeable and start to appear only after the disturbance has started. Such situations can still be analysed (Chapman *et al.*, 1995; Glasby & Underwood, 1996), but, again, there may be uncertainty about the cause of the impact. Without previous data from before the event, there is less certainty that the impact started after the disturbance.

There is now a substantial body of reviews of literature on sampling and analysis of environmental impacts, starting from Green (1979) and, more recently, Spellerberg (1991), the papers in Schmitt and Osenberg (1996) and Sparks (2000). There are still many problems in analyses of complex samples for multivariate measures, but interactions caused by impacts can, in the simplest cases, be analysed by permutation procedures (Anderson, 2001).

1.10 Precautionary principles and errors in interpretations

Precaution has become a guiding goal for decision-making in a framework of ecological sustainability (see Cameron & Abouchard, 1991; Wynne, 1992). Despite different views by lawyers, managers and scientists on what precautionary

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Table 1.4 Two types of error in conclusions from statistical tests.

	As a result of sampling, the null hypothesis is:	
	Rejected	Retained
	You believe there has been an environmental impact	You believe there has been no environmental impact
Unknown to you, the null hypothesis is:		
TRUE	TYPE I ERROR With probability α	CORRECT DECISION
FALSE	CORRECT DECISION	TYPE II ERROR With probability β

principles mean (Dovers & Handmer, 1995), there is nevertheless consensus that precaution can be defined in terms of environmental decisions. Given uncertainty about the scientific information underpinning managerial decisions (and, although often forgotten, the uncertainty in all the architectural, economic, engineering, legal, political and social information), it is important to remember that mistakes do occur in the interpretation of analyses of quantitative ecological data. In statistical terms, there are two major types of mistake (Table 1.4). Type I errors occur when a null hypothesis is rejected by a statistical procedure, but which is, in fact, true. Type II errors occur when a null hypothesis is false (i.e. some alternative is true), but is retained. In environmental decision-making, the hypothesis put forward is that there will be an impact due to some disturbance. For other types of ecological studies, hypotheses include predictions such as the following:

(i) Based on the observation that faunal assemblages vary with depth and composition of sediments varies with depth, it can be proposed that differences in composition of sediments cause the difference in assemblages. Therefore, a relevant hypothesis is that, for any particular composition of sediments, similar fauna will be found at all depths. Also, at any depth, the fauna in a particular type of sediment will be similar to that in the same type of sediment at other depths.

(ii) Given previous observations of fewer amphipods in areas where rays feed, the model can be proposed that predation or disturbance by the rays decreases the number of amphipods. This leads to the hypothesis that areas where rays are experimentally prevented from entering will develop larger numbers of amphipods than in corresponding control areas.

Sampling is then done to test the hypothesis (hypotheses) and data are analysed statistically. A statistical procedure includes choosing a probability (α) of Type I error – the probability that the data collected cause rejection of the null hypothesis as the outcome of the test, even though the hypothesis is, in fact, correct. So, an impact could apparently be found where there is no impact; different fauna seem to be present in similar sediments, whereas in reality, there is no difference. The

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presence of rays seems to make a difference, but in fact, the numbers of amphipods are not influenced by rays.

Type II errors exhibit the opposite situation – no impact is found, even though one has occurred. A Type II error occurs when fauna do actually differ, but no difference is found in the samples; or if the sampled numbers of amphipods are not significantly different in the presence and absence of rays, even though rays are, in fact, important predators. The probability of making Type II errors (β) cannot be assessed in most studies.

In environmental studies, failure to detect an impact (a Type II error) is much more serious than erroneously claiming to find one (Type I) where there is none. Where an impact is believed to have occurred, more work will usually be done (to check, to determine its extent, to test further hypotheses about its consequences). In such a case the mistake will almost certainly be detected. In contrast, where a real impact has not been detected, no particular follow-up will occur and environmental degradation will continue. Precautionary principles, therefore, are based on the assumption that errors should be of Type I and not of Type II (Gray, 1990, 1996; Peterman & M'Gonigle, 1992; Underwood, 1997b).

There is not enough space here to demonstrate how to reduce the probability of Type II errors. Details can be found in Green (1989), Peterman and M'Gonigle (1992) and Underwood (1997b). The probability of not making a Type II error, i.e. of correctly rejecting a null hypothesis (finding an impact) when it is false (there *is* an impact), is $(1 - \beta)$ and is called the power of a test (see Table 1.4). Power is increased where:

(i) α, the probability of Type I error is increased;
(ii) the intrinsic variance, or 'noise', in the measurements is small;
(iii) the size of the effect is large, i.e. large differences are expected if the null hypothesis is false;
(iv) the sizes of samples are large.

Of these, the variance is a property of the system being measured (but see Section 1.11). α and the sizes of samples (numbers of replicate sites, times of sampling, sample units in each site) are chosen by the experimenter. Thus, they should be chosen to achieve large power, i.e. great capacity to reject null hypotheses (to find differences where they exist).

The key to power analysis is the effect-size. To calculate the power of any sampling and analysis requires that the amount of difference among times, places, habitats, experimental treatments, etc., be specified in advance. Thus, the hypothesis that rays influence amphipods is devoid of real information. What is required is a statement, based on available knowledge, that, if rays decrease the numbers of amphipods, removing them from some experimental areas will cause an increase of X amphipods per unit area of habitat. Where the original observations provoking the study were that fewer amphipods are present where rays feed (see above), X is

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known from these observations (it is the observed difference hypothetically caused by rays).

For the assessment of environmental impacts, effect-sizes are more difficult to establish. A good yardstick is that the effect-sizes (how much difference in the densities of the affected species) or how much change in any other relevant variable is expected (if there really is an impact) should be based on what responses would be triggered by the discovery of that size of impact. So, suppose that an impact causes a reduction of 10% in numbers of crustaceans in some mud flat near an industrial outfall and constitutes an impact, but there is no proposed change in regulation or management. In contrast, suppose that a reduction of 60% would trigger immediate regulatory responses. It is, therefore, very important to be able to detect a 60% difference, but of limited or no value to be able to find much smaller impacts.

In this case, a sensible effect-size would be somewhere around 50% change and the power of the study should be made large (say $>90\%$) in order to have a good chance of finding the impact if it occurs (Green, 1989; Underwood, 1997a). Where resources (money, time, equipment (i.e. money, money, money!)) are insufficient to allow adequate replication to achieve large power, α (the probability of Type I error) should be increased in order to achieve reductions in β (the probability of Type II error). Mapstone (1995) has described in detail how to trade off the lack of resources (increasing β) by increasing the chance of mistakenly finding impacts (increasing α) in the assessment of environmental impacts.

1.11 Precision and the size of samples

As indicated earlier (see Section 1.10), the power of tests is a function of the sizes of samples used. In general, the precision of an estimate from a certain sample is increased as a function of the size of sample. A typical measure of precision is the standard error, which is (sample variance/size of sample)$^{1}/_{2}$ and clearly decreases (so precision increases) as size of sample increases. Wherever possible (and it is always desirable), a maximal acceptable imprecision should be specified. It is possible to estimate the variance of the variable being measured and thereby to calculate how many replicates should be included in a sample to achieve the necessary precision.

There are, however, several features of design of sampling that help to increase precision of estimates of abundances of organisms. The first is stratification. Wherever it is possible to make a 'map' of abundances (from previous studies in the literature or from pilot studies) stratification of sampling will often substantially reduce imprecision. As a simple example, let us suppose that it is generally known that a particular species of sea urchin is generally more abundant (per box core) in areas of very coarse sediment than where sediments are finer. Let us suppose that in the study area, about 25% of the seafloor is composed of coarse sediments, in several large patches. The remaining areas are finer sediments. There are sufficient

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Table 1.5 Sampling urchins in an estuary with two types of sediments. In (a), $n = 16$ cores are taken at random over the whole area; some are in coarse, some are in finer sediments. In (b), $n = 4$ cores are taken in the areas of coarse sediment and $n = 12$ in areas of finer sediment. Stratification substantially increases the precision of the estimate of mean number per core.

(a) Numbers of urchins in 16 cores
19, 100, 77, 13, 1, 15, 20, 17, 90, 77, 8, 22, 8, 78, 14, 29
Mean = 36.8; variance = 1156; SE = 8.5
(b) Numbers of urchins
Coarse sediment: $n = 4$
102, 80, 100, 95
Mean = 15.2; variance = 289; SE = 8.5
Finer sediment: $n = 12$
8, 10, 5, 6, 26, 23, 25, 26, 17, 8, 1, 27
Mean = 15.2; variance = 81.1; SE = 2.8
Combined sample:
Mean = 35.6; SE = 2.6

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funds to take a total of 16 cores to estimate the average numbers of urchins per m^2 of seafloor, as part of an ongoing study of disturbances due to trawling. Suppose you now take the samples at random positions across the area, resulting in the data in Table 1.5a. This gives an estimate of mean abundance of 36.8, with Standard Error (SE) = 8.5.

If, instead, the sampling were to be stratified – i.e. you took 25% (or 4 of the 16 samples) in the areas of coarse sediment and the remainder in the other habitat, then you would have the data in Table 1.5b. Now, the mean number of urchins is estimated separately in each habitat, with separate estimates of imprecision, which are then combined for the whole area to give a mean abundance of 34.9, with SE = 2.5. This latter method is much more precise because it removes the variation among replicate cores that is due to the systematic differences between the two types of habitat. Of course, it also gives explicit information about the densities of urchins in each of the two habitats, which may or may not be useful (depending on the actual hypotheses being tested).

Combining the samples from each habitat, as done here, is only valid if the variances in numbers of urchins in the two areas are measured independently. Alternative methods, in particular how to stratify samples so that the number of replicates taken in each stratum is proportional to the variance in the stratum, have been described in detail (Cochran & Cox, 1957; Cox, 1958) and other issues in marine ecology were reviewed by Andrew and Mapstone (1987).

A different method of expressing imprecision is to calculate the Confidence Interval (CI) for a particular mean estimated from a sample. A 95% CI indicates a range of values that have a 95% probability of including the true mean being estimated. If the CI is small, the unknown mean has been estimated with a fair degree of precision.

Table 1.6 Sampling an amphipod in three habitats to demonstrate precision of combined samples. Data (hypothetical) are numbers in $n = 5$ cores in each habitat. Variances were homogeneous (Levene's test, F with 2, 12 df = 1.18, $P > 0.30$).

		Habitat		
		1	2	3
Replicate	1	10	28	12
	2	14	31	9
	3	3	33	3
	4	5	26	0
	5	2	19	1
Mean		6.8	27.4	5.0
Variance		25.7	293	31.2
SE		2.3	2.4	2.3
95% CI (4 df)		6.3	6.7	6.5
Combined variance		28.7	28.7	28.7
Combined SE		2.4	2.4	2.4
95% CI (12 df)		5.2	5.2	5.2

A 95% CI for a sample mean $\bar{X}$ from a sample of size n is

$$\bar{X} \pm t_{0.05,(n-1)\text{df}} \times \sqrt{\text{Sample variance}/n}.$$

As size of sample (n) increases, the CI will decrease because the SE (i.e. (variance/mean)$^1/_2$) is smaller and because there are more degrees of freedom (df) (i.e. $(n - 1)$ increases), giving a smaller value of t (from Student's t-distribution, in this case choosing $\alpha = 0.05$, the probability of Type I error).

In any study of differences in means through time (seasons, before/after events, etc.) or across space (habitats, patches, depths, etc.), it makes sense to combine samples, so that the estimates of variance around each mean come from a combined estimate of variance, with more df than from each sample alone. Thus, putting the samples together as in an analysis of variance improves the precision of sampled estimates of means, as illustrated in Table 1.6. For this to be valid the variances of the various samples should be similar and this can be tested by various procedures. The example in Table 1.6 considers samples of $n = 5$ cores from each of three habitats. The combined CI is much smaller than the individual ones. To achieve the same precision (i.e. the same small CI) for each habitat separately would have required samples of $n = 7$ for each habitat, thus increasing the required work 1.4 times.

1.12 Gradients and hierarchies in sampling

There has been debate about appropriate sampling designs to use for analyses of influences along an environmental or other gradient. For example, when testing a hypothesis about the influence of discharge of fresh water from an estuary, it is appropriate to sample along a gradient from the mouth of the estuary. Similarly

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and more obviously, sampling down a depth gradient requires the sites to be along the gradient.

In other cases, the situation is not so clear-cut and whether or not sampling along a gradient is the best option depends entirely on the hypothesis being tested. Furthermore, even if a gradient is to be sampled, the spacing of samples is dependent on the precise issue under examination.

To provide an illustration of the issues, consider a simple case of discharges of contaminants from an outfall on the coast (see Underwood, 2000, for more details). In all three situations considered here, it is proposed that pollution due to the contaminants will be revealed by differences in the assemblages of animals in sediments near to, as opposed to far away from, the outfall.

In the first case, the design of the outfall included modelling of the probable dispersal of contaminants and their dilution away from the pipe (Fig. 1.12a). The requirement of the ecological sampling is to test the hypothesis that concentrations of contaminants in the animals in sediments do, indeed, conform to the modelled pattern of distribution. In this case, samples must be taken along the gradient, at fairly regular intervals, to have the greatest chance of detecting departures from the prediction.

In the second case (Fig. 1.12b), the issue of concern is not the actual spatial pattern of contamination, but the distance from the outfall at which concentrations of contaminants fall below a certain critical value. For example, if the outfall is discharging pesticides in run-off from housing, there may be, for human safety, a maximal allowable concentration of contaminants in scallops on the seafloor. It is, therefore, important to focus attention on the limits within which it is unsafe to harvest scallops for human consumption. In this case, sampling effort should be entirely focused on the areas where modelling indicates that concentrations of contaminants are below the critical threshold. The resources and effort should be used to identify the 'safe' boundary rather than the actual spatial gradient of contamination.

Mostly, however, there are major disconnections between gradients of contamination and actual biological responses, i.e. pollution (Phillips, 1978; Spellerberg, 1991; Keough & Black, 1996; Raimondi & Reed, 1996). Such disconnections are due to the inertia of biological systems, i.e. animals or assemblages not being affected by that contaminant, so that there is no response despite chemical signals. Alternatively, biological responses can occur when concentrations of contaminants are minimal, or undetectable, because of bioaccumulation in individual animals. Finally, populations may be unaffected by pollution due to widespread dispersal of their larvae, maintaining populations from elsewhere (Underwood & Peterson, 1988). In such cases, analyses along gradients are not likely to be the best approach, because there is no clear indication of the course or extent of the gradient. An effective alternative is to sample at sites near the outfall to make comparisons with distant sites that are chosen to be control sites because they are located far enough away to be unaffected by the outfall (Fig. 1.13a). As discussed above (see

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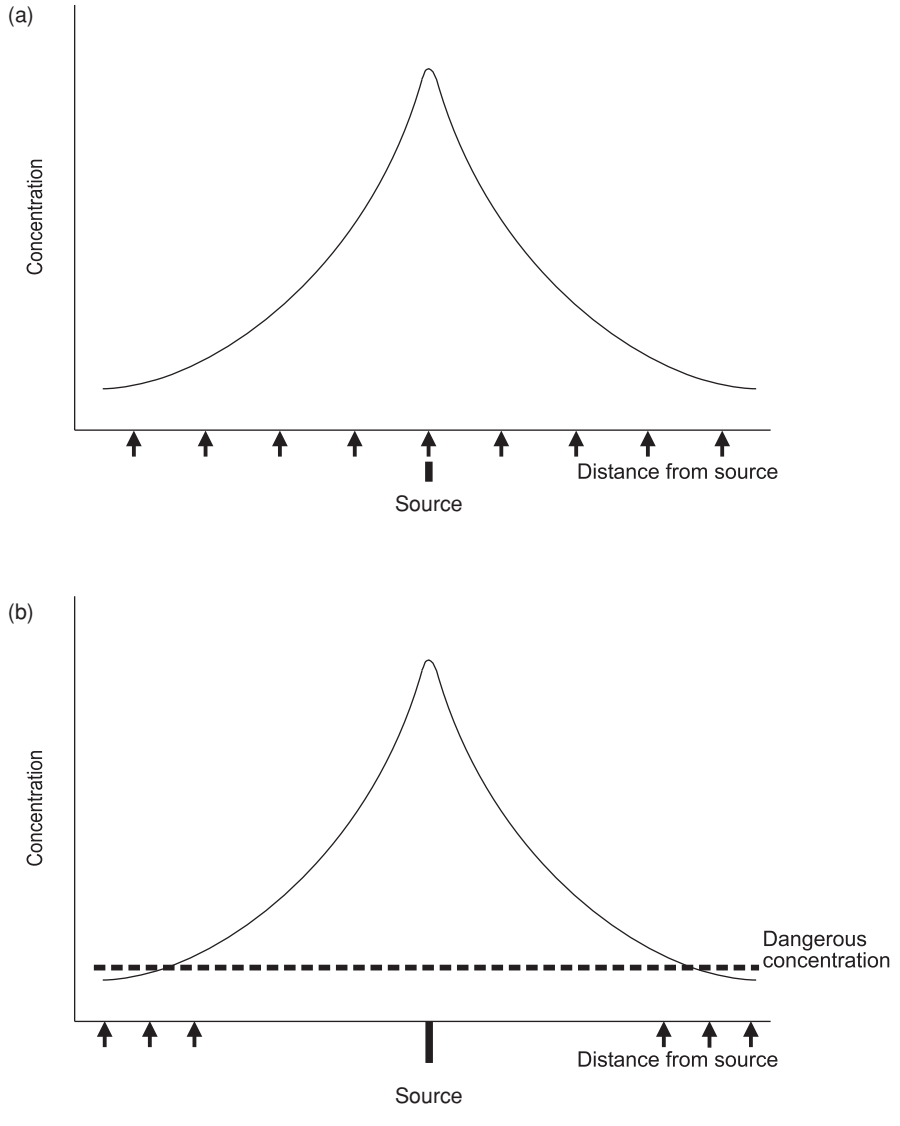

Fig. 1.12 Sampling relevant to gradients of some influence (e.g. pollution from an outfall pipe). (a) The issue is the spatial pattern of decreasing impact based on predicted spatial dilution of contaminants, so sampling along the gradient away from the outfall is necessary. (b) The major concern is risk to health due to pollution being over a defined 'safe' limit in animals at some distance from the outfall. Sampling is, therefore, concentrated around the areas where this safe limit is supposed to occur.

the section entitled 'Appropriate spatial replication'), uncertainty about the scale of the potential pollution requires sampling at more than one spatial scale.

Another design that may be useful concerns tests of hypotheses about differences in assemblages resulting from some installation or intrusion into a habitat. For example, it may be proposed that fauna in sediments near to rocky reefs are different

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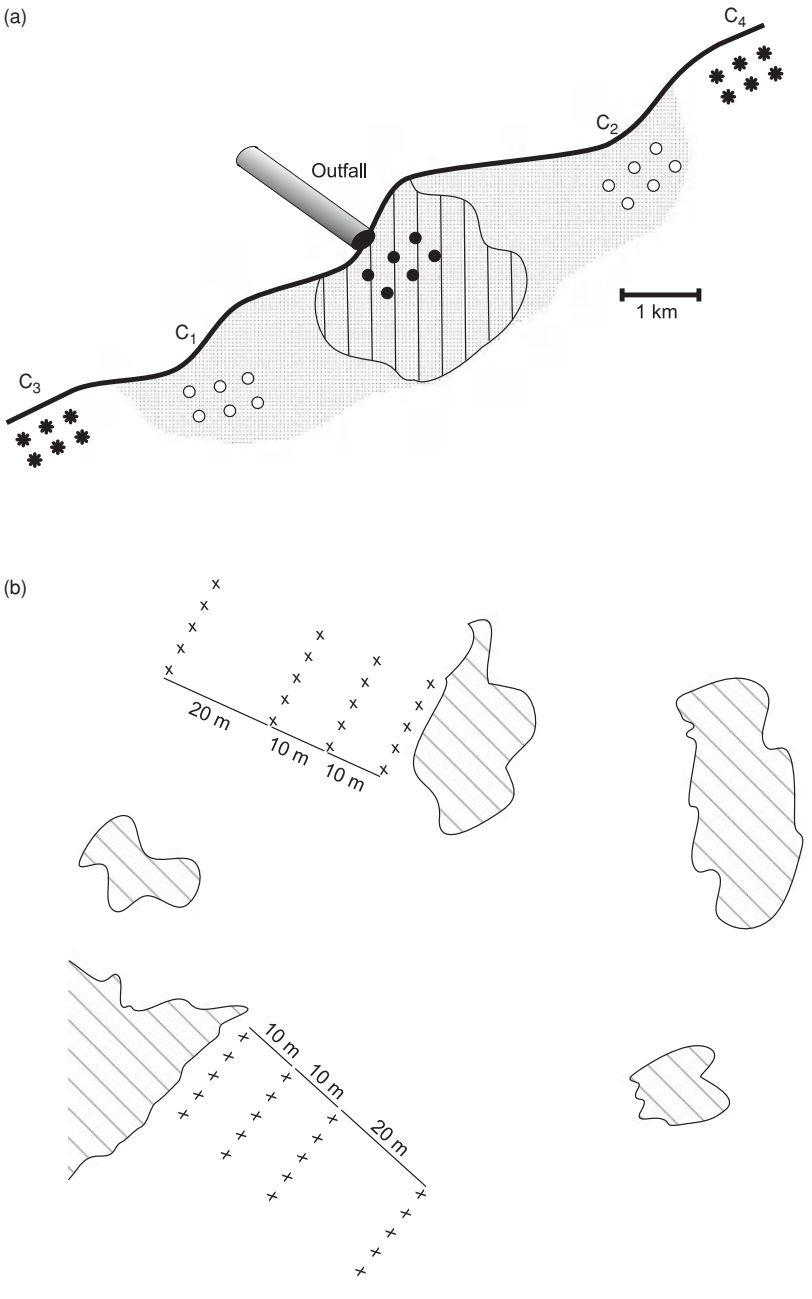

Fig. 1.13 (a) Sampling to handle uncertainty about the scale of an impact due to an outfall. It has been proposed that any impact would only extend over a small distance (stippled), so samples are taken from that area and from controls on either side, outside the area expected to be affected. The area influenced may, however, be much larger (shaded), so other control samples are taken at much greater distances from the outfall. (b) Sampling to test the hypothesis that assemblages in sediments are influenced by the presence of a rocky reef (reefs are shaded). For two replicate reefs, $n = 5$ cores are taken next to the reef (0 m) and at 10, 20 and 40 m from it. The reef will cause samples at 0–10 to differ more than 10–20 or 0–20 to differ more than 20–40 (i.e. more than similar distances where there are no reefs; for more details, see the text).

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from those further away. The reasons for these differences may be physical (waves, water flow, sediments may all be affected by the presence of a reef) or biological (predatory fish are associated with reefs and therefore eat animals close to a reef, but not those further away). In this case, sampling is needed to test the hypothesis that assemblages close to a reef are different from those further away. Samples should then be taken near a reef and at some distance away, say, 10 m (Fig. 1.13b).

The hypothesis is that any difference is greater than normal spatial differences, so a further set of samples is taken 20 m in from the reef. If the reef influences the fauna, the difference in uni- or multivariate measures of a species or the assemblage from the samples at the reef and at 10 m will not be the same as the natural variation at that scale, i.e. the difference from 10 to 20 m. If the scale of potential influences is not known in advance, other distances can be sampled. Thus, a further sample at 40 m allows comparison of the differences between samples at the reef and at 20 m with the natural variation between 20 and 40 m (Fig. 1.13b). A detailed example of the use of this design was described by Kelaher *et al*. (1998) for infauna in sediments near boardwalks in mangrove forests.

The point to be understood is that sampling designs must be modified for each particular situation and the general principle to guide designs must be to have a flexible framework (Green, 1993), responsive to the needs of the particular hypotheses being tested (Underwood, 1997a).

1.13 Combining results from different places or times

Sometimes, a particular study may be quite small so that, taken on its own, it cannot reveal much about a process under investigation. It is, therefore, important to think about mechanisms for combining the outcomes of such studies in a meta-analysis, i.e. a combination of results of similar tests of the same hypothesis in different places and times and conditions. General issues about ecological meta-analyses were pioneered by Gurevitch *et al*. (1992).

Here, some procedures are introduced. First, in many experimental studies, the samples are quite small and the outcomes are not clear, because the tests are not powerful enough to provide unambiguous interpretations. There are two general procedures available for combining the results of several small tests. Let us suppose that the outcomes of experimental treatments (say, mean numbers of some polychaete at five different depths) are available from several small studies (perhaps six different cruises have collected such data, but each only had two or three samples from each depth). The means from the five depths can be ranked from smallest to largest for each study and summed. This gives the frequency out of six studies of each depth having the smallest, second, third, fourth or the largest number of worms. Such data can be analysed by a test devised by Anderson (1959) to determine non-random patterns in the rankings (see Underwood & Chapman, 1992, for a detailed example).

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Second, there may be a test of a certain hypothesis in several studies. Each gives a probability of the data being likely to be due to chance errors in sampling, but only a few (or none) are significant. The probabilities from these small tests can be combined using Fisher's (1935) test

$$C = -2\sum_{i=1}^{k} \log_e (P_i)$$

where k is the number of tests and P_i is the probability from each test ($i = 1, \ldots, k$). C is distributed as χ^2 with $2k$ df if there are, in fact, no differences in the set of tests. Thus, large values of χ^2 would cause the rejection of the null hypotheses over a set of tests. An example is given in Table 1.7.

Finally, in some situations, several small tests have been done, but the meta-analysis requires them to be weighted, for example, to reflect different amounts of habitat across a study area. Suppose, for example, it is proposed that numbers of species of crustaceans are greater in some estuary in areas with seagrasses than in patches of open, sandy sediments among the seagrass beds. Three species of seagrass form beds in the estuary and one of them (*Posidonia*) occurs in densely or sparsely covered beds. Thus, there are four habitats of interest. In the estuary, there are different areas of each type of habitat.

Samples of $n = 5$ replicate cores were taken among each type of seagrass and $n = 5$ cores were taken in bare patches within the bed. For each such pair of samples, a 1-tailed t-test was done. There were significantly more crustaceans among seagrass in only one of the four tests (Table 1.7), but the probabilities were quite small in several tests. To test the hypothesis of a difference between seagrass and bare areas over the whole estuary, the tests were combined by Fisher's (1935) method, giving $C = 18.29$, with 8 df. This was significant at $P < 0.02$, indicating greater numbers of worms among seagrasses across the whole set of data. This test does not take into account the different areas occupied by the different habitats. So, a Stouffer–Liptak procedure (Folks, 1984) was used, which weights the outcomes

Table 1.7 Example (using hypothetical data) of meta-analysis of numbers of worms per core ($n = 5$ cores per sample) among seagrass and in empty patches in seagrass in an estuary. The proportion of all seagrass made up by each habitat is given and is the weighting for the Stouffer–Liptak procedure (for all other details, see text).

Habitat	Proportion (= weight)	Seagrass	Bare patches	$t_{1\text{-tail, 8 df}}$	P	Z_I
Dense *Posidonia*	0.40	62 (10.1)	51 (9.7)	0.79	0.227	0.749
Sparse *Posidonia*	0.30	15 (2.7)	11 (2.9)	1.01	0.170	0.954
Zostera	0.13	23 (3.0)	18 (3.2)	1.13	0.146	1.054
Halophila	0.17	15 (1.5)	9 (1.9)	2.49	0.019	2.075
$C = 18.28$						
$Z_w = 1.98$						

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of tests so that large habitats count for more than do smaller ones in a test of the estuary as a whole. The test statistic is

$$Z_W = \sum_{i=1}^{k} W_i . Z_i \Big/ \sqrt{\sum_{i=1}^{k} W_i^2}$$

where Z_i is the standard normal score for P_i, the probability in each test and W_i, is the weighting for the ith habitat. The weighting is the proportional area of the estuary occupied by that habitat, so that widespread, common habitats count for more in the outcome. The probability of getting a value as large or larger than Z_W can be found from the standard normal distribution. In this case, $Z_W = 1.98$, with $P < 0.03$. There is a major difference between areas with seagrass and empty areas across all habitats. A more detailed example from marine ecological data may be found in McDonald *et al*. (1993).

Thus, even where resources do not allow for large amounts of replication and adequate power, it is possible to combine the outcomes of repeated or similar studies, provided they are designed in logically comparable ways and have specific, well-identified hypotheses so that the procedures for combining them are valid.

1.14 Conclusions

It should be clear from the above that there are important and close connections between biological knowledge about systems, logical development of ideas, models and hypotheses, design of sampling, analysis of data and interpretations of the information. Of course, like everything else in science (and life itself), there will be continuing disagreements about the role and purpose of hypothesis-testing in marine research (see Stewart-Oaten, 1996; Suter, 1996). This can be a rich debate or a sterile one. Here, it would be a distraction, so it will receive no mention. Suffice it to say that those who favour risk analysis in environmental assessments and those who favour estimation of magnitudes of differences among sites, times, habitats, conditions, rather than structured tests of formally explicit hypotheses would surely all agree about the need for care in developing sampling programmes. Without advance thought about appropriate scales of replication, it is no more possible to put valid CI around estimates of mean numbers of animals (or any other parameter being estimated) than it is to do a valid statistical test of some hypotheses about a parameter. Estimators and other describers of the world have the same issues for design as do experimentalists testing hypotheses in advancing scientific understanding.

The issues are similar, whatever problem is being investigated. Biological systems are variable in space and time at many scales, due to many interacting processes. It is the responsibility of biologists and ecologists to understand the

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consequences of such variation and the ensuing interactions and non-independent patterns so that sampling can be planned to take all of the issues into account. Where 'ideal' sampling is not possible, what is uncertain in the data can and must be considered before any conclusions are reached. This will ensure that unplanned difficulties or accidental loss of samples do not prevent interpretations being of value. If major problems are inevitable with some design, alter the design or investigate more preliminary hypotheses in order to unravel the problems.

This introductory assessment of some of the issues is selective and seriously incomplete – there are many other issues. Its purpose is to identify the sorts of issues that should be considered when planning any programme of sampling in marine benthic systems, whatever the issue of concern in the study. Understanding the themes discussed should, at least, provide warnings about pitfalls and some of the vocabulary needed to translate a particular study into meaningful questions to ask statistical advisors.

The problems of pollution, fragmentation and destruction of habitat, overharvesting of resources, restoration of degraded habitats, conservation of biodiversity, control of introduced species, global warming and rises in sea level, etc., are vast and urgent. Never has there been such a need for good scientific understanding and advice about what to do and how, when and where. This science deserves the best scientific practice, so improving logic, design, analysis and interpretation of studies is an urgent and ongoing task for marine scientists. Complacency and unprofessionalism will continue to undermine the role of science and will continue to slow down the implementation of solutions to currently urgent problems. Getting designs of sampling right entails getting problems identified and understood and provides links to valid analysis and interpretation. Improved sampling designs are the key to improved scientific contributions to social needs.

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References

Anderson, M.J. (2001) A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, **26**, 32–46.

Anderson, R.L. (1959) Use of contingency tables in the analysis of consumer preference studies. *Biometrics*, **15**, 582–590.

Andrew, N.L. & Mapstone, B.D. (1987) Sampling and the description of spatial pattern in marine ecology. *Annual Review of Oceanography and Marine Biology*, **25**, 39–90.

Bender, E.A., Case, T.J. & Gilpin, M.E. (1984) Perturbation experiments in community ecology: theory and practice. *Ecology*, **65**, 1–13.

Benedetti-Cecchi, L., Airoldi, L., Abbiati, M. & Cinelli, F. (1996) Estimating the abundance of benthic invertebrates: a comparison of procedures and variability between observers. *Marine Ecology Progress Series*, **138**, 93–101.

Bernstein, B.B. & Zalinski, J. (1983) An optimum sampling design and power tests for environmental biologists. *Journal of Environmental Management*, **16**, 335–343.

Box, G.E.P. & Jenkins, G.M. (1976) *Time Series Analysis: Forecasting and Control*. Holden Day Inc., San Francisco, CA.

Brown, J.H. & Gibson, A.C. (1983) *Biogeography*. C.V. Mosby Co., St Louis, MO.

Caffey, H.M. (1985) Spatial and temporal variation in settlement and recruitment of intertidal barnacles. *Ecological Monographs*, **55**, 313–332.

Cameron, J. & Abouchard, J. (1991) The precautionary principle: a fundamental principle of law and policy for the protection of the global environment. *Comparative Law Review*, **14**, 1–27.

Chapman, M.G. & Underwood, A.J. (1999) Ecological patterns in multivariate assemblages: information and interpretation of negative values in ANOSIM tests. *Marine Ecology Progress Series*, **180**, 257–265.

Chapman, M.G., Underwood, A.J. & Skilleter, G.A. (1995) Variability at different spatial scales between a subtidal assemblage exposed to the discharge of sewage and two control assemblages. *Journal of Experimental Marine Biology and Ecology*, **189**, 103–122.

Clarke, K.R. (1993) Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology*, **18**, 117–143.

Clarke, K.R. & Gorley, R.N. (2006) *Primer V6: User Manual*. Primer-E Ltd, Plymouth.

Clarke, K.R., Somerfield, P.J. & Chapman, M.G. (2006a) On resemblance measures for ecological studies, including taxonomic dissimilarities and a zero-adjusted Bray-Curtis coefficient for denuded assemblages. *Journal of Experimental Marine Biology and Ecology*, **330**, 55–80.

Clarke, K.R., Chapman, M.G., Somerfield, P.J. & Needham, H.R. (2006b) Dispersion-based weighting of species counts in assemblage analyses. *Marine Ecology Progress Series*, **320**, 11–27.

Cliff, A.D. & Ord, J.K. (1973) *Spatial Autocorrelation*. Pion Ltd, London.

Clifford, D.H.T. & Stephenson, W. (1975) *An Introduction to Numerical Classification*. Academic Press, New York.

Cochran, W.G. & Cox, G. (1957) *Experimental Designs*, 2nd edn. John Wiley & Sons, New York.

Cox, G. (1958) *The Planning of Experiments*. John Wiley & Sons, New York.

Dayton, P.K. (1971) Competition, disturbance and community organization: the provision and subsequent utilization of space in a rocky intertidal community. *Ecological Monographs*, **41**, 351–389.

Diggle, P.J. (1990) *Time Series: A Biostatistical Introduction*. Clarendon Press, Oxford.

Dovers, S.R. & Handmer, J.W. (1995) Ignorance, the precautionary principle, and sustainability. *Ambio*, **24**, 92–97.

Durbin, J. & Watson, G.S. (1951) Testing for serial correlation in least-squares regression. *Biometrika*, **38**, 159–178.

https://telegram.me/Geologybooks

Elliott, J.M. (1977) *Methods for the Statistical Analysis of Samples of Benthic Invertebrates*. Freshwater Biological Association Scientific Publication No. 25, Reading.

Fisher, R.A. (1935) *The Design of Experiments*. Oliver and Boyd, Edinburgh.

Folks, J.R. (1984) Combination of independent tests. In: *Handbook of Statistics 4. Non-parametric Methods* (eds P.R. Krishnaiah & P.K. Sen), North-Holland, New York, pp–72–94.

Foster, M.S., Harrold, C. & Hardin, D.D. (1991) Point versus photo quadrat estimates of the cover of sessile organisms. *Journal of Experimental Marine Biology and Ecology*, **146**, 193–204.

Gaines, S.D. & Bertness, M.D. (1992) Dispersal of juveniles and variable recruitment in sessile marine species. *Nature*, **360**, 579–580.

Glasby, T.M. & Underwood, A.J. (1996) Sampling to differentiate between pulse and press perturbations. *Environmental Monitoring and Assessment*, **42**, 241–252.

Gray, J.S. (1990) Statistics and the precautionary principle. *Marine Pollution Bulletin*, **21**, 174–176.

Gray, J.S. (1996) Environmental science and a precautionary approach revisited. *Marine Pollution Bulletin*, **32**, 532–534.

Gray, J.S., Aschan, M., Carr, M.R., Clarke, K.R., Green, R.H., Pearson, T.H., Rosenberg, R. & Warwick, R.M. (1988) Analysis of community attributes of the benthic macrofauna of Frierfjord/Langesundfjord and in a mesocosm experiment. *Marine Ecology Progress Series*, **46**, 151–165.

Green, R.H. (1979) *Sampling Design and Statistical Methods for Environmental Biologists*. John Wiley & Sons, Chichester.

Green, R.H. (1989) Power analysis and practical strategies for environmental monitoring. *Environmental Research*, **50**, 195–205.

Green, R.H. (1993) Application of repeated measures designs in environmental impact and monitoring studies. *Australian Journal of Ecology*, **18**, 81–98.

Green, R.H. & Hobson, K.D. (1970) Spatial and temporal structure in a temperate intertidal community, with special emphasis on *Gemma gemma* (Pelecypoda: Mollusca). *Ecology*, **51**, 999–1011.

Gurevitch, J., Morrow, L.L., Wallace, A. & Walsh, J.S. (1992) A meta-analysis of competition in field experiments. *American Naturalist*, **140**, 539–572.

Hall, S.J. & Harding, M.J. (1997) Physical disturbance and marine benthic communities: the effects of mechanical harvesting of cockles on non-target benthic infauna. *Journal of Applied Ecology*, **34**, 497–517.

Hurlbert, S.H. (2004) On misinterpretations of pseudoreplication and related matters: a reply to Oksanen. *Oikos*, **104**, 591–597.

Hurlbert, S.J. (1984) Pseudoreplication and the design of ecological field experiments. *Ecological Monographs*, **54**, 187–211.

Kelaher, B.P., Chapman, M.G. & Underwood, A.J. (1998) Changes in benthic assemblages near boardwalks in temperate urban mangrove forests. *Journal of Experimental Marine Biology and Ecology*, **228**, 291–307.

Keough, M.J. (1984) Dynamics of the epifauna of the bivalve *Pinna bicolor*: interactions among recruitment, predation, and competition. *Ecology*, **65**, 677–688.

Keough, M.J. (1998) Responses of settling invertebrate larvae to the presence of established recruits. *Journal of Experimental Marine Biology and Ecology*, **231**, 1–19.

https://telegram.me/Geologybooks

Keough, M.J. & Black, K.P. (1996) Predicting the scale of marine impacts: understanding planktonic links between populations. In: *Detecting Ecological Impacts: Concepts and Applications in Coastal Habitats* (eds R.J. Schmitt & C.W. Osenberg), Academic Press, San Diego, CA, pp 199–234.

Lawton, J.H. (1999). Are there general laws in ecology? *Oikos*, **84**, 177–192.

Leduc, A., Prairie, Y.T. & Bergeron, Y. (1994) Fractal dimension estimates of a fragmented landscape: sources of variability. *Landscape Ecology*, **9**, 279–286.

Lindegarth, M., Valentinsson, D., Hansson, M. & Ulmestrand, M. (2000) Interpreting large-scale experiments on effects of trawling on benthic fauna: an empirical test of the potential effects of spatial confounding in experiments without replicated control and trawled areas. *Journal of Experimental Marine Biology and Ecology*, **245**, 155–169.

Mapstone, B.D. (1995) Scalable decision rules for environmental impact studies: effect size, Type I, and Type II errors. *Ecological Applications*, **5**, 401–410.

McDonald, L.L., Erickson, W.P. & Strickland, M.D. (1993) Survey design, statistical analysis, and basis for statistical inference in coastal habitat injury assessment: Exxon Valdez oil spill. In: *Exxon Valdez Oil Spill: Fate and Effects in Alaskan Waters* (eds P.G. Wells, J.N. Butler & J.S. Hughes), American Society for Testing and Materials, Philadelphia, PA, pp 296–311.

Morrisey, D.J., Howitt, L., Underwood, A.J. & Stark, J.S. (1992a) Spatial variation in soft-sediment benthos. *Marine Ecology Progress Series*, **81**, 197–204.

Morrisey, D.J., Underwood, A.J., Howitt, L. & Stark, J.S. (1992b) Temporal variation in soft-sediment benthos. *Journal of Experimental Marine Biology and Ecology*, **164**, 233–245.

Olsgard, F., Somerfield, P.J. & Carr, M.R. (1997) Relationships between taxonomic resolution and data transformations in analyses of a macrobenthic community along an established pollution gradient. *Marine Ecology Progress Series*, **149**, 173–181.

Peterman, R.M. & M'Gonigle, A.B. (1992) Statistical power analysis and the precautionary principle. *Marine Pollution Bulletin*, **24**, 231–234.

Peters, R.H. (1991) *A Critique for Ecology*. Cambridge University Press, Cambridge.

Phillips, D.J.H. (1978) The use of biological indicator organisms to quantitate organochlorine pollutants in aquatic environments: a review. *Environmental Pollution*, **16**, 167–229.

Raimondi, P.T. & Reed, D.C. (1996) Determining the spatial extent of ecological impacts caused by local anthropogenic disturbances in coastal marine habitats. In: *Detecting Ecological Impacts: Concepts and Applications in Coastal Habitats* (eds R.J. Schmitt & C.W. Osenberg), Academic Press, San Diego, CA, pp 179–198.

Resetarits, W.R. & Bernardo, J. (eds) (1998) *Experimental Ecology: Issues and Perspectives*. Oxford University Press, Oxford.

Schmitt, R.J. & Osenberg, C.W. (eds) (1996) *Detecting Ecological Impacts: Concepts and Applications in Coastal Habitats*. Academic Press, San Diego, CA.

Shanks, A.L. & Wright, W.G. (1986) Adding teeth to wave action: the destructive effects of wave-borne rocks on intertidal organisms. *Oecologia*, **69**, 420–428.

Skilleter, G.A. (1992) Recruitment of cerithiid gastropods (*Rhinoclavis* spp.) in sediments at One Tree Reef, Great Barrier Reef. *Journal of Experimental Marine Biology and Ecology*, **156**, 1–21.

https://telegram.me/Geologybooks

Smith, E.P., Orvos, D.R. & Cairns, J. (1993) Impact assessment using the before-after-control-impact (BACI) model: concerns and comments. *Canadian Journal of Fisheries and Aquatic Science*, **50**, 627–637.

Sousa, W. (1979) Disturbance in marine intertidal boulder fields: the nonequilibrium maintenance of species diversity. *Ecology*, **60**, 1225–1239.

Sparks, T. (ed.) (2000) *Statistics in Ecotoxicology*. John Wiley & Sons, Chichester.

Spellerberg, I.F. (1991) *Monitoring Ecological Change*. Cambridge University Press, Cambridge.

Stewart-Oaten, A. (1996) Goals in environmental monitoring. In: *Detecting Ecological Impacts: Concepts and Applications in Coastal Habitats* (eds R.J. Schmitt & C.W. Osenberg), pp. 17–28. Academic Press, San Diego, CA.

Stewart-Oaten, A. & Bence, J.R. (2001) Temporal and spatial variation in environmental impact assessment. *Ecological Monographs*, **71**, 305–339.

Stewart-Oaten, A., Murdoch, W.M. & Parker, K.R. (1986) Environmental impact assessment: 'pseudoreplication' in time? *Ecology*, **67**, 929–940.

Suter, G.W. (1996) Abuse of hypothesis testing statistics in ecological risk assessment. *Human and Ecological Risk Assessment*, **2**, 331–347.

Thrush, S.F. (1999) Complex role of predators structuring soft-sediment macrobenthic communities: implications of changes in spatial scale for experimental studies. *Australian Journal of Ecology*, **24**, 344–354.

Underwood, A.J. (1981) Structure of a rocky intertidal community in New South Wales: patterns of vertical distribution and seasonal changes. *Journal of Experimental Marine Biology and Ecology*, **51**, 57–85.

Underwood, A.J. (1989) The analysis of stress in natural populations. *Biological Journal of the Linnean Society*, **37**, 51–78.

Underwood, A.J. (1990) Experiments in ecology and management: their logics, functions and interpretations. *Australian Journal of Ecology*, **15**, 365–389.

Underwood, A.J. (1991) Beyond BACI: experimental designs for detecting human environmental impacts on temporal variations in natural populations. *Australian Journal of Marine and Freshwater Research*, **42**, 569–587.

Underwood, A.J. (1992) Beyond BACI: the detection of environmental impact on populations in the real, but variable, world. *Journal of Experimental Marine Biology and Ecology*, **161**, 145–178.

Underwood, A.J. (1993) The mechanics of spatially replicated sampling programmes to detect environmental impacts in a variable world. *Australian Journal of Ecology*, **18**, 99–116.

Underwood, A.J. (1994) On beyond BACI: sampling designs that might reliably detect environmental disturbances. *Ecological Applications*, **4**, 3–15.

Underwood, A.J. (1997a) *Experiments in Ecology: Their Logical Design and Interpretation Using Analysis of Variance*. Cambridge University Press, Cambridge.

Underwood, A.J. (1997b) Environmental decision-making and the precautionary principle: what does this mean in environmental sampling practice? *Landscape and Urban Planning*, **37**, 137–146.

Underwood, A.J. (1998) Grazing and disturbance: an experimental analysis of patchiness in recovery from a severe storm by the intertidal alga *Hormosira banksii* on rocky shores in New South Wales. *Journal of Experimental Marine Biology and Ecology*, **231**, 291–306.

https://telegram.me/Geologybooks

Underwood, A.J. (2000) Trying to detect impacts in marine habitats: comparisons with suitable reference areas. In: *Statistics in Ecotoxicology* (ed. T. Sparks), John Wiley & Sons, Chichester, pp 279–308.

Underwood, A.J. & Chapman, M.G. (1992) Experiments on topographic influences on density and dispersion of *Littorina unifasciata* in New South Wales. *Proceedings of the Third International Symposium on Littorinid Biology* (eds J. Grahame, P.J. Mill & D.G. Reid), The Malacological Society of London, London, pp 181–195.

Underwood, A.J. & Denley, E.J. (1984) Paradigms, explanations and generalizations in models for the structure of intertidal communities on rocky shores. In: *Ecological Communities: Conceptual Issues and the Evidence* (eds D.R. Strong, D. Simberloff, L.G. Abele & A. Thistle), Princeton University Press, Princeton, NJ, pp 151–180.

Underwood, A.J. & Peterson, C.H. (1988) Towards an ecological framework for investigating pollution. *Marine Ecology Progress Series*, **46**, 227–234.

Whitlatch, R.B., Lohrer, A.M., Thrush, S.F., Pridmore, R.D., Hewitt, J.E., Cummings, V.J. & Zajac, R.N. (1998) Scale-dependent benthic recolonization dynamics: life stage-based dispersal and demographic consequences. *Hydrobiologia*, **375/376**, 217–226.

Winer, B.J., Brown, D.R. & Michels, K.M. (1991) *Statistical Principles in Experimental Design*, 3rd edn. McGraw-Hill, New York.

Wynne, B. (1992) Uncertainty and environmental learning: reconceiving science and policy in the preventative paradigm. *Global Environmental Change*, **2**, 111–127.

Ysebaert, T. & Herman, P.M.J. (2002) Spatial and temporal variation in benthic macrofauna and relationships with environmental variables in an estuarine, intertidal soft-sediment environment. *Marine Ecology Progress Series*, **244**, 105–124.

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Chapter 2

Characterising the Physical Properties of Seabed Habitats

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Abstract

This chapter describes some of the most important practical methods for characterising the seabed physical environment, the habitats and their sedimentary properties. It does this on two levels: (i) describing the principal remote acoustic sensing techniques that have the ability to characterise the seabed environment over large spatial scales and (ii) habitat and sediment properties determined by point sampling typically with cores, grabs and underwater cameras.

The chapter aims to provide basic practical guidance compatible with biological studies and their implementation along with references to sources of more detailed information. Therefore, its primary aim is to allow workers to define recent surface sediment substrates, associated with biological studies and, possibly, to infer ecological information from a knowledge of the sediment properties in a particular environment. It also provides some guidance on acoustic survey design considerations based upon the power of acoustic systems to detect and discriminate seabed features of different spatial scales and patchiness.

Keywords bathymetry, sediment, acoustic characterisation, seabed, habitat

2.1 Introduction

The physical nature of the seabed environment (defined by its geological origin, sediment and substrate type, topography, depth, light availability, temperature and exposure to the combined effects of wave and tidal action) plays a vital role in determining the structure and function of benthic communities. The importance of such abiotic factors in determining the status of benthic communities is well

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known, to the extent that the physical characteristics of the seabed environment can often be used to provide a good estimate of the types of benthic community likely to be found inhabiting the seabed. This chapter focuses on describing practical methods for characterising the seabed physical environment, the habitats and their sedimentary properties. It does this on two levels: (i) describing the principal remote acoustic sensing techniques that have the ability to characterise the seabed environment over large spatial scales and (ii) habitat and sediment properties determined by point sampling typically with cores, grabs and underwater cameras. Both approaches complement one another: point sampling provides direct information for comparison with biological data in samples as well as 'ground truth' for the remotely sensed methods, whereas remotely sensed surveys put 'point sample' information into perspective, i.e. in relation to the broad spatial distribution of sediment properties, benthic habitats and their patchiness.

This chapter is not intended to provide a thorough critique of acoustic surveying or sedimentological analytical methods, but rather it aims to provide basic practical guidance compatible with biological studies along with references to sources of more detailed information. Its primary aim is to allow workers to define recent, surface sediment substrates associated with biological studies and, possibly, to infer ecological information from a knowledge of the sediment properties in a particular environment.

The sediment composition and nature of the seabed (e.g. topography) at a given site depends on the source material, whether geological or biological in origin or both. It depends crucially on the energy (waves, tides, currents) of the environment and the balance between erosion and accumulation (or accretion). For a discussion of physical processes that govern sediment transport and the bedforms they create, see Dyer (1979) and Soulsby (1997). However, a key factor affecting sediment properties is the biota itself. Various components of the biota can destabilise sediments through burrowing and feeding, while others act to stabilise and modify the substrate through root development, construction of worm tubes, faecal pelletisation and secretion of Extracellular Polymeric Substances (EPS) (Decho, 1990; Paterson, 1997). Seasonal and climatic changes in the balance between these processes can significantly influence sediment erosion and accretion processes in estuaries (Widdows *et al.*, 2000). Both physical and biological aggregations of grains modify the size distribution and properties of sediment. Furthermore, particularly in silt-rich sediments, the adsorption of organic compounds at particle surfaces and electro-static, inter-particle attractions means that these sediments behave cohesively rather than as individual grains. In classical sediment petrology (where most of the practical techniques for sediment grain size analysis were developed), the analyst generally seeks to remove organic material from samples in order to describe the population of primary mineral grains within the sediment. However, there is now a growing appreciation that for biological and environmental sedimentology, the *in situ*, natural properties of the sediment when measured at a range of spatial scales are more relevant for interpreting the physical behaviour

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of the sediment and animal–sediment relationships than those derived from the petrology-based analysis of mineral grain sizes and properties alone.

2.2 Remote acoustic methods for surveying the seabed

Acoustic sampling techniques can generate images of the seabed that reveal their geo-physical characteristics and allow inferences to be drawn regarding their geological origins and modern day (Holocene) sedimentary processes. The interpretation of the acoustic images can be used to generate maps of seabed features, which can make an important contribution to studies of benthic communities, survey planning and sampling design as well as allowing the evaluation of natural and man-made disturbances of the seabed over large spatial scales. When used in conjunction with 'ground-truthing' methods (e.g. grabs, corers and underwater photography), remote acoustic sensing enables the delineation of habitats (mainly physical attributes) and their associated biological communities. This section describes the principal techniques for remote acoustic sensing of seabed topography (bathymetry) and of the physical properties of seabed sediments.

Background

In general, acoustic remote seabed mapping or sensing instruments fall into one of two generic categories, namely (i) low-grazing angle *swathe* systems (e.g. SideScan Sonars (SSS); Newton & Stefanon, 1975; Fish & Carr, 1990; Green & Cunningham, 1998; Kenny, 1998; Coggan *et al.*, 2007) and (ii) *normal* incidence beam formation echo-sounders (single- or multi-beam systems; Burns *et al.*, 1985; Chivers *et al.*, 1990; Loncarevic *et al.*, 1994; Magorrian *et al.*, 1995; Greenstreet *et al.*, 1996; Foster-Smith & Gilliland, 1997; Hughes Clarke, 1998; Fernandes & Chakraborty, 2009). Systems that process normal incidence beam data for habitat classification purposes are generally referred to as Acoustic Ground Discrimination Systems (AGDS). The distinction between the two types of sonar is very important since they insonify the seabed in different ways and, therefore, the outputs between the systems require different interpretation methods. For example, the low-grazing angle (broad-beam) swathe systems insonify, as the name implies, a wide swathe of seabed due to their low-grazing angle, but the beam is narrow in azimuth (i.e. as you look down on the beam it is narrow). In order to achieve the low-grazing angle the sonar transducer/receiver has to be relatively close to the seabed and is generally towed rather than mounted on the ship's hull. The advantage of this configuration is that small objects protruding from the seabed cast large acoustic shadows. Therefore, the acoustic geometry of the sonar footprint makes 'swathe' sidescan systems most suitable for detecting small objects on the seabed including changes in bed roughness. The echo-sounder systems, or AGDS, by contrast, have normal incidence beams to accurately quantify changes in bed level or bathymetry.

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To achieve good object detection the beam geometry must be narrow, which is in direct contrast to the SSS systems.

Recent developments in swathe system post-processing are enabling classification of the returned echoes into seabed types and, therefore, can be termed swathe-based AGDS, but for the purpose of comparison and to distinguish between the two systems, we describe AGDS as single beam echo-sounders, with swathe classification systems described separately.

Each of the different systems has their advantages and disadvantages and the most suitable technique or combination of techniques depends upon the requirements and constraints of each specific survey. Increasingly, the 'collect once, use many times' philosophy is being adopted and this enables multiple systems to be deployed in combination with each other as data formats and processing techniques allow for much greater integration of system level outputs.

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Echo-sounders (single beam systems)

Single beam echo-sounders vary considerably in their sophistication and cost, yet all function essentially in the same way: the transducer converts an electrical pulse into a mechanical pulse that creates a sound wave that is directed towards the seabed (Figs. 2.1a, b). The pulse is reflected off the seabed and the transducer acts as a receiver converting the mechanical energy into electrical energy. The elapsed time is converted into a measure of distance. The echo-sounder leaves enough time to receive the echo before transmitting another pulse and, since this will vary with depth, many echo-sounders automatically adjust the ping rate to the depth range set. Since signal strength is dissipated as it spreads out and becomes weaker with depth, echo-sounders also adjust signal strength according to the depth range. This is not an issue when depth alone is recorded, but it does affect measurement of the echo return signal, which has implications for processing the signal if quantitative sediment discrimination is required.

The beam angles are designed into the transducer and vary considerably between systems. The beam can conveniently be thought of as a cone of sound whose base (the footprint) increases with depth. Echo-sounders primarily designed for depth measurement have narrow beams with minimal sidelobes resulting in a relatively narrow footprint, while those suitable for AGDS (see the section entitled 'Acoustic ground discrimination systems based on single beam echo-sounder') might have a wider beam angle and sidelobes to increase the backscatter of the signal from the seabed (Fig. 2.1b).

Acoustic ground discrimination systems based on single beam echo-sounder

Acoustic ground discrimination systems based upon single beam echo-sounders are designed to detect different substrates by measuring differences in their acoustic reflectance and absorption properties. For example, hard surfaces are good reflectors

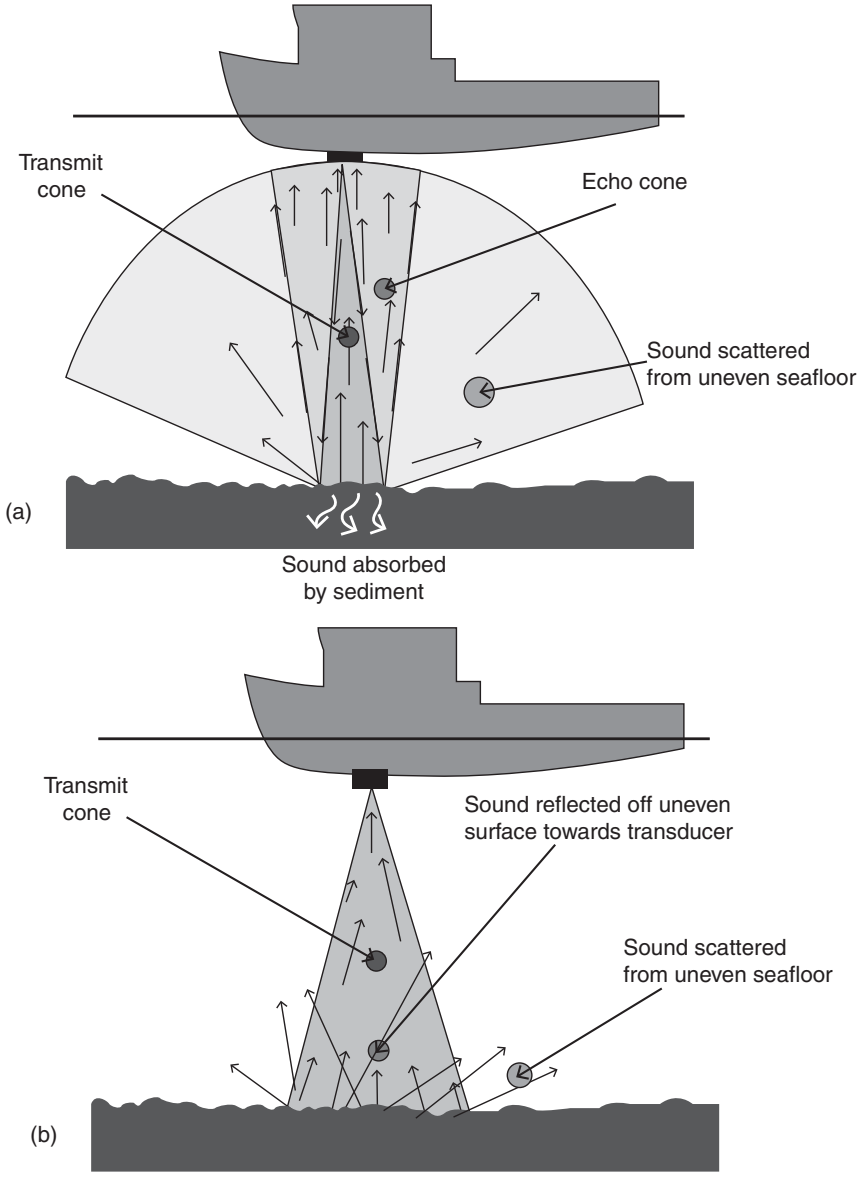

Fig. 2.1 (a) The transducer transmits a 'cone' of sound into the water. Some of this energy is absorbed by the sediment but most is reflected back, still maintaining the geometry of the transmit cone. However, some of the sound is scattered in all directions by the uneven surface of the seabed. The sound received by the transducer is only a small proportion of the original energy from the centre of the cone. (b) Sound energy from echo-sounders with a wide beam angle and sidelobes have the potential to reflect off uneven surfaces of the seabed at the outer margins of the footprint and for this energy to be directed back towards the transducer.

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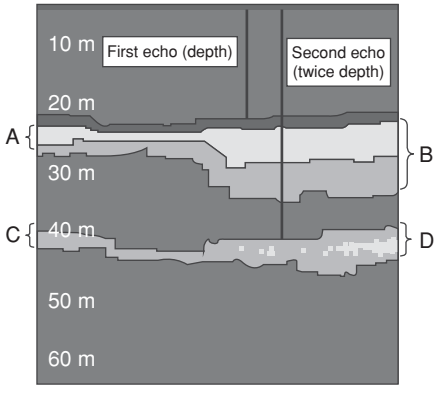

Fig. 2.2 A typical screen display of an echo-sounder (signal strength decreasing from red to blue) as though a vessel had passed over soft, smooth ground onto hard, rough ground. 'A' is the small tail of the first echo (smooth surface); 'B' is a more extended tail (rough surface); 'C' is a weak second echo (soft material) and 'D' is a stronger second echo (hard material). Overall this output describes a transition from a smooth soft seabed to one that is relatively rough and hard. (For a colour version of this figure, see Plate 2.1.)

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resulting in strong echoes, while soft surfaces absorb sound and give weak echoes. Additionally, smooth surfaces reflect sound away from the vertical line of travel between seabed and transducer so that the echo detected by the transducer is of short duration. Conversely, rough surfaces will reflect some of the sound travelling away from vertical back towards the transducer. Rough surfaces, therefore, can give an echo with a long duration (or 'tail') (Fig. 2.2).

The manner in which AGDS respond to hard or soft, and rough or smooth ground varies between types of system. The first AGDS was *RoxAnn*™ (Sonavision, Aberdeen), which is an analogue system that detects the tail of the first signal (first echo, E1), which is a measure of roughness (see Fig. 2.1b). Hardness is measured not by the strength of the peak of the first echo but instead by the full strength of the second echo. This results from the echo rebounding from the sea surface and back to the seabed for a second time to be received by the transducer (second echo, E2).

RoxAnn is designed to be a real-time prospecting tool with the capability of predicting sediment type and users can segment the XY plot to represent different sediments. However, it is probably more successfully used as a tool that provides empirical data requiring ground-truthing. Real-time display is still a useful check on performance during the survey, but full analysis on E1 and E2 is undertaken post-survey.

Other AGDS are also available. *Echo Plus*™ (SEA Ltd, Bath) is designed to be a digital version of *RoxAnn*. Another system in common use is *QTC View*™ (Questor Tangent Corp, Sidney, Canada). This converts the echo into a digital format and the software then uses a number of algorithms to extract parameters of the echo.

When AGDS are deployed, care should be taken to ensure that no other acoustic systems deployed interfere with the system and that any gains, ranges and pulse lengths are not altered during the survey. The data are essentially point records that are collected as the vessel tracks across the area of interest. Track spacing, direction, speed and orientation should be planned to best encompass the area and features of interest and allow for consistency.

Sub-bottom profiling

Sub-bottom profiling systems obtain data on the vertical layers of sediment or bedrock strata below the seabed. They are, essentially, powerful low-frequency echo-sounders that create enough energy to penetrate the sediment. The sediment consists of different density layers and at each boundary some of the sound will be reflected back. Eventually, all the energy is reflected or absorbed. The transducer samples the returning echo and differences between their signal strengths and timing provides a real-time display of the sediment vertical structure or profile.

Sub-bottom profilers can be towed, hull- or pole-mounted either on a surface vessel or attached to a Remotely Operated Vehicle (ROV). They typically use frequencies ranging between 3 and 50 kHz with high intensity (or energy). Lower frequencies have greater ability to penetrate the sediment than higher frequencies. For environmental studies where the surface layers of sediment (<1 m) are of greater ecological significance than deeper sediment (>1 m), frequencies between 100 and 150 kHz are most appropriate. Lower-frequency systems typically involve towing arrays of pneumatic air guns and 'streams' of hydrophones to detect the reflected sound. The air guns emit pulses of predominantly low-frequency (10–300 Hz), high-intensity (215–250 dB) sound.

Sidescan sonar

Sidescan sonar (SSS) gives an image analogous to a monochrome photograph of the seabed. Because the sound transmitted from the sidescan hits the sea at an oblique angle, the image reveals topographic features with objects casting acoustic shadows. Sediments with different reflectance properties can also be distinguished by changes in the strength of the return signal. A hard, reflective sediment (such as gravel, rock, shells, etc.) will result in strong acoustic backscatter, while softer substrates with higher porosity (fine sand and mud) will generate a weaker acoustic return. SSS systems have two transducers, one on either side, and each emits a single pulse that is fan-shaped: wide across the track (typically 120°) and narrow along the direction of travel (Fig. 2.3).

The transducers then continuously sample the returning echo over time and record the signal strength. Each successive 'acoustic ping' adds a new line to the top of the display so that the image of the swathe scrolls downwards – termed a 'waterfall' display (Fig. 2.4). Although the images seem very similar to photographs,

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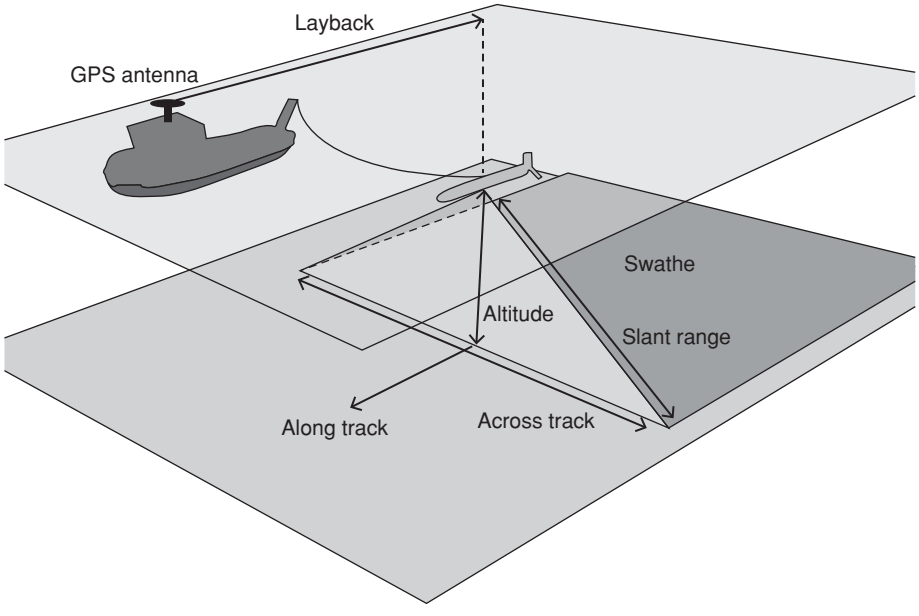

Fig. 2.3 Standard deployment and geometry for a typical sidescan sonar system. (For a colour version of this figure, see Plate 2.2.)

differences in the way sound behaves in water compared to light result in many subtle differences that require careful interpretation. It is important to understand the geometry of the signal to appreciate that objects may appear very different depending upon towfish height above the seabed (altitude), the pitch and yaw of the fish.

The ability of the sidescan to detect objects depends on the height of the object and the grazing angle of the sound to that object. Close to the nadir (the position

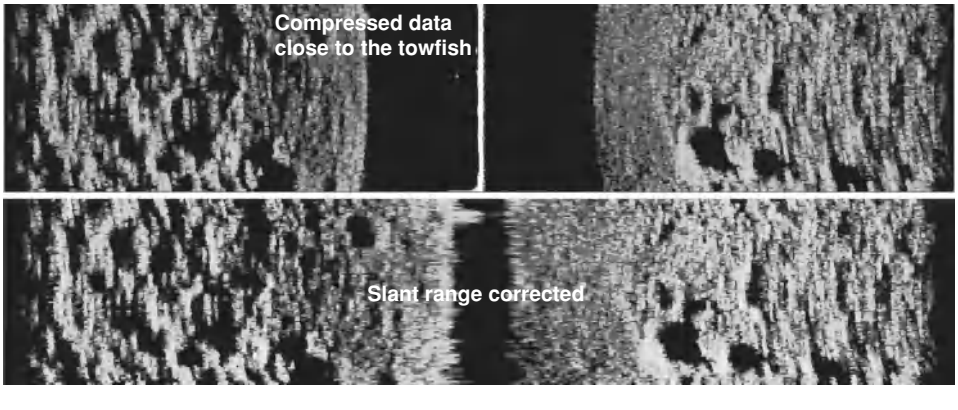

Fig. 2.4 Illustration of a waterfall display without bottom tracking and slant range correction (top) and with correction applied (bottom). (Klein Associates.)

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Table 2.1 The object resolution versus range for two sidescan sonar systems.

Range (m)	Spacing between soundings (m) at 4 knots	120 kHz sidescan 0.75° beam width (m)	330 kHz sidescan 0.3° beam width (m)
25	0.07	0.33	0.13
50	0.13	0.65	0.26
100	0.26	1.30	0.52
200	0.52	2.60	1.00
500	1.30	6.50	n/a

Source: Adapted from Kenny *et al*. (2003).

on the seabed directly under the fish), the grazing angle is high and objects throw small shadows or none at all. The grazing angle reduces further towards the edges of the swathe and longer shadows are thrown. Additionally, there will be distortion due to the geometry of the slant range. Objects close to the nadir are displayed closer together than objects towards the edges of the swathe.

Sidescan sonar systems are manufactured to operate at a set frequency or combination of frequencies. Frequencies range from 50 to 500 kHz or higher. In general, there is a trade-off between the area that can be mapped in a given time and the resolution or detectability of seabed features within the mapped area (see Table 2.1). The new generation of multi-beam and synthetic aperture SSS greatly reduce the trade-off between resolution and coverage by using beam steering and focusing techniques to generate several acoustic beams on each side of the sonar fish. Adjustments to the range, pulse width and gain of the systems can be made to obtain the image best suited to the survey purpose and local conditions of operation (e.g. sea state). These parameters should all be recorded as part of the metadata to support post-survey data processing.

Most sidescan systems are towed at depth with the ideal height of the fish above the seabed (the altitude) for target detection being between 6 and 15 m. Towing at a low altitude means the cable must be paid-out or taken-in according to changes in depth, with a log kept of cable length in order to calculate the layback of the fish.

Advanced sidescan and towed 'fish' systems have heading sensors and can be fitted with an Ultra-Short Baseline acoustic Locator (USBL) to accurately position the fish, but these are not used as standard on many environmental sidescan surveys (James (2007), cited in Coggan *et al.* (2007)). Thus, accurate location of the sidescan image is usually limited to the geometric calculations based on layback, vessel heading and position. Tow speeds are relatively slow in comparison with other acoustic systems, as faster speeds reduce the quality of data collected. Surface conditions influence sidescan data, and deteriorating sea state reduces the quality due to heaving of the towfish cable. Full coverage is obtained by running parallel lines with some overlap to ensure that there are no gaps in the data. However, some surveys may not have the resources for full coverage and widely spaced tracks may be used. Orientation of the lines is important for the detection of features:

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linear features (such as sand waves) oriented across the tracks may go undetected, but will be very conspicuous when tracks are run in line with them; therefore, including cross-track lines is important when designing a sidescan survey to ensure that features are not missed.

Sidescan data have traditionally been recorded on paper records with notation but electronic data storage is becoming standard, which means a detailed written log should be kept during the survey.

Swathe bathymetry

Swathe bathymetric systems are essentially multiple single beam echo-sounders that are capable of ensonifying a swathe of seabed with a 100% footprint comparable to high-resolution SSS (Fig. 2.5). Essentially, there are two main types of swathe bathymetric systems: (i) Multi-Beam Echo-Sounders (MBES), also known as 'beamforming' systems, and (ii) interferometric systems, also known as interferometric bathymetric sidescan, multi-beam SSS or Phase Differencing Bathymetric Sonar (PDBS).

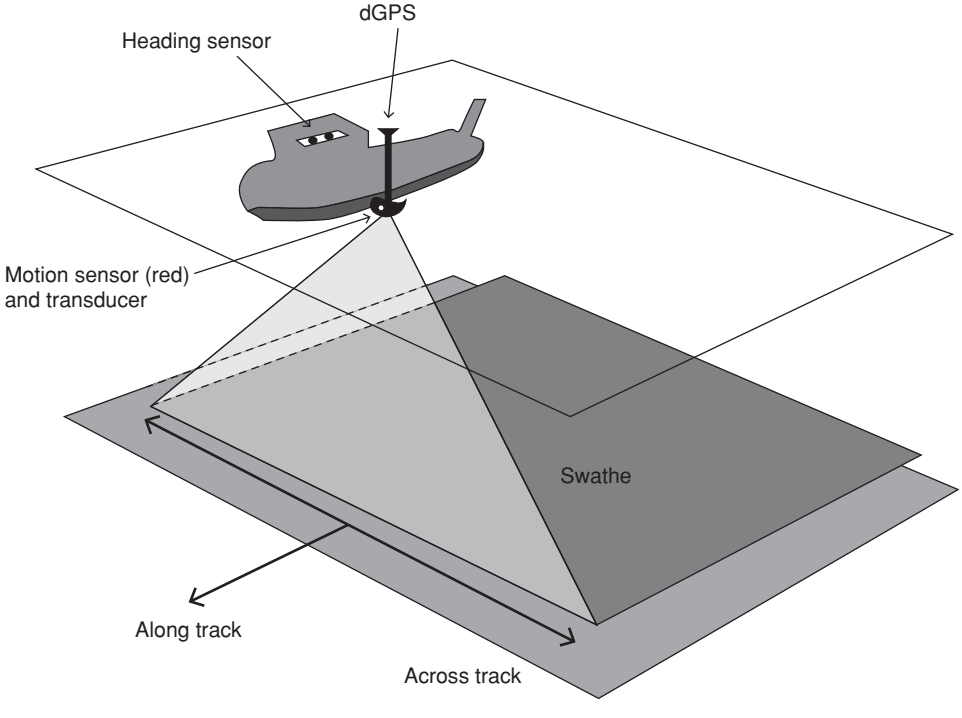

Fig. 2.5 A typical set-up for a pole-mounted swathe bathymetric system. Offsets are measured for heading/dGPS sensors and transducers. Motion sensors are precisely positioned within the transducer V-plate. (For a colour version of this figure, see Plate 2.3.)

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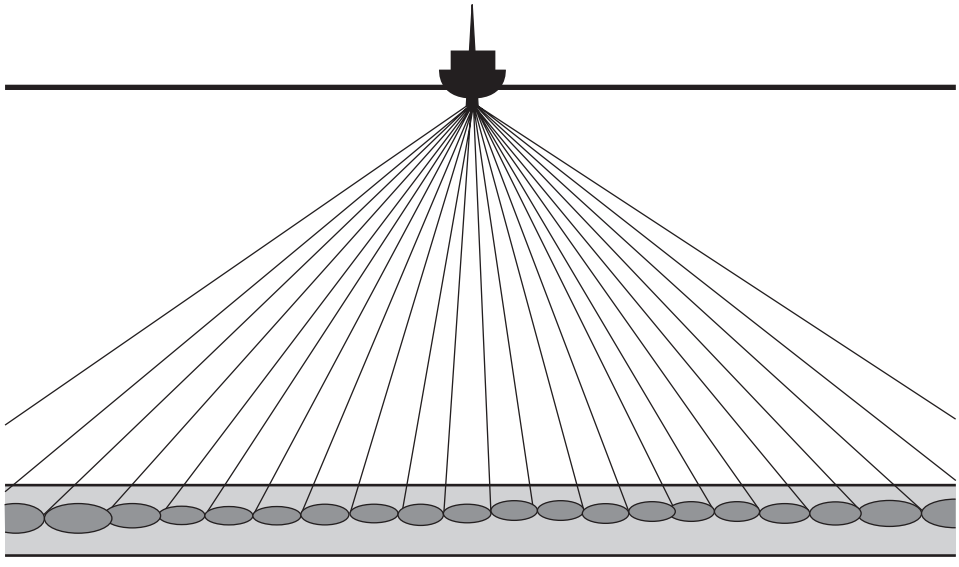

Fig. 2.6 Beams with equal angles arranged in a fan shape in a typical Multi-Beam Echo-Sounders (MBES) system. The soundings and backscatter values are measured from their footprints and these become more widely separated towards the outside of the swathe; for example, horizontal target precision tends to decrease at increasing distance from the nadir (centre line).

Multi-beam echo-sounder systems

Multi-beam echo-sounder systems function using separate beams (formed electronically from the transducer elements) that fan out on either side of the vessel perpendicular to the direction of travel at precise angles (Fig. 2.6). These beams reflect from the seabed and are received by the sonar head and the slant range is calculated from the time lag between transmission and receiving signal. The true range is then calculated by knowing the beam angle and slant range. However, due to the relatively high angle of incidence (low sonar grazing angle) of the multiple sonar beams, the target recognition and ability to resolve small seabed features (<1 m in horizontal distance) is generally less than a high-resolution SSS system. Nevertheless, the primary advantage of using an MBES is the quantitative nature of the data, which can resolve relatively small (<30 cm) vertical (bathymetric height) differences over wide areas. Such precision in bathymetric measurements across a wide swathe of seabed makes MBES systems ideal for generating high-resolution maps of bathymetry. More recently, the nature of the returning echo or 'backscatter' has been used to describe certain characteristics of the seabed environment (Fernandes & Chakraborty, 2009) such as sediment type. In many cases, combined high-resolution bathymetry with backscatter analysis provides a detailed representation of the seabed environment suitable for survey planning, data overlay and habitat mapping purposes.

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Table 2.2 The relative performance of two multi-beam echo-sounder systems.

Water depth (m)	Spacing between soundings at 12 knots (m)	EM 1000 3.3° beam			EM 1000 1.5° beam		
		Footprint (m) nadir	Footprint (m) 30°	Footprint (m) 75°	Footprint (m) nadir	Footprint (m) 30°	Footprint (m) 75°
50	1.6	2.9	3.3	12	1.3	1.5	5.0
100	3.2	5.8	6.6	24	2.6	3.0	10.0
200	6.4	11.6	13.2	48	5.2	6.0	n/a
500	16	29	33	n/a	n/a	n/a	n/a
1000	32	58	66	n/a	n/a	n/a	n/a

Source: Adapted from Kenny *et al*. (2003).

In most MBES systems, the beams are arranged with equal angles between adjacent beams so that the density of soundings across the swathe are highest directly under the vessel (the nadir) and decrease with distance from the vessel (as the grazing angle increases). However, some modern MBES account for this in the beam formation to ensure even density and ensonification of the seabed. The data can also be less accurate at the edges of the swathe due to the accentuation of the uncertainties in motion of the beam at the outer margins of the swathe. The arc of the fan and the geometry of the beams vary between systems but generally the swathe width can be four times the water depth.

Multi-beam echo-sounders are available at a variety of frequencies, which affect the performance of the systems (Table 2.2), with the most appropriate system being used for survey requirements.

Interferometric systems

Multi-beam sidescan sonar or Phase Differencing Bathymetric Sonar (PDBS) operates like standard SSS, ensonifying the seabed with a narrow, fan-shaped beam, and recording the reflected echoes as a uniformly sampled time series. The array consists of a number of parallel elements (or 'staves'); on each side of the sonar head there is usually one transmit stave and two or more receive staves (Fig. 2.7). The time taken for the return signal to reach the receiver is used to calculate the range. Comparing the phase of the return signal at each stave relative to the others is used to calculate the angle between the direction of the acoustic signal and the transducer head. The corrected true range of each sounding can then be calculated in the same way as for MBES.

Interferometric systems provide 100% coverage of the seabed; however, as with MBES, the quality and precision of bathymetric data decreases with increasing angle of incidence, i.e. towards the farther extents of the swathe. Interferometric swathe widths tend to range between 10 to 12 times water depth, thereby providing very efficient data acquisition in shallow water (<40 m), which more closely resembles the type of swathe width obtained by SSS systems. A simple way to compare and contrast MBES with PDBS systems is to think of the MBES systems as mainly providing high-resolution bathymetric data with some acceptable

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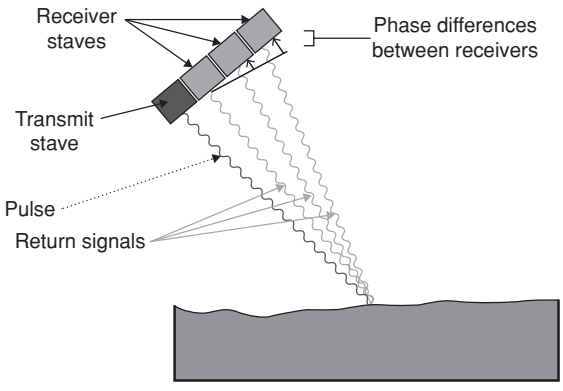

Fig. 2.7 An interferometric array with three receiver staves and the transmit stave. The diagram shows just one pathway of sound (dotted-line arrow) and the paths of reflectance (full-line arrows) that reach the receiver staves from among the whole fan of transmitted sound. There is a phase shift at each receiver and this is measured to calculate the grazing angle and combined with the elapsed time for that particular sound wave to determine the horizontal distance of the sounding.

quality sidescan 'backscatter', whereas PDBS systems provide good-quality sidescan 'backscatter' with some acceptable quality bathymetry.

Both MBES and PDBS systems are mounted on the vessel, either installed permanently or temporarily rigged to a vessel's bow or side. Swathe systems require peripheral equipment to record the vessel's position, heading and motion so that the soundings can be accurately located in their true geographic location. The speed of sound is recorded and used to compensate for the bending of the sonar beams through the water column. Tidal corrections must also be applied. Detailed operational guidelines and procedures are defined for the use of these systems for hydrographic surveys (General Instruction for Hydrographic Surveyors (GIHS), International Hydrographic Organisation (IHO) and national or regional organisations) and these can be used for benthic environmental surveys, especially if the resulting data are intended to meet hydrographic standards as well as environmental survey. Note that the IHO standards can be relaxed for purely environmental surveys and this allows surveys to continue to collect adequate data, which might otherwise halt a hydrographic survey.

Speed of survey is important for data quality: fast vessel speeds will result in a lower density of soundings than slow speeds. Thus, speed is a trade-off against data quality and will need to be considered when allocating resources.

Speed, track spacing and orientation should be planned to provide adequate or better data for survey requirements, as data coverage and resolution are affected, and the most appropriate plan should be adopted.

Acoustic survey design considerations

As a general rule, the survey effort (per unit area) will vary according to the desired resolution and size of area to be sampled (Fig. 2.8), which in turn are dependent on the objectives and requirements of the survey.

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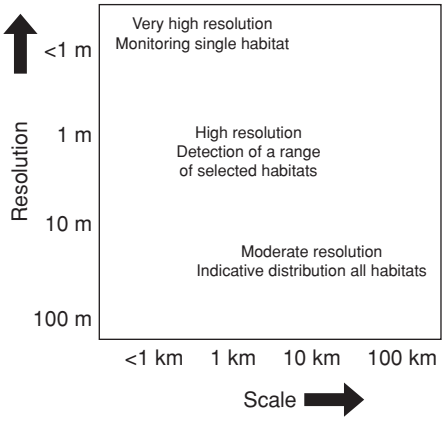

Fig. 2.8 Relationship between survey sample resolution and assessment area or scale of survey to be undertaken.

However, comprehensive (100%) coverage of large areas may not be efficient with some instruments due to their mode of operation unless compromises are introduced into the design of the survey such as representative but widely spaced survey lines. The trade-offs between the unit area surveyed and resolution for different acoustic gear types is shown in Table 2.3.

The main consideration will be what sediment properties and features are of primary interest. For general biological habitat mapping, these will be depth (and slope), bulk sediment properties (e.g. grain size and silt content), habitat patchiness and fine-scale features and the presence of larger features that might indicate particular hydrodynamic regimes of importance. A summary of the main techniques with respect to feature detection is given in Table 2.4, which highlights that no single technique can provide all the information that might be needed in all situations. Therefore, it may be entirely justifiable to use all multiple techniques (e.g. SSS, MBES and AGDS) at the same time.

Table 2.3 Area of seabed mapped (per unit of effort) versus resolution for different remote acoustic methods.

System	Area mapped ($km^2\ h^{-1}$)	Horizontal resolution (m)					
		1000	100	10	1	0.10	0.001
Interferometric swathe	15	•	•	•	•	•	
Chirp SSS	10		•	•	•		
MBES	5	•	•	•	•	•	
SSS	3.5		•	•	•	•	•
Line bathymetry	1.5		•	•	•	•	
AGDS	1.5		•	•	•	•	
High-resolution sub-bottom profiler	0.8		•	•	•	•	
Scanning sonar	0.1			•	•	•	•

Source: Adapted from Kenny *et al.* (2003).
SSS, SideScan Sonar; MBES, Multi-Beam Echo-Sounder; AGDS, Acoustic Ground Discrimination Systems.

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Table 2.4 The powers of systems to detect and discriminate depends upon the nature of the features or properties and their scale and patchiness.

Scale	Feature	Echo-sounder	Acoustic Ground Discrimination Systems (AGDS)	Sidescan	Multi-beam
All scales	Sediment bulk properties (homogeneous)	No	Good powers to discriminate, but needs adequate ground-truthing. Not recommended as a prospecting tool without sampling	Can discriminate broad sediment types where these contrast distinctly, but difficult where gradual change. Can be confused by changes in topography	Backscatter can discriminate broad sediment types. Possibly suitable for automated classification
	Patterns or 'texture' of mixed sediment (heterogeneous)	No. However, fisheries sonar may detect vegetation	Can detect along-track variability at a low resolution	Good at detecting fine-scale variability, but needs ground-truthing as different ground types can result in similar texture	Speckling of backscatter may indicate fine-scale texture, but near limits of resolution
Fine scale (m)	Ripples	No	Not unless ripples change roughness index. Any apparent change would need ground-truthing	Yes if tracks suitably oriented. Best resolution without slant range correction	May be near limits of resolution
	Small waves	If tracks oriented across features	If tracks oriented across features	Yes if tracks suitably oriented	Yes. Small linear waves make distinctive pattern
	Patchy biogenic mounds	Unlikely	If features linked to changes in roughness, but require adequate ground-truthing	Yes if tracks suitably oriented and features clearly delineated from background sediment. May be confused with other fine-scale features	Yes if features larger than variability in soundings (20 cm). Backscatter resolution at limits for detection of textures of patchy biogenic structures

(*Continued*)

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Table 2.4 (*Continued*)

Scale	Feature	Echo-sounder	Acoustic Ground Discrimination Systems (AGDS)	Sidescan	Multi-beam
Medium scale (10s m)	Large waves and troughs	Yes if tracks cross features. May be seen in interpolated grids	Yes if tracks cross features and especially if features are associated changes in sediment. May be seen in interpolated grids	Yes if tracks suitably oriented in line with features. Profile in waterfall display may reveal waves if tracks cross features	Yes. Sun illumination will display these features well
	Patches: riffles	No	Yes if associated with sediment changes and tracks happen to cross feature. Tracks may straddle individual feature	Yes if associated with sediment changes	Yes if associated with slight changes in depth and/or sediment changes (backscatter)
	Linear gravel/sand streaks	No	Yes if tracks sufficiently close to follow streaks across intertrack spaces	Yes	Yes. Backscatter will detect changes in sediment
Broad scale (100s km)	Banks, basins and troughs	Yes. Interpolation useful	Yes. Interpolation useful	May not detect large features associated with gradual changes in depth	Yes

However, this may not be practical for a number of reasons. Firstly, there is a cost attached to the use and analysis of each data acquisition system. Secondly, it may be difficult to assemble a survey team that can combine all the expertise needed. Thirdly, the scope for deployment might be even more limited by other survey demands (such as seismic survey).

Multi-beam and high-quality sidescan along with associated processing software is probably equally expensive, though AGDS is substantially cheaper. Sidescan has the highest resolution, but performs only marginally better than multi-beam for most habitats. AGDS has the lowest resolution, but is good at discriminating bulk sediment properties. However, advances in the analysis of multi-beam backscatter may rival AGDS in this aspect. Lastly, multi-beam and AGDS are hull- or pole-mounted and can be used at speeds greater than 5 knots, while sidescan is towed at slower speeds and runs a greater risk of snagging fishing gear. The use of towed gear is particularly fraught for surveying in the hours of darkness.

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2.3 Particle (grain) size analysis

Grain size analyses define the sedimentary environment and give an insight into the physical regime. Although there is a wide range of techniques available for grain size analysis (Lloyd, 1985; McCave & Syvitski, 1991), there are fewer techniques that are appropriate for aquatic sediment samples (Kramer *et al*., 1994). Grain size analysis attempts to define the dimensions of a particle, or population of particles, using a single parameter and, except for spherical particles, this always involves compromise since cylinders, rectangles, pyramids, plates and irregular shapes, all require more than one dimension to define their size. Additionally, various analytical methods measure different characteristics of particles in attempting to define their size. For example, with microscopy, the long and short axes of a plan view are averaged to give a diameter. The vertical dimension is assumed to be similar though it is probably less than the cross-section because the particle will tend to rest in its most stable attitude. Electro-sensing devices measure the true particle volume irrespective of the shape of the particle and assume the particle to be spherical in assigning a dimension. This value is the Effective Spherical Diameter (ESD). Sieves measure the smallest section of a particle since elongated particles can pass through a mesh lengthwise. For sand, sphericity is a reasonable assumption but for silt-sized material containing clay platelets and irregular biogenic particles and debris, this assumption is less valid. There is a range of definitions for particles that attempt to accommodate shape factors (Allen, 1990), but for most purposes the assumption of sphericity and the use of the ESD is the only practical option. The diameter, or ESD, is the standard definition of the size of a particle.

For sediment with significant silt content, particle size analysis is operationally-dependent and consistent sample preparation and handling are required. Sand grains, however, are straightforward to analyse and good size distribution

information can be obtained easily with a set of certificated tests. Although electro-optical methods can now measure sand size particles of up to 2 mm diameter, the characterisation of larger particles will generally require the use of sieves. For many purposes, sieves are the most practical option for describing gravel and sand sizes down to 63 μm. Sieves are arranged sequentially in a tower, largest apertures uppermost, and used with a mechanical shaker. Sieve analyses are 'low tech', and the equipment is inexpensive and readily comparable between laboratories. On the debit side, sieving is labour-intensive and slow; sieves also need to be well maintained by washing, brushing and drying between uses to clean the meshes and avoid corrosion. Mesh sizes and condition should be checked periodically using a suitable microscope and calibrated eyepiece graticule. Before progressing to the methodology employed for sands and silts, it is worth studying the concept of grade scales and the conventions used in sedimentology.

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Sample collection and storage

The design of sampling schemes to define a sediment/biotic environment depends very much on the questions that are to be addressed by the study, and by the nature and scale of the patchiness of the particular substrate, as well as by practical and economic considerations; these aspects are covered more fully in Chapter 1. In practical terms, sampling techniques and apparatus must be consistent with the purpose for which the samples are required. To take an extreme example, there is no point in attempting to quantify silt content in samples obtained from an open grab system where unknown and variable quantities of fines have been washed out of the sample during collection. Useful information on sampling equipment is given by Kramer *et al*. (1994). For information on the practical efficiency of various sampling devices, readers are referred to Chapter 5 and to Blomqvist (1991), Somerfield and Clarke (1997) and Rumohr (1999). Several studies point to the efficiency of coring systems such as the Craib corer (Craib, 1965) and the Barnett–Watson multicorer (Barnett *et al*., 1984) (see Chapter 6) that maintain the integrity of the sample, along with its surface 'fluff' and overlying water. The key principle of both of these systems is that they employ a bed frame and hydraulically-damped core penetration to minimise disturbance of the mobile surface layer (fluff), which, if lost due to bow-wave effects, can bias surface sediment composition (Bale & Morris, 1998). However, these corers are really only suitable for silt-rich (cohesive) sediments and do not work well in compacted sands or sediments containing gravel or stones. Furthermore, core samples from these instruments are generally too small (50–100 mm diameter) to sample macrofauna adequately. The Reineck box corer (square cores of 0.5 m sides and 0.7 m deep) takes representative samples (good retention of fines) that are large enough for macrofaunal sampling. Unfortunately, coring equipment of this size requires a substantial vessel with winches and a gantry capable of hauling the 2–3 tonne suction load as the corer is pulled out of the sediment. If such a vessel is not available then compromises will have to be

made and the choice of sampler will have to take account of the capabilities of the vessel. None of the coring systems performs well in the presence of stones and gravel and, in such cases, large mechanical or gravity-operated grabs may be the only option. Clearly, operators should acquaint themselves with the limitations of each device and be aware of the variations in sample integrity that can be introduced through instrument wear and tear and by changes in weather conditions and sea state. Mudroch and Azcue (1995) have produced a comprehensive manual of the practical aspects of sediment sampling.

Video cameras in suitable underwater housings attached to the frame of sampling equipment can sometimes provide valuable insights into the operation of the sampling device as well as allow the nature of the bed to be assessed. Another approach in relatively shallow waters is to employ divers (see Chapter 4) to collect sediment samples with hand corers. On intertidal sediments, samples can be collected by hand using plastic core tubes or by manual excavation, and in these cases, there can be a high degree of confidence in the integrity of the sample as long as appropriate care is taken.

If sediment samples are to be used for analyses of chemical or biological constituents then preservation must be considered to minimise the bacterial degradation of organic material. For work on redox-sensitive chemical species, core samples may need to be processed and stabilised under nitrogen in a glove box. In unpreserved samples, the death of animals and subsequent degradation of organic material can quickly alter sediment parameters such as organic content and redox potential. Conversely, many common biological preservatives may compromise subsequent chemical analyses. There are several options for sample preservation that fall into the categories of poisons (e.g. mercuric chloride, sodium azide, chloroform and antibiotics) or fixatives (e.g. formaldehyde and glutaraldehyde) and selection would depend on the purpose for which the sediment was subsequently required. The relative merits of various preservatives are reviewed by Knauer and Asper (1989). In some cases, representative subsamples for granulometric and chemical analyses should be removed from the main sample before treatment with preservatives, and stored differently. Preservation may be achieved by freezing at –20°C or lower, in which case glass containers should not be used. Preliminary experiments to assess the influence of containers and storage conditions on the substances of interest are strongly recommended for analyses of many sediment constituents.

Efficient labelling of samples is essential as this is the only factor relating the subsequent analyses to a specific sampling site. During sample collection, most workers adopt a sequential numbering system added to the container by indelible marking pen or similar tool. However, most pens quickly become inefficient during inclement weather and external labels can also become defaced by abrasion during transport. Therefore, it is very good practice to have a secondary label, on waterproof paper, placed within the container. Log sheets should be used to record all relevant data at the time of sampling: i.e. position, weather/sea conditions,

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visual observations of sediment character and the sample identification. Buller and McManus (1979) advise that each new batch of samples received in a laboratory should be checked for numbering consistency against the log sheets to ascertain that all the samples expected are present. It is much easier to rectify any anomalies such as mis-numbered or unreadable labels or to identify missing samples at this early stage rather than after a number of samples have been removed for analysis.

In summary, two aspects should be highlighted. Firstly, make use of the best statistical advice available for determining sampling patterns and sample numbers (see Chapter 1). Secondly, try to make sure, where possible, that sample containers, storage conditions and preservation techniques are compatible with all the proposed determinations.

Sediment grade scales

The object of particle size analysis is to characterise the sediment as a frequency distribution of grain sizes. In this process, grain size is the independent variable in a continuous distribution, i.e. natural particles have potentially an infinite number of sizes ranging from the sub-micron to many millimetres. To be able to define such a distribution and to generate descriptive statistics, an arbitrary set of finite intervals is usually employed to convert the continuous distribution to a discrete series. The Udden/Wentworth scale (Wentworth, 1922), which combines numerical intervals with rational definitions (fine sand, coarse sand, etc.; see Table 2.5), has been almost universally adopted among marine ecologists and geologists.

The scale is geometric, based on a dimension of 1 mm and a ratio of 2 and has the advantage of increasing the resolution of information with decreasing particle size. If required, the resolution can be increased using a ratio of the square root of 2 or the 4th root of 2 (Table 2.5). For further information on grade scales, workers should consult a standard sedimentology text such as Lindholm (1987). A logarithmic transformation of the Wentworth scale gives the phi (ϕ) notation (Krumbein, 1938):

$$\text{phi} = (-\log_{10}(\text{diameter in mm}))/\log_{10} 2$$

The phi notation stemmed from the need for a graphical mechanism for data manipulation in the days before electronic calculation. However, in the words of Lindholm (1987), 'the phi scale is almost meaningless to most biologists, archaeologists and engineers who report grain sizes, as measured, in metric units'. He goes on to suggest that there are now good arguments for abandoning phi notation particularly since the calculation of measures of central tendency (median, skewness, sorting, etc.) graphically has been superseded by computerised methods. Nevertheless, as environmental scientists, we need to be aware of the phi notation because it appears in sedimentology literature and because of the degree to which it is embedded in the methods of calculating sediment distribution parameters. The

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Table 2.5 The Udden/Wentworth grade scale based on powers of 2 and $\sqrt{2}$ and equivalent phi values.

Broad description	Description	2 scale (mm)	$\sqrt{2}$ scale (mm)	ϕ (phi)
Gravel	Cobble	256		−8
		128		−7
		64		−6
	Pebble	32		−5
		16		−4
		8		−3
	Granule	4		−2
Sand	Very course sand	2		−1
			1.41	−0.5
	Course sand	1		0
			0.71	0.5
	Medium sand	0.5		1.0
			0.351	1.5
	Fine sand	0.25		2.0
			0.177	2.5
	Very fine sand	0.125		3.0
			0.088	3.5
Silt		0.062		4.0
			0.044	4.5
		0.031		5.0
			0.022	5.5
		0.0156		6.0
			0.011	6.5
		0.0078		7.0
			0.0055	7.5
		0.0039		8.0
Clay		<0.0039	<0.0039	

use of phi scales is discussed by Buller and McManus (1979) and considered further in this chapter within the section on data presentation (entitled 'Presentation and analysis of grain size data').

Sample aggregation status

Before looking at the sediment analytical techniques, it is worth revisiting the sample 'handling' dilemma, particularly for samples with high silt or clay content. In natural silts, primary mineral grains are the exception rather than the norm. In practice, a high percentage of the sediment will have been digested and evacuated as faecal pellets many times over by deposit-feeding organisms. Additionally, surface sediment particles will have been deposited primarily as particle aggregations (flocs) resulting from particle-to-particle collisions in the water column. All these structures will have different degrees of resistance to breakage; flocs break at the

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slightest disturbance and faecal pellets can be initially quite resistant to breakage but become more fragile with time. Thus, in effect, the size distribution determined for a silt fraction generally reflects the degree of mechanical and chemical dispersion applied to the sample. This process of disturbance will have started from the moment the sample was collected and the extent of disruption will vary with the operational procedures in use. There are really only two options, neither of which is ideal. One option is to apply methods that completely disperse every structural element so that the size distribution of primary particles is measured. This would need experimentation to optimise but might include, for example, chemical 'digestion' of organic material with hydrogen peroxide followed by ultrasonic dispersion and the use of sodium hexametaphosphate dispersant as described previously. The other option is to adopt a carefully standardised procedure so that a minimal but consistent degree of disruption is applied to each sample. This approach probably best reflects the true nature of the sediment silt properties *in situ* at the time of sampling.

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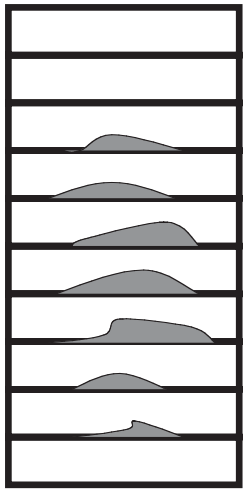
Analytical techniques

Dry sieving clean sands

Clean sands, e.g. with less than 5% silt and negligible organic matter, are easy to deal with in the dry state and are straightforward to analyse by sieving. The analysis requires a stack of Wentworth grade sieves within the range 2000–62 μm. The stack should be closed at the bottom with a pan and covered at the top with a lid. A mechanical shaker is essential when several samples have to be analysed and each sample should be shaken for a fixed interval – typically 15 minutes. A pre-weighed sample, e.g. 25 g of oven-dried sand, is introduced to the top sieve. After 15 minutes of agitation, the weight of material retained by each sieve, and in the bottom pan (<63 μm) must be determined (Fig. 2.9). Characterising larger particles such as gravels and pebbles can also be done with appropriate sieve screens, but prohibitively large samples are often required. For materials in this range and for larger pebbles and boulders, it is often adequate to estimate sizes visually.

Rapid partial analysis of silt (or mud) content

For many purposes, including benthic ecology, sedimentology and evaluation of chemical contaminant data, a single determination of the silt content (sometimes termed mud content) is an extremely good indicator of the overall character of a sediment sample and has a predictive capability for determining mechanical properties (Flemming & Delafontaine, 2000). This single determination greatly reduces the work compared with a complete spectral analysis of size. The object is to determine the silt fraction as a percentage of the total sediment weight by sieving at 63 μm. This method is fairly robust in that as long as all the silt is washed out of



0.5 phi sieve stack	Mesh size mm	wt %	Cumulative % coarser	Cumulative % finer
	1.00	0	0	100
	0.71	0	0	100
	0.50	4	4	96
	0.351	11	15	85
	0.25	18	33	67
	0.177	30	63	37
	0.125	21	84	16
	0.088	10	94	6
	0.062	6	100	0
	pan	0	100	0

Fig. 2.9 Schematic and table showing the results obtained from sieving a sand sample with a series of Wentworth interval sieves.

the sand remaining on the sieve, the results are largely independent of the sample preparation and the sieving operation; i.e. it does not matter how much the silts/clay structures are degraded as the objective is merely to remove them from the sand to determine the sample weight loss. If, however, it is important to maintain the integrity of the silt/clay fraction, then this process can also be undertaken on undried (natural) sediment.

Method for silt content – dry sediment

Take an accurately weighed sample of oven-dried sediment of about 25 g. Place the sample with about 250 ml tap water in a beaker and add 10 ml of 6.2 g/litre sodium hexametaphosphate ($(NaPO_3)_6$) to aid dispersion of clay particles. Break up the sediment with a glass rod and then stir mechanically for 10–15 minutes. Allow the sediment to soak overnight and then stir again for an additional 10–15 minutes.

Wash the dispersed sediment suspension from the beaker on to a 63 μm sieve. This can be done manually with the sieve partially immersed in a white basin containing clean water so that the sediment is submerged. The sample is then sieved by 'puddling' so that the fines fall through to the basin. Replace the water in the basin at intervals and continue sieving and washing the sediment until no further fines are washed out. Alternatively, the sample can be sieved using a mechanical shaker connected to a water supply. In this apparatus, the lid of the sieve has transparent viewing windows and is fitted with a water inlet generating a spray

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and the bottom tray has a drain outlet that can either be led to a large receiver or to waste. During the sieving operation, water is introduced at mains water tap pressure at intervals until the effluent from the bottom pan runs clear and the sand in the sieve can be seen to be clean.

Finally, transfer the sieve and contents to an oven and dry rapidly at 100°C. Successive weights should be checked to determine the time required to achieve a constant weight, i.e. no further weight loss. At this stage the weight of sand remaining in the sieve can be determined and subtracted from the original dry weight of sediment to allow a percentage silt to be calculated:

$$\text{Silt content}\,\% = ((\text{wt sample} - \text{wt sand}) \times 100)/\text{wt sample}$$

Some workers like to carry out further dry sieving of the dried sand over white paper to check for any further silt loss as a result of drying. If required, the sand fraction can be graded through the sand sieve series. In some environments, the sand fraction may include large organic debris (leaves, twigs). This might be evaluated by further sieving at, for example, 2 mm or, alternatively, evaluated as weight loss on combustion (see the section entitled 'Loss on ignition').

Method for silt content of wet sediment

A variation on the previous method that allows water content to be determined as well as avoiding initial drying of the sample is to sieve a representative portion of the original wet sediment. This may be important if, for example, part of the silt fraction is required for further analysis. To determine silt content in this way, approximately 50–60 g of the wet sediment should be weighed accurately and then dispersed in 250 ml of tap water with sodium hexametaphosphate. The method is identical to that used for oven-dried sediment but the silt fraction must be quantified. This will require a large receiver, e.g. a 10 litre plastic measuring cylinder, to retain the silt and washings. The dry weight of sand is determined as before but the weight of silt and clay must also be determined and this is achieved using gravimetry.

Method for gravimetry

Gravimetry is an essential method for anyone engaged in sediment work; it is also used for the determination of suspended solids concentration and the calibration of optical suspended load meters.

Measure and record the total volume of the silt suspension collected. Thoroughly mix the suspension by shaking and/or mechanical stirring so that a representative volume of subsample can be withdrawn for filtration. Ideally, the volume chosen should provide the maximum load on the filter without significant clogging.

Transfer the subsample onto a pre-weighed GF/C or GF/F glass fibre filter in a vacuum filtration unit. For maximum accuracy, filters need to be pre-washed

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by filtering 50–100 ml of particle-free, distilled water and then dried to constant weight. Weights need to be determined to five decimal places of grammes.

When the sample has been filtered, add two 50 ml volumes of distilled water to wash salt and sodium hexametaphosphate from the sediment. When this has been drawn through, remove the funnel from the filter unit but leave the filter on the sinter under vacuum and use a distilled water wash bottle to rinse salt water from the rim of the filter. The volume filtered must be recorded.

Using flat tip forceps, remove the loaded filter from the sinter while still under vacuum so that excess water is drawn out. Then dry to constant weight and determine the filter load by difference:

$$\text{Silt load (g litre}^{-1}) = ((\text{wt filter 2} - \text{wt filter 1})(\text{g}) \times 1000)/\text{volume filtered (ml)}$$

To determine the total weight of silt in the original sample, simply multiply the silt load by the volume of suspension.

To calculate the silt content as a percentage of the weight of sand plus silt/clay:

$$\text{Silt content \%} = (\text{wt silt} \times 100)/(\text{wt sand} + \text{wt silt})$$

Method for water content

From the data obtained for the wet silt content, the water content can be calculated as a percentage of the original wet weight by subtracting the total weight of sand plus silt/clay from the original sample wet weight:

$$\text{Water content (\%)} = ((\text{wt wet sample} - (\text{wt silt} + \text{wt sand})) \times 100)/\text{wt wet sample}$$

Analysis of the silt–clay fraction

No single analytical method covers the entire size range of sediment particles. Sieving tends to be efficient for particles down to 63 μm; below this, alternative methods are required. Although sets of electro-formed 'micro-sieves' are available (e.g. Fritch and Veco with apertures down to 3 and 7 μm, respectively), they are not suitable for routine analyses of natural sediments that often contain colloidal organic material and mucous substances that rapidly clog small pores. Similarly, filtration membranes can be obtained with a range of pore sizes (0.1–10 μm) (e.g. Millipore™ and Nuclepore™) that could be used to screen particles for research purposes but, as with micro-sieves, they are not a practical (or economic) option for routine analysis of sediment samples.

If grading of the silt–clay fraction is required, there are a number of options. Until the early 1980s, the only practical method of sizing silt/clays was the Andreasen

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pipette technique. This involved determining sedimentation rates by allowing dispersed particles to settle through a known distance/time, employing Stoke's Law and assuming a constant particle density. This technique had numerous drawbacks being very time-consuming, extremely operationally-dependent and rather unsuitable for natural sediments that can contain a range of materials with different densities. The problem of variable densities applies equally to instruments that measure the decrease of X-ray density during sedimentation (e.g. Sedigraph) and to sedimentation balance instruments. Additionally, Stokes settling assumes spherical particles and a significant proportion of particles in this fraction are non-spherical biogenic debris or clay platelets that settle differently. Since electro-resistive and optical analytical methods are now widely available, no further consideration will be given to these sedimentation-based sizing techniques. Readers who require information on this technique are referred to earlier editions of this handbook.

Electro-resistance particle counters

Developed in the 1940s for blood cell counting, electro-resistance counters are one of the most efficient particle sizing techniques available because they both count and size particles in a given sample volume. Examples are manufactured by Beckman-Coulter and Micromeritics. The method is based on measuring changes in electrical resistance across a small aperture in a glass barrier separating two electrodes immersed in an electrolyte (Fig. 2.10). The increase in resistance between the two electrodes when a particle passes through the orifice, when calibrated, is a measure of the particle volume. Reduced pressure generated by a mercury manometer is employed to draw samples through the orifice from the stirred beaker and the volume of suspension drawn through the aperture is accurately measured to allow the system to count and size particles on a per volume basis. Several thousand

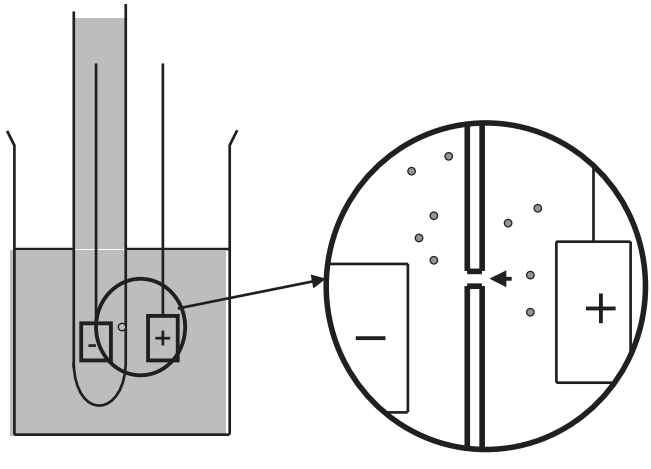

Fig. 2.10 Schematic diagram of the sensing zone in an electro-resistive particle sizer.

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Table 2.6 Particle size ranges that can be measured with each Coulter aperture.

Nominal aperture diameter (μm)	Nominal particle diameter range (μm)
15	0.4–9
20	0.5–12
30	0.6–18
50	1.0–30
70	1.4–42
100	2.0–60
140	2.8–84
200	4.0–120
280	5.6–168
400	8.0–240
560	11–336
1000	20–400
2000	40–1200

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particles per second are individually counted and sized with great accuracy and the method is independent of particle shape, colour and density.

Although almost unique in its ability to both count and size particles automatically, the electro-resistance method has some limitations. Firstly, this method detects the volume of a particle irrespective of its shape and assigns each particle the diameter of a spherical particle with the equivalent volume (the ESD). Thus, cylindrical particles are given a dimension that is shorter than the long axis and greater than the actual cross-section. However, it should be remembered that similar problems also apply to sieves in that cylindrical particles can pass through a mesh lengthways. Most importantly, the suspension of particles has to be sufficiently dilute that only individual particles pass through the aperture. An individual aperture is only sensitive over a limited size range of 2–60% of the aperture diameter. The maximum theoretical size range that can be measured is 0.4–1200 μm, but this can only be achieved by several sequential analyses of each sample using different apertures (see Table 2.6).

In practice, it is usually possible to find a single aperture that covers the required size range, e.g. for examining silt samples screened at 63 μm, a 100 μm aperture allows measurements in the range 2–60 μm. The presence of particles in the sample that are larger than the aperture will lead to blockages so all samples will need to be pre-screened in some way. Work with the smallest particles (aperture of 50 μm and less) is extremely demanding in terms of minimising background counts in the suspending medium and in reducing electrical noise to an absolute minimum. Although the Coulter Multisizer™ can size grains up to 1200 μm and should, in theory, be capable of sizing sand, in practice the problems of maintaining heavy particles in suspension make sand difficult to analyse. An upper limit of 100 μm is perhaps reasonable, though the possibility exists of increasing this with the addition of glycerol to the suspending electrolyte to increase viscosity and density. Additionally, with large grain sizes, the large sample volumes required to achieve

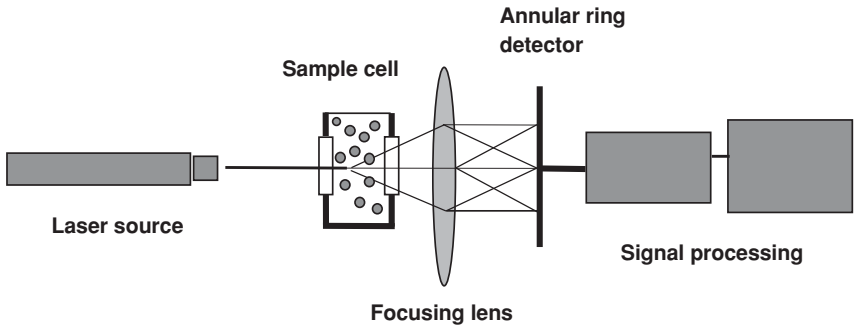

Fig. 2.11 Schematic diagram of a laser diffraction instrument based on the Malvern Instruments apparatus.

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statistically significant counts also become a limiting factor. Detailed operating procedures and methods are provided in the manuals for particular instruments.

The Coulter Multisizer can resolve a particle population into 256 size intervals. Using spreadsheets, electro-resistive data can easily be recalculated into groups to approximate Wentworth grade intervals. However, it must be remembered that sieve data generates mass in intervals and the electro-resistive techniques generate volume distributions. For sediments, volumes could be converted crudely to mass by the assumption of a typical density.

Laser diffraction particle sizing

Particle sizing by laser diffraction has been developed over the last 30 years by a number of instrument manufacturers (Malvern, Cilas, Beckman-Coulter). The method relies on the measurement of near Forward Angle Light Scattering (FALS), also called Low-Angle Light Scattering (LALS), which is generated when a particle suspension is illuminated with a laser source (Fig. 2.11). The systems then employ Mie and/or Fraunhofer theory to deconstruct the radially symmetrical diffraction pattern into a size distribution. Early instruments operated over restricted size ranges, but current instruments can now handle large size ranges, e.g. 0.05–3500 μm (Malvern Instruments) and 0.04–2000 μm (Beckman-Coulter) in one measurement. But, as mentioned in relation to the electro-resistance method, the problems of keeping heavy sand grains suspended in the optical sensing zone in representative numbers may cause errors. This is because in a sand/silt sample, sand comprises relatively low numbers, but a significant proportion of the mass or volume. The large dilutions required for laser diffraction quickly introduce errors in the sampling of sand grains causing a large bias in the mass distribution. For this reason, experience suggests that for samples composed of sand and silt, pre-screening at 63 μm with a sieve is an advantage from the sample handling viewpoint. Laser diffraction can then measure the silt material at low dilution. With the appropriate sample handling systems, laser diffraction systems can also measure the size distribution of sands

of up to about 1 mm diameter, though not if silt is present. It is relatively easy, using either spreadsheets or the instrument manufacturers software, to recombine the data into one size distribution.

Laser diffraction, like the electro-resistance technique, requires dilute suspensions, otherwise the laser beam is completely obscured or multiple scattering (particle–particle scattering) occurs. Therefore, a major consideration is the preparation of representative subsamples. Subsampling sand is relatively easy through standard cone and quartering techniques or using sample splitters. If sediment has been wet-sieved at 63 μm, the silt fraction will be in suspension and a representative volume may need to be taken for further dilution before analysis. Settled material needs to be uniformly suspended but care needs to be taken when stirring suspensions to make sure that the mixing is gently turbulent and not laminar, otherwise centrifugal effects can lead to grading of material within the container. One answer is for the container holding the sample to be fitted with baffles or vanes. Some instruments are linked to sample handling systems that allow the optimum dilution of a suspension to be obtained before it is presented to the diffraction instrument. Replicates can be analysed to test reproducibility but care needs to be exercised to ensure that the samples are not consistently biased as well. Wet mud samples that are not going to be screened by wet sieving must be thoroughly mixed before subsamples are taken for analysis.

Microscopic examination and characterisation

There are a number of microscopic approaches to particle characterisation that can usefully be applied to sediment analysis. Microscopy can be used either as an adjunct to sample preparation prior to other methods of analysis (i.e. to assess the degree of aggregation or the nature of particles) or as a direct method of counting and sizing.

With an eyepiece graticule calibrated against a stage micrometer, sand size particles can be observed easily at 100 × or 200 × magnification (obviously a calibration is required for each magnification). Ensuring that samples are representative is an important factor. Dry sand grains are effectively small marbles and are often difficult to mount for microscopic examination. One approach is to take a small piece of transparent adhesive tape (Sellotape™), e.g. 15 mm × 25 mm, and use forceps to pass the tape through the sample so that a single layer of particles adheres to the sticky side. The tape can then be placed on a slide, particles upwards, with a coverslip laid over the top to keep the tape flat. Silt size particles are best viewed wet using a cavity slide and require higher magnification to resolve. If allowed to dry, samples of silt and clay can aggregate and individual particles become difficult to resolve. Filtration of small particles on polycarbonate membranes (e.g. Whatman's Cyclopore™ and Millipore's Isopore™ range) is another method of presentation for microscopy, these membranes being sufficiently transparent to light for microscopy and inert to most stains.

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While microscopy is an invaluable aid in determining the nature of samples, sizing of particles by this method is time-consuming because of the number of particles that need to be counted to achieve reliable information. Experiments by Kennedy and Mazzullo (1991) suggested that measuring 50–60 particles from a well-sorted sample with a mean diameter in the region of 100 μm could define the mean within 10%. However, in multi-modal samples or where information on the distribution is required, more particles need to be counted: possibly 200–300 or even a 1000 for some samples.

Image analysis

Automation of optical microscopy can be achieved, either partially or completely, using image analysis software packages combined with digital imagery (Kennedy & Mazzullo, 1991; Soille, 1999). Being automated, the task of characterising sufficient particles to allow the size distribution to be determined with confidence is easier and the results more objective. Bernard *et al*. (1999) employed Scanning Electron Microscopy (SEM) and a motorised sample stage with image analysis to automatically accumulate particle size characteristics. Parallel elemental composition gathered for each particle using an X-ray fluorescence facility allowed relationships between the size and composition to be explored.

Particle stains

There are various stains and fluorophores that can be employed in conjunction with microscopy to allow a wide range of specific components within the sediment mixture to be identified. For example, Periodic and Schiff (PAS) (Whitlatch, 1981) and Rose Bengal allow organic components to be distinguished from mineral grains. A stain specific to ligneous plants can allow terrestrial material to be distinguished from marine plants (Pocklington & Hardstaff, 1974). Bacteria can be visualised using acridine orange (Daley, 1975) or DAPI (Porter & Feig, 1980) and epifluorescence microscopy.

Presentation and analysis of grain size data

The results of sediment analyses can be portrayed in a number of ways. In the simplest form, sediment can be assigned a verbal description based on the Wentworth grade scale, e.g. ‘coarse sand’ or ‘pebbles and greater’. On the basis of analytical information, this can be taken a stage further and given a numerical value using criteria relevant to the study, e.g. 85% (by weight) >1 mm (coarse sand and larger). At the other extreme, sediment size and compositional properties can be incorporated with environmental and biological information using multivariate statistical methods such as MultiDimensional Scaling (MDS) to describe and differentiate between environments (Warwick *et al*., 1991). Falling within this spectrum, there are several approaches to the presentation of sediment properties, some of which are discussed later.

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Ternary diagrams

Ternary diagrams allow a sediment sample to be characterised with respect to three variables, for example the percentages of sand, silt and clay determined by sieving. Equally, the three variables could be pebbles, gravel and sand. Using triangular graph paper, it is possible to present the percentage content of three variables as a single point. In practice, each of the three vertices of the triangular graph is labelled for one of the three variables. The distance from each base to its corresponding vertex is taken as 100%. The location of a sample with 60% sand, 30% silt and 10% clay would be as shown in Fig. 2.12a. When several sediments are plotted on the same graph, it is often possible to classify the sediments into groups that have regional ecological significance. The triangular graph can then locate field samples into 'classes' for descriptive purposes. Fig. 2.12b shows an example of an arbitrary but intuitive classification scheme by Shepard (1954). Pejrup (1988) has proposed a revised set of classes (Fig. 2.12c) where sediments are split in four classes A–D depending on their sand–silt composition. The corresponding silt–clay gradient is split into four equal classes (I–IV) indicative of hydrodynamic conditions. This concept is further expanded by Flemming (2000).

Histograms

Histograms are by far the most visually illustrative method of portraying grain size distributions and are employed as a matter of routine by most of the instrumentation manufacturers (e.g. Coulter and Malvern). However, they do not lend themselves to further statistical analysis, except, perhaps, that the mode, or modes (most common sizes) can be observed. Typically, size intervals used by instrumentation manufacturers, and those of the Wentworth grade sieves, employ geometric rather than arithmetic intervals to increase resolution in the smaller sizes. Since the cubic relationship between diameter and mass (or volume) skews mass–frequency relationships towards the largest particles, the use of logarithmic scales tends to transform sediment distributions into bell-shaped distributions that can be easily compared. Conventionally, size information is plotted on the *x*-axis and frequency (wt%) on the *y*-axis. Historically, phi intervals were plotted increasing to the right so that the true (millimetre) size decreased to the right but this somewhat anomalous convention is currently being questioned. McCave and Syvitski (1991) promote the plotting of log size in μm or mm, increasing from left to right on the lower side of the diagram and phi parameter (if required) increasing to the left along the top (see Fig. 2.13). This convention will be followed from here on.

Cumulative frequency curves and distribution statistics

In almost every sedimentology text of the last few decades, the use of cumulative frequency curves was described. This form of presentation, effectively stacking each bar of the histogram vertically upwards by the amount of the previous bar, has virtually no presentational value in that even quite significant differences in a histographical presentation are difficult to resolve visually from a cumulative plot.

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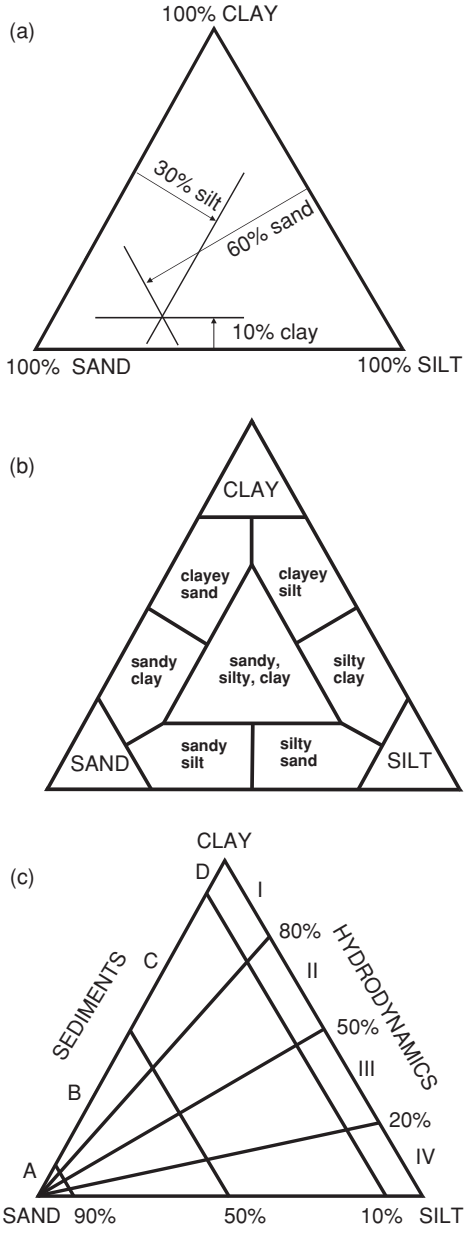

Fig. 2.12 Examples of ternary diagrams for plotting three variables describing sediments. (a) Showing how a sample with a 60%, 30% and 10% (sand, silt, clay) composition is located within the plot. (b) Example of classification after Shepard (1954). (c) Classification including an interpretation of the hydrodynamic environment after Pejrup (1988).

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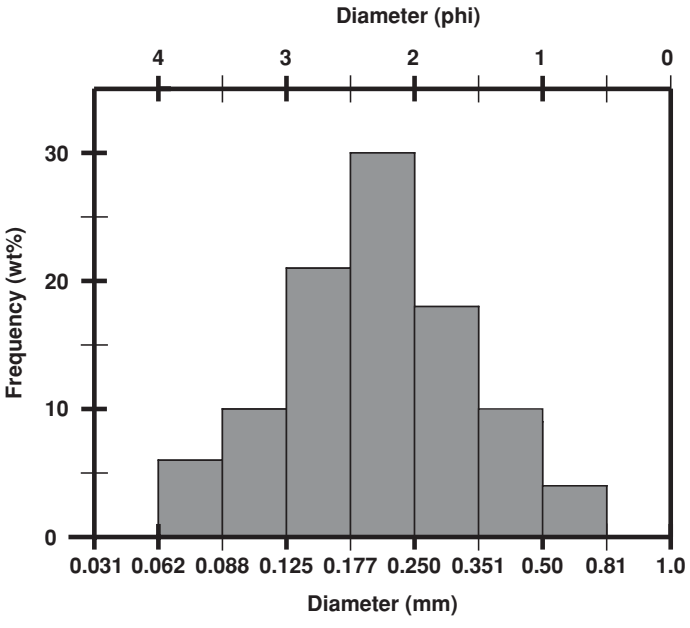

Fig. 2.13 A histogram, frequency–weight plot of the data from Fig. 2.9 employing both grain diameter in mm on a logarithmic scale and phi units on the *x*-axes.

The principal advantage of this construction is that it allows percentile values to be extracted (see Fig. 2.14) in order that statistical parameters such as coefficients of sorting and measures of skewness and kurtosis (Buller & McManus, 1979; Lindholm, 1987) can be calculated.

One factor that needs to be considered here, and it stems from the convention to plot histograms with particle size increasing left to right, is that to achieve a conventional (increasing from left to right) sigmoid, frequency distribution, the plot needs to be cumulative 'percent finer' rather than the 'percent coarser' that is invariably presented in older text books. A percent finer plot is the inverse of the percent coarser and is the reverse of the way in which, for example, sieve data are collected (see Fig. 2.9).

Although the graphical construction of the cumulative frequency curve allows manual extraction of percentile values, modern data processing routines using computing technology can automate the fitting of a curve to the data and the subsequent calculation of the statistical parameters. The standard, sigmoid, cumulative frequency curve is well fitted by the expression

$$y = a/(1 + b\mathrm{e}^{-cx})$$

Using a spreadsheet system such as Microsoft's® Excel, for example, a set of particle size data pairs (x = size, y = cumulative percent finer) can be fitted

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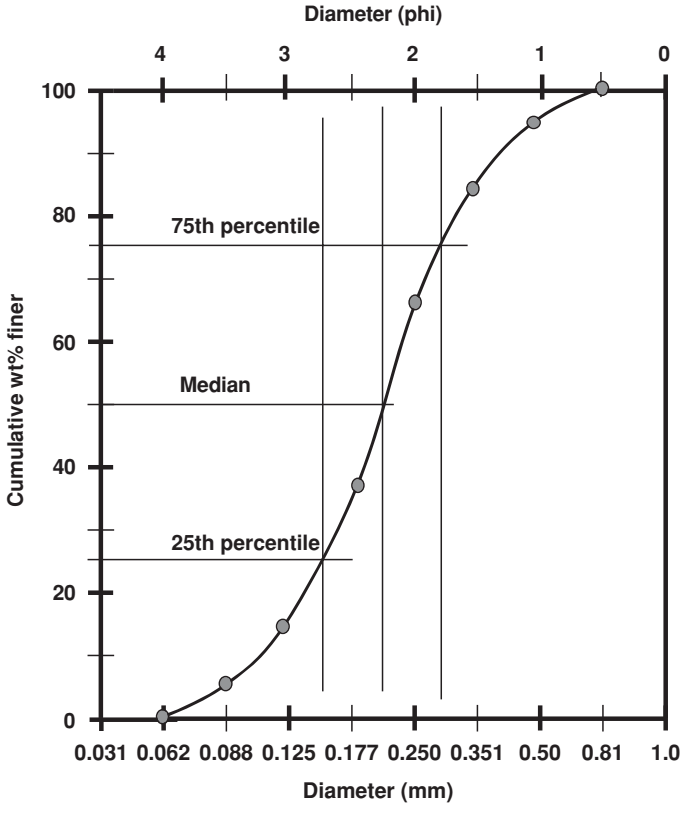

Fig. 2.14 A cumulative, 'percent finer', frequency–weight plot of the data from Fig. 2.9 showing how percentile values are extracted.

to the above expression using Solver® to refine the fitting parameters (*a*, *b* and *c*) iteratively and minimise the 'least squares' error. Once the best fit has been derived, *x* values equivalent to specific percentiles can be extracted automatically and inserted into the chosen statistical parameters (Buller & McManus, 1979).

Alternatively, software such at the US Geological Survey's SEDSIZE programme, which is freely available on the Internet (http://water.usgs.gov/software/sedsize.html; accessed 8 November 2012), will perform these calculations. The programme takes size distribution data derived from sieves or other analytical sources and outputs conventional statistical parameters based on millimetre (Trask, 1930) or phi notation (Krumbein, 1938; Folk, 1966); it will also give percentages in the Wentworth grades of sand, silt and clay.

Statistical parameterisation of particle size data

The descriptive parameters that have been developed to describe features of sediment size distributions are analogous to the mean and standard deviation employed

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with the normal distribution in conventional statistics. The parameters fall into the following categories:

- Measures of central tendency (median, mean, mode).
- Measures of scatter about a central value (dispersion, deviation, sorting).
- Measures of the degree of asymmetry (skewness).
- Measures of the degree of peakedness (kurtosis).

Methods for calculating a number of descriptive statistics are given in Table 2.7. Clearly, values derived by one equation, e.g. for dispersion, are not comparable with values calculated using another expression for dispersion and certainly, except for median values, metric and phi-based parameters are not interchangeable. Buller and McManus (1979) provide a thorough overview. These methods are limited when dealing with multi-modal distributions that arise when a number of processes, possibly biological and physical, are affecting a sediment. It may be that the principal modes are the only useable descriptors in these circumstances.

Another approach to the calculation of statistical parameters is based on the calculation of moments, which lends itself to automated, computerised methods but requires information over the full size spectra (i.e. closed distributions). In sediment analysis, the information in the 'tails' typically has the greatest uncertainty, or is lacking altogether. This method is described by Lindholm (1987) and the limitations reviewed by Buller and McManus (1979).

Geographical information system mapping and correlation of parameters

The spatial mapping of sediment properties on a geographical basis, particularly employing Geographical Information Systems (GIS) to overlay numerous parameters (e.g. sediment characteristics, biological information, chemical information, bathymetry and hydrographic data) in a coordinated spatial reference can be very informative (DeMers, 1997; Burrough & McDonnell, 1998). Distributions mapped in this way often provide good visual relationships between sediment characteristics and physical or biological factors such as bathymetry and currents in deep water or wave action, desiccation and algal distributions on intertidal sediments. With spatially referenced data storage and visualisation techniques, remotely sensed data (aircraft or acoustic imagery) can be incorporated and used to inform process models that draw on a number of components within the GIS to generate further insight (Gittings, 1999).

2.4 Other important sediment properties

Bulk and dry density, water content and porosity

Measurements of the mass physical properties (e.g. bulk and dry density) and the porosity of sediment, i.e. in submerged sediment, the space between the grains that

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Table 2.7 Examples of statistical measures used to define grain size distributions.

System	Median	Mean	Dispersion/sorting	Skewness	Kurtosis
Metric	$Md = P_{50}$	$M = \frac{P_{25} + P_{75}}{2}$	$QD_a = \frac{P_{25} - P_{75}}{2}$	$Sk_a = \frac{P_{25} + P_{75} - 2Md}{2}$	$Kq_a = \frac{P_{25} - P_{75}}{2(P_{10} - P_{90})}$
			$So = (P_{25} / P_{75})^{\frac{1}{2}}$	$Sk = (P_{25}\ P_{75}) / (Md)^2$	
Phi	$Md = \varphi_{50}$	$M_\varphi = \frac{\varphi_{84} + \varphi_{16}}{2}$	$\sigma_\varphi = \frac{\varphi_{16} - \varphi_{84}}{2}$	$\alpha_\varphi = \frac{M_\varphi - Md_\varphi}{\sigma_\varphi}$	$\beta_\varphi = \frac{{}^1/_2(\varphi_{95} - \varphi_5) - \sigma_\varphi}{\sigma_\varphi}$
		$M_z = \frac{\varphi_{84} + \varphi_{50} + \varphi_{16}}{3}$	$\sigma_I = \frac{\varphi_{16} - \varphi_{84}}{4} + \frac{\varphi_5 - \varphi_{95}}{6.6}$	$Sk_I = \frac{\varphi_{84} + \varphi_{16} - 2\varphi_{50}}{2(\varphi_{16} - \varphi_{84})} + \frac{\varphi_{95} + \varphi_5 - 2\varphi_{50}}{2(\varphi_5 - \varphi_{95})}$	$K_G = \frac{\varphi_{95} - \varphi_5}{2.44(\varphi_{25} - \varphi_{75})}$

Source: Adapted from Buller and McManus (1979) with the permission of Cambridge University Press.
NB: These equations have been adapted to take account of the fact that plotting cumulative frequency against size in millimetre increasing from left to right (McCave & Syvitski, 1991) requires a percent finer plot, rather than percent coarser, in order to generate a 'conventional' sigmoid curve *increasing* from left to right.
Md, Median; *M*, mean; QD_a, arithmetic quartile deviation; *So*, Trask sorting coefficient; Sk_a, arithmetic quartile skewness; *Sk*, geometric quartile skewness; Kq_a, arithmetic quartile kurtosis (Trask, 1930; Krumbein & Pettijohn, 1938; Krumbein, 1939); M_φ, phi mean diameter; σ_φ, phi deviation measure; α_φ, phi skewness measure; β_φ, phi kurtosis measure (Inman, 1952); M_z, mean size; σ_I, inclusive graphic standard deviation; Sk_I, inclusive graphic skewness; K_G, inclusive graphic kurtosis (Folk & Ward, 1957).

is occupied by water, are closely inter-related. Porosity is affected by compaction and grain size and is one of the factors 'measured *in situ*' by geo-physical methods of sediment characterisation (Dunn *et al*., 1980). The mass physical properties of a sediment are related to its mechanical strength and behaviour. For example, critical erosion shear stress is related to sediment dry density (Delo, 1988). Systematic relationships between bulk density, water content and sediment composition often reflect environmental conditions.

Care must be taken when examining sediment properties as a function of core depth. Bird and Duarte (1989) and Flemming and Delafontaine (2000) show how vertical distributions of properties of sediments such as Particulate Organic Carbon (POC) and bacterial numbers can be misinterpreted when related to sediment mass rather than volume. This is because the bulk density of sediment (mass per unit volume) changes with compaction (water content) and composition (sand/silt ratio). These authors recommend that parameters associated with sediments, such as contaminants, should be quoted as per unit volume of sediment rather than per unit mass of sediment. Flemming and Delafontaine (2000) give the following definitions:

Wet bulk density

Wet bulk density (BD) reflects the relationship between the total water-saturated mass (M_t) and the volume of the water-saturated sample (V_t) and is given by

$$BD_w = M_t / V_t$$

Dry bulk density

Dry bulk density (BD_d) is the relationship between the mass of dry solids (M_s) and the volume of the water-saturated sample (V_t) is calculated as

$$BD_d = M_s / V_t$$

Water content

Water content (absolute water content, W_a) is given as the mass of water (M_w) in a sample as a percentage of the original wet mass (M_t) of sediment and is the preferred unit, thus

$$W_a = (M_w / M_t) \times 100$$

Therefore, absolute water contents never exceeds 100%, unlike relative water contents (W_r, given by $(M_w/M_s) \times 100$) that can result in water contents of several hundred percent (which is illogical). These values can all be measured directly using gravimetric means. Absolute water content (W_a) has been shown to be a universal master variable by which differences between sediments from various environments can be normalised (Flemming

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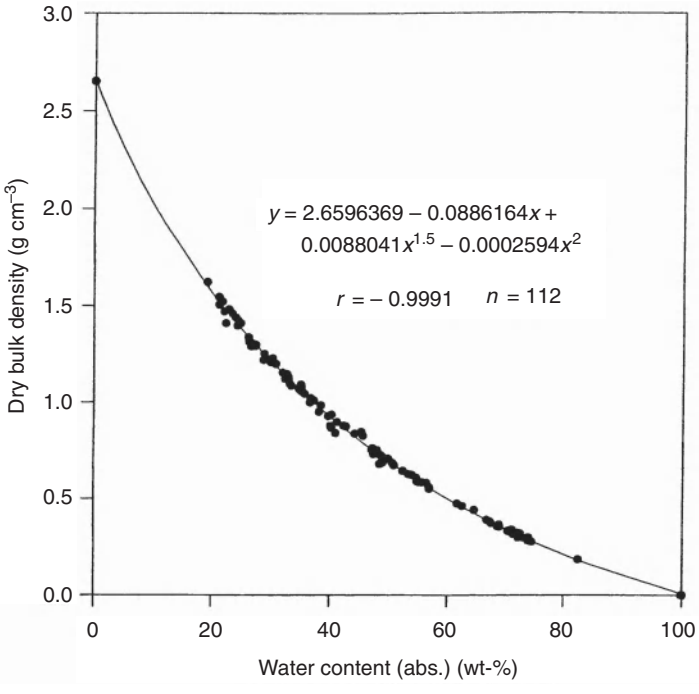

Fig. 2.15 Dry bulk density as a function of water content (W_a) for pooled data from the Wadden Sea, Griefswalder Bodden and Bay of Mont Saint Michel. (Reproduced from Flemming and Delafontaine (2000), with permission from Elsevier Science.)

& Delafontaine, 2000). For example, the relationships between water content and dry bulk density from a wide range of environments are described by a single regression with a high correlation (Fig. 2.15).

Porosity

Porosity (ϕ), also termed voids ratio, is directly related to water content in that it represents the fractional pore space in a sediment but is presented in terms of volume:

$$\phi = V_p / V_b$$

where V_p is the volume of pores and V_b is the bulk volume of the sediment. The values are dimensionless and usually quoted as decimals but may be multiplied by 100 to give percentages. Determination of porosity requires a measurement of the volume of water in a sediment sample and the volume of the dry sediment. Just as with water content, both of these can be measured gravimetrically. The weight (and thus volume) of water in a sediment can be determined by subtracting the weight of washed (to remove salt) and dried (to constant weight) sediment from the weight of the wet sediment. Washing can be achieved by repeated centrifuging, decanting

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and resuspension in distilled water. Dialysis in running water is an alternative method of removing the salt. For approximate results washing can be omitted by calculating the weight contribution of salt from seawater density tables by assuming that the salinity of the interstitial water is the same as the overlying water. The volume of the dry sediment can be estimated from its weight by assuming a density of 2.65, but for accurate work, the sediment volume of the sample needs to be determined from its actual density. The underlying method for this is the same as the laboratory determination of specific gravity of any material.

The representative sample of wet sediment is placed in a tightly stoppered, pre-weighed, weighing jar and the weight of wet sediment determined. The sample is then transferred to a centrifuge tube that has been previously weighed in air and weighed suspended but completely immersed in water. Remove salt by adding distilled water, shaking, centrifuging and pouring away the overlying water. Repeat this two or three times.

The sediment and tube should then be weighed suspended, immersed in water.

Afterwards, the tube is dried to constant weight and the dry weight of sediment determined. The difference between the dry and immersed weights of the sediment is equivalent to its volume (V_s) by displacement of water.

The difference between the wet and the dry weight of sediment is the volume of interstitial water (V_p).

Thus,

$$\phi = V_p/(V_s + V_p)$$

Permeability

Permeability is a concept taken from petrology, which is important for hydrocarbon yield/flow and to ground water percolation. Permeability is not necessarily related to porosity because the extent of interconnectivity between interstices is not known nor are the sizes of throats or pores that link them known. For sediments, permeability is understood as the rate at which water under a constant head, h, passes through a cylindrical core of sediment of length L and cross-section A. By applying a constant head of water to a core in a sealed apparatus, the coefficient of permeability, P, is given by

$$P = QL/hAt$$

where Q is the volume of water flow in time t.

Organic matter content

Examination of the compositional elements of sediments will require some knowledge of analytical chemistry or the facility to draw on the services of an analytical

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chemist. However, since the parameters discussed in the following text are indicators of the biology residing within, or on, the sediment system, they constitute valid topics for this chapter. The organic content of sediment can provide insight into sediment cohesion, information on the potential nutritional value to deposit-feeders, and on the oxygen demand within the sediment.

High-temperature oxidation (elemental analysers)

Several manufacturers (Perkin Elmer, Thermo, Leco) produce elemental analysers (C, N, O, H, S) in which mg samples of oven-dried (90–100°C) sediment are oxidised at high temperature (1000–1300°C). This is currently the method of choice for determining carbon, which is detected as CO_2. For standards, and total carbon, the precision can be excellent. However, for sediments, the method is complicated by the difficulty in distinguishing between organic and inorganic carbon. There are two approaches: the first is to use thermal oxidation at a temperature that drives off all the organic material as CO_2 and then measure the remaining (unaffected?) inorganic carbon with an elemental analyser and determine the organic carbon as the difference between total carbon and inorganic carbon. This is not straightforward because temperatures sufficiently high to oxidise refractory organic material are probably too high to prevent the decomposition of magnesium carbonate. The second (recommended) approach is to use a non-oxidising acid to dissolve the carbonates, ideally without solubilising or volatilising the organic material. Suitable candidates are hydrochloric (HCl, 2-6N), phosphoric (H_3PO_4, 1 M) and sulphurous (SO_2, 5% w/v) acids. HCl is recommended by King *et al*. (1998) following an intercomparison between ten laboratories. The remaining organic fraction of the carbon can then be determined directly.

Loss on ignition

A simple, if crude, estimate of the organic content of sediments can be derived from the mass Loss On Ignition (LOI) in a muffle furnace. Pre-weighed, oven-dried (90–100°C) samples are typically ignited at 450°C for six to eight hours (or to constant weight). Ignition temperatures ranging from 300°C to 600°C are found in the literature but it is generally considered that carbonates (principally shells) start to decompose at temperatures above 400°C (Weliky *et al*., 1983). The presence of carbonates can be assessed by looking for effervescence on the addition of dilute acid (e.g. 1 molar HCl). Even for carbonate-free sediment, however, the LOI method is operationally dependent on temperature. Mook and Hoskin (1982) showed that for organic-free sediments with an appreciable clay content (83%), significant weight loss (10–20%) at temperatures over 400°C could be attributed to loss of 'structural water' within the clay matrices. For samples with lower clay contents, 'structural' or 'lattice' water may be insignificant and several workers have achieved very good relationships between LOI values and POC (e.g. Craft *et al*., 1991; $R^2 = 0.990$). Typical factors relating POC to LOI fall between 0.5 and

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Table 2.8 Examples of conversion factors determined from Particulate Organic Carbon (POC) and Loss On Ignition (LOI) measurements showing that the relationship is site-specific; POC = LOI multiplied by factor.

Reference	Factor	LOI values	Temperature
Craft *et al*. (1991) Estuarine marsh	0.50	0–10%	450°C, 8 h
Byers *et al*. (1978) Sediment spiked with chitin compound	0.436	22.9%	475–500°C, 4–6 h
Wirth and Weisner (1988) North Sea sediments	0.24	0–10%	500°C
Morris *et al*. (1982), Stephens *et al*. (1992) Turbid region of Tamar Estuary	0.29	8.2%	300°C, 2 h
Leong and Tanner (1999) Tolo Harbour, Hong Kong	0.197 and 0.165	12.3% and 12.6%	440°C
Sutherland (1998) Fluvial sediments (silt & clay), Hawaii	0.14	15–20%	450°C, 16 h

0.2 (Table 2.8) depending on the balance between the supply and degradation of organic matter, though often with a large spread at a given site.

Wet oxidation

Wet oxidation using chromic acid–sulphuric acid mixtures and either titration or coulometric analyses of evolved CO_2 have been employed routinely and are straightforward but they are time-consuming, use noxious reagents and require a reasonable degree of analytical chemical skill. Wet oxidation methods also underestimate organic carbon by 20–30% in oxic samples (Byers *et al*., 1978; Leong & Tanner, 1999) and over-estimate it (140% of example) in anoxic sediments (Leong & Tanner, 1999) due to the presence of other reduced species (e.g. Fe and Mn) that 'consume' oxidant during the titration. This method is now superseded by the high-temperature oxidation methods described above.

Chlorophyll

Chlorophyll is the key photosynthetic molecule and is measured as a proxy for photosynthetic biomass both in the water column and, more relevant to this chapter, on the surface of intertidal sediment or subtidal sediment within the photic zone. The microphytobenthic population provides much of the energy for epibenthic grazers and also influences the stability of the sediment (Paterson, 1989, 1997) through the production of mucopolysaccharides, also known as EPS (see the section entitled 'EPS carbohydrate').

There are three key stages in the determination of chlorophyll: (i) sampling and preservation, (ii) extraction of the chlorophyll from the cells and (iii) quantification of the chlorophyll in the extract. Sampling water column chlorophyll usually

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involves filtering a known volume of water through a GF/C or GF/F filter pad. Samples of sediment are usefully acquired using a small core of known dimensions, which can be extruded so that a known depth (e.g. 2 mm) and area of sediment can be taken. Because corers tend to compact soft sediment, the wet weight of the sample should be determined. Water content and bulk density values from replicate samples can then be used to convert wet weight to dry weight or sediment volume. Samples for chlorophyll analysis should be kept in the dark and stored deep frozen at the lowest temperature available. The chlorophyll molecule degrades rapidly to phaeopigments at ambient temperatures, especially in the presence of sunlight. Mantoura *et al*. (1997) recommend storage of samples in liquid nitrogen (−196°C) immediately after collection, though for short periods (e.g. up to 4 weeks) −80°C is sufficient. Various solvents can be used for the extraction of chlorophyll, though acetone is the least toxic and is preferred (Wright *et al*., 1997). Samples should be disrupted for 15–25 seconds using an ultrasonic probe in 5 or 10 ml of 90% acetone in a plastic centrifuge tube, left for 10–20 minutes and then centrifuged at 3000 g for five minutes to separate the particles from the solvent. Samples should be kept in the dark and as cold as possible during these operations and extracts stored in a freezer until analysis. In a given sample, a number of other chloropigments, carotenoids and chlorophyll degradation products will be present and interfere with chlorophyll when analysed spectrophotometrically. Sediments in particular are typically high in chlorophyll degradation products (phaeopigments) because of the accumulation of detrital plant material. Therefore, the recommended method of analysis for chlorophyll is to use High-Performance Liquid Chromatography (HPLC) to separate and quantify chlorophyll *a* from within the mixture of pigments. Spectrophotometric methods of quantifying chlorophyll in extracts are a more practical prospect for the sediment chemist but users must be aware that the results will be compromised by the presence of the other pigments. Cross-calibration of spectrophotometric measurements with some HPLC analyses may provide a means of assessing the reliability of these data. Various spectrophotometric equations employing up to three absorption measurements at specific wavelengths are detailed by Jeffrey and Welschmeyer (1997). Fluorometric analyses of extracts enable greater sensitivity at low chlorophyll concentrations but this is generally not needed for sediment pigments.

EPS carbohydrate

It is now widely accepted that the microphytobenthic population, especially epipelic diatoms, have a large impact on the stability of intertidal and shallow sediments through the exudation of mucopolysaccharide material that effectively binds surficial sediment particles together. Several workers (Black, 1997; Tolhurst *et al*., 2000) have shown that the critical erosion threshold of cohesive sediment can be dramatically reduced (virtually to the point of zero cohesion) by chemically removing organic material (peroxide digestion), or by poisoning the biota (mercuric chloride, copper sulphate). The erosion threshold can be increased again

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in direct proportion to EPS added to organic-free sediment (Tolhurst *et al*., 2000). Not surprisingly, colloidal EPS and chlorophyll are well correlated (Underwood & Smith, 1997, 1998). However, EPS is now considered a valuable measure of intertidal sediment character in its own right and the measurement of colloidal EPS, as carbohydrate, is not difficult. A quantity of wet sediment (e.g. 0.2–0.3 g) (for which the water content should be determined separately) or of dried or freeze-dried sediment (e.g. 0.1–0.2 g) is weighed into a plastic centrifuge tube and 5 ml of carbohydrate-free water is added. The resultant slurry is agitated vigorously for 10 seconds using a vortex stirrer and then left to 'extract' for 15 minutes at 20°C. Following this, the slurry is centrifuged at 3000 g and 1 ml of the supernatant analysed for carbohydrate using the phenol-sulphuric acid assay outlined by Underwood *et al*. (1995). This method measures total colloidal carbohydrate (which is all that most workers do), but in order to determine the true EPS, the polymeric material within an extract must be separated from the low molecular weight carbohydrate. This is achieved by precipitating the polymer fraction in 70% ethanol and decanting the low molecular weight carbohydrate. After a second wash with 70% ethanol, the polymer is 'resolubilised' in distilled water and assayed for carbohydrate as before. EPS is typically 20–40% of the total carbohydrate.

Temperature

The temperature of sediments will be closely related to that of the overlying water, though significant deviations can be experienced on intertidal sediments, which can heat or cool considerably depending on ambient weather and insolation. For submerged sediments, a 'high-tech' approach to measuring temperatures can be achieved with digital thermometer probes mounted on bottom-landing vehicles. For most practical purposes, a standard thermometer inserted into the centre of a grab or core sample immediately on recovery will provide a good indication of *in situ* temperature. For fieldwork, a digital thermometer with a metal probe is much more robust than a mercury-in-glass thermometer.

Eh and pH

Indicators of acidity and redox balance (reduction–oxidation status) in sediments, pH and Eh, respectively, are geo-chemically inter-related. Microbial decomposition of organic material firstly consumes oxygen and then reduces nitrate to nitrite and ammonia. If oxygen or nitrate are not replaced through diffusion, continued oxidation of organic material results in the reduction of metal oxides such as Fe and Mn to an ionic form, and of sulphate to hydrogen sulphide giving rise to the familiar 'rotten eggs' smell of some anaerobic sediments. Microbial respiration of organic carbon produces CO_2, which, in solution, is weakly acidic. These processes of early sediment diagenesis are described fully by Berner (1971). Since the sedimentation of organic material tends to be associated with relatively quiescent environments, and the scope for oxygenation of sediments through physical

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disturbance or diffusion is consequently reduced, anaerobic conditions tend to be associated more with silts and clays than sands.

The pH of the interstitial liquids in cores can be measured using conventional glass electrodes in combination with $Hg–HgCl_2$ or $Ag–AgCl_2$ reference electrodes. Several manufacturers make pH probes that are suitable for insertion into semi-solid materials. Redox potential is usually measured using an inert Pt electrode in conjunction with a standard hydrogen electrode or a reference electrode of known, fixed Eh. Suitably formed electrodes can be profiled through soft sediment cores using a rack and pinion system to achieve mm depth resolution (Mortimer *et al*., 1998). An important consideration when working with anaerobic or anoxic sediments is that reduced species will quickly oxidise in the present of atmospheric oxygen. This can be controlled by storing and manipulating sediment samples in a nitrogen-filled glove box.

In Situ Sediment Characterisation Methods

Because of the problems associated with sediment disturbance during sampling and the labour-intensive aspect of manual analyses, a growing number of devices are being developed, or are available, for *in situ* measurements and observations. Brooke Ocean's, Free Fall Cone Penetrometer (FFCPT) can determine sediment structure and shear strength in the surface 2 m of soft sediments. Geotek Ltd's SAPPA (Sediment Acoustic and Physical Properties Apparatus) will measure geotechnical properties using a heavy, bottom-landing device that inserts acoustic probes 1.3 m into the sediment. Directly relevant to benthic biology, several *in situ* imaging systems allow surface sediments to be visualised under water so that the biota can be assessed and sediment grain sizes and even redox conditions estimated (Rumohr & Schomann, 1992). Examples of these are Benthos's REMOTS^{TM} and NOAA's SPI (Sediment Profiling Imagery). Both these systems employ a bottom-landing frame to insert a prism and mirror system slowly into the surface 20 cm of the sediment. A camera contained within an underwater housing then photographs the section of sediment exposed by the vertical face of the prism.

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References

Allen, T.E. (1990) *Particle Size Measurement*. Chapman and Hall, London.

Bale, A.J. & Morris, A.W. (1998) Organic carbon in suspended particulate material in the North Sea: effect of mixing resuspended and background particles. *Continental Shelf Research*, **18**, 1333–1346.

https://telegram.me/Geologybooks

Barnett, P.R.O., Watson, J. & Connelly, D. (1984) A multiple corer for taking virtually undisturbed samples from shelf, bathyal and abyssal sediments. *Oceanologica Acta*, **7**, 399–408.

Bernard, P., Eisma, D. & Van Grieken, G. (1999) Electron micro-probe analysis of suspended matter in the Angola Basin. *Journal of Sea Research*, **41**, 19–33.

Berner, R.A. (1971) *Principles of Chemical Sedimentology*. McGraw-Hill, New York.

Bird, D.F. & Duarte, C.M. (1989) Bacteria-organic matter relationship in sediments: a case of spurious correlation. *Canadian Journal of Fisheries and Aquatic Sciences*, **46**, 904–908.

Black, K.S. (1997) Microbiological factors contributing to erosion resistance in natural cohesive sediments. In: *Cohesive Sediments* (eds N. Burt, R. Parker & J. Watts), John Wiley & Sons, Chichester, 231–244 pp.

Blomqvist, S. (1991) Quantitative sampling of soft-bottom sediments: problems and solutions. *Marine Ecology*, **72**, 295–304.

Buller, A.T. & McManus, J. (1979) Sediment sampling and analysis. In: *Estuarine Hydrography and Sedimentation* (ed. K.R. Dyer), Cambridge University Press, Cambridge, 87–130 pp.

Burns, D., Queen, C.B. & Chivers, R.C. (1985) An ultasonic signal processor for use in underwater acoustics. *Ultasonics*, **23**, 189–191.

Burrough, P.A. & McDonnell, R.A. (1998) *Principles of Geographical Information Systems*. xiii, 2nd edn, Oxford University Press, Oxford, 333 pp.

Byers, S.C., Mills, E.L. & Stewart, P.L. (1978) A comparison of methods of determining organic carbon in marine sediments, with suggestions for a standard method. *Hydrobiologica*, **58**, 43–47.

Chivers, R.C., Emerson, N. & Burns, D. (1990) New acoustic processing for underwater surveying. *Hydrographic Journal*, **42**, 8–17.

Coggan, R., Populus, J., White, J., Sheehan, K., Fitzpatrick, F. & Piel, S. (eds) (2007) *Review of Standards and Protocols for Seabed Habitat Mapping*. MESH project document.

Craft, C.B., Seneca, E.D. & Broome, S.W. (1991) Loss on ignition and Kjeldahl digestion for estimating organic carbon and total nitrogen in estuarine marsh soils. *Estuaries*, **14**, 175–179.

Craib, J.S. (1965) A sampler for taking short, undisturbed cores. *Journal du Conseil Permanent International pour l'Exploration de la Mer*, **30**, 34–39.

Daley, R.J. (1975) Direct counts of aquatic bacteria by a modified epi-fluorescence technique. *Limnology and Oceanography*, **20**, 875–882.

Decho, A.W. (1990) Microbial exopolymer secretions in ocean environments: their role(s) in food webs and marine processes. In: *Oceanography and Marine Biology, Annual Review*, **28** (ed. H. Barnes), Aberdeen University Press, Aberdeen, 73–153 pp.

Delo, E.A. (1988) *Estuarine Muds Manual*. Report SR 164. Hydraulics Research, Wallingford.

DeMers, M.N. (1997) *Fundamentals of Geographic Information Systems*, xvii. John Wiley & Sons, Chichester, 486 pp.

Dunn, I.S., Anderson, L.R. and Kiefer, F.W. (1980) *Fundamentals of Geotechnical Analysis*. John Wiley & Sons, New York.

Dyer, K.R. (1979) Estuaries and estuarine sedimentation. In: *Estuarine Hydrography and Sedimentation* (ed. K.R. Dyer), Cambridge University Press, Cambridge, 1–18 pp.

https://telegram.me/Geologybooks

Fernandes, W.A. & Chakraborty, B. (2009) Multi-beam backscatter image data processing techniques employed to EM 1002 system. In: *Proceedings of the International Symposium on Ocean Electronics (SYMPOL-2009)*, 18–20 November (eds P.R.S. Pillai & M.H. Supriya), Cochin University of Science and Technology, Cochin, 93–99 pp.

Fish, J.P., & Carr, A.H. (1990) Sound underwater images; a guide to the generation and interpretation of side-scan sonar data. *American Underwater Search and Surveys Ltd*, Lower Cape Publishing, Orleans, MA, 189 pp.

Flemming, B.W. (2000) A revised textural classification of gravel-free muddy sediments on the basis of ternary diagrams. *Continental Shelf Research*, **20**, 1125–1138.

Flemming, B.W. & Delafontaine, M.T. (2000). Mass physical properties of muddy intertidal sediments: some applications, misapplications and non-applications. *Continental Shelf Research*, **20**, 1179–1197.

Folk, R.L. 1966. A review of grain-size parameters. *Sedimentology*, **6**, 73–93.

Folk, R.L. & Ward, W.C. (1957) Brazos River bar: a study in the significance of grain size parameters. *Journal of Sedimentary Petrology*, **27**, 3–26.

Foster-Smith, R. & Gilliland, P. (1997). Mapping the seabed. *CoastNET*, **2**, 1.

Gittings, B.M. (ed.) (1999) *Integrating Information Infrastructures with Geographical Information Technology* (Innovations in GIS 6), xv. Taylor and Francis, London, 280 pp.

Green, M. & Cunningham, D. (1998) Seabed imaging techniques. *Hydro International*, **2**, 4 pp.

Greenstreet, S.P.R., Tuck, I.D., Grewar, N.G., Armstrong, E., Reid, D.G. & Wright, P.J. (1996) An assessment of the acoustic survey technique, RoxAnn, as a means of mapping sea-bed habitat. *ICES Journal of Marine Science*, **54**, 939–959.

Hughes Clarke, J.E. (1998) Detecting small seabed targets using high-frequency multibeam sonar. *Sea Technology*, **6**, 87–90.

Inman, D.L. (1952) Measures for describing the size distribution of sediments. *Journal of Sedimentary Petrology*, **22**, 125–145.

Jeffrey, S.W & Welschmeyer, N.A. (1997) Spectrophotometric and fluorometric equations in common use in oceanography. In: *Phytoplankton Pigments in Oceanography* (eds S.W. Jeffrey, R.F.C. Mantoura and S.W. Wright), UNESCO, Paris, 597–615 pp.

Kennedy, K & Mazzullo, J. (1991) Image analysis method of grain size measurement. In: *Principles, Methods, and Application of Particle Size Analysis* (ed. J.P.M. Syvitski), Cambridge University Press, Cambridge, 76–87 pp.

Kenny, A.J. (1998) A biological and habitat assessment of the sea-bed off Hastings, southern England. ICES, CM., 1998/E:5, 21 pp.

Kenny, A.J., Cato, I., Desprez, M., Fader, G., Schüttenhelm, R.T.E. & Side, J. (2003). An overview of seabed mapping technologies in the context of marine habitat classification. *ICES Journal of Marine Science*, **60**, 411–418.

King, P., Kennedy, H., Newton, P.P., Jickells, T.D., Brand, T., Calvert, S., Cauwet, G., Etcheber, H., Head, R., Khripounoff, A., Manighetti, B. & Miquel, J.C. (1998) Analysis of total and organic carbon and total nitrogen in settling oceanic particles and a marine sediment: an inter-laboratory comparison. *Marine Chemistry*, **60**, 203–216.

Knauer, G. & Asper, V. (1989) *U.S. GOFS Working Group on Sediment Trap Technology and Sampling*. U.S. GOFS Planning Report Number 10.

Kramer, K.J.M., Brockman, U.H. & Warwick, R.M. (1994) *Tidal Estuaries: Manual of Sampling and Analytical Procedures*. Balkema, Rotterdam.

https://telegram.me/Geologybooks

Krumbein, W.C. (1938) Size frequency distributions of sediments and the normal phi curve. *Journal of Sedimentary Petrology*, **8**, 84–90.

Krumbein, W.C. (1939). Graphic presentation in statistical analysis of sedimentary data. In: *Recent Marine Sediments. A Symposium* (ed. P.D. Trask), American Association of Petroleum Geology, Tulsa, OK. Murby, London, 558–591 pp.

Krumbein, W.C. & Pettijohn, F.J. (1938). *Manual of Sedimentary Petrography*. Appleton-Century-Crofts, New York.

Leong, L.S. & Tanner, P.A. (1999) Comparison of methods for determination of organic carbon in marine sediment. *Marine Pollution Bulletin*, **38**, 875–879.

Lindholm, R.C. (1987) *A Practical Approach to Sedimentology*. Allen & Unwin, London.

Lloyd, P.J. (ed.) (1985) Particle size analysis. *Proceedings of the 5th Particle Size Analysis Conference*, University of Bradford, 16–19 September. John Wiley & Sons, Chichester.

Loncarevic, B.D., Courtney, R.C., Fader, G.B.J., Piper, D.J.W., Costello, G., Hughes Clarke, J.E. & Stea, R.R. (1994). Sonography of a glaciated continental shelf. *Geology*, **22**, 747–750.

Magorrian, B.H., Service, M. & Clarke, W. (1995) An acoustic bottom classification survey of Strangford Lough, Northern Ireland. *Journal of the Marine Biological Association of UK*, **75**, 987–992.

Mantoura, R.F.C., Wright, S.W., Jeffrey, S.W., Barlow, R.G. & Cummings, D.E. (1997) Filtration and storage of pigments from microalgae. In: *Phytoplankton Pigments in Oceanography* (eds S.W. Jeffrey, R.F.C. Mantoura & S.W. Wright), UNESCO, Paris, 283–306 pp.

McCave, I.N. & Syvitski, J.P.M. (1991) Principles and methods of geological particle size analysis. In: *Principles, Methods, and Application of Particle Size Analysis* (ed. J.P.M. Syvitski), Cambridge University Press, Cambridge, 3–21 pp.

Mook, D.H. & Hoskin, C.H. (1982) Organic determinations by ignition; caution advised. *Estuarine, Coastal and Shelf Science*, **15**, 697–699.

Morris, A.W., Loring, D.H., Bale, A.J., Howland, R.J.M., Mantoura, R.F.C. & Woodward, E.M.S. (1982) Particle dynamics, particulate carbon and the oxygen minimum in an estuary. *Oceanologica Acta*, **5**, 349–353.

Mortimer, R.J.C, Krom, M.D., Watson, P.G., Frickers, P.E., Davey, J.T. & Clifton, R.J. (1998) Sediment-water exchange of nutrients in the intertidal zone of the Humber estuary, UK. *Marine Pollution Bulletin*, **37**, 261–279.

Mudroch, A. & Azcue, J.M. (1995) *Manual of Aquatic Sediment Sampling*, Lewis, Boca Raton, FL, 224 pp.

Newton, R.S. & Stefanon, A. (1975) Application of side-scan sonar in marine biology. *Marine Biology*, **31**, 287–291.

Paterson, D.M. (1989) Short-term changes in the erodibility of intertidal cohesive sediments related to the migratory behaviour of epipelic diatoms. *Limnology and Oceanography*, **34**, 223–234.

Paterson, D.M. (1997) Biological mediation of sediment erodibility: ecology and physical dynamics. In: *Cohesive Sediments* (eds N. Burt, R. Parker, & J. Watts), pp. 215–230. John Wiley & Sons, Chichester.

Pejrup, M. (1988) The triangular diagram used for classification of estuarine sediments: a new approach. In: *Tide-influenced Sedimentary Environments and Facies* (eds P.L. de Boer, A. van Gelder & S.D. Nio), Reidel Publishing, Dortrecht, 289–300 pp.

https://telegram.me/Geologybooks

Pocklington, R. & Hardstaff, W.R. (1974) A rapid semiquantitative screening procedure for lignin in marine sediments. *Journal du Conseil. Conseil International pour l'Exploration de la Mer*, **36**, 92–94.

Porter, K.G. & Feig, Y.S. (1980) Use of DAPI for identifying and counting aquatic microflora. *Limnology and Oceanography*, **25**, 943–948.

Rumohr, H. (1999) Soft bottom macrofauna: collection, treatment, and quality assurance of samples. *ICES Techniques in Marine Environmental Sciences*, No **27**, ICES, Denmark, 26 pp.

Rumohr, H. & Schomann, H. (1992) REMOTS sediment profiles around an exploratory drilling rig in the Southern North Sea. *Marine Ecology Progress Series*, **91**, 303–311.

Shepard, F.P. (1954) Nomenclature based on sand-silt-clay ratios. *Journal of Sedimentary Petrology*, **24**, 151–158.

Soille, P. (1999) *Morphological Image Analysis; Principles and Applications*. Springer, Berlin.

Somerfield, P.J. & Clarke, K.R. (1997) A comparison of some methods commonly used for the collection of sublittoral sediments and their associated fauna. *Marine Environmental Research*, **43**, 145–156.

Soulsby, R. (1997) *Dynamics of Marine Sands*. Thomas Telford, London.

Stephens, J.A., Uncles, R.J., Barton, M.L. & Fitzpatrick, F. (1992) Bulk properties of intertidal sediments in a muddy, macro-tidal estuary. *Marine Geology*, **103**, 445–460.

Sutherland, R.A. (1998) Loss-on-ignition estimates of organic matter and relationships to organic carbon in fluvial beds. *Hydrobiologia*, **389**, 153–167.

Tolhurst, T., Paterson, D.M. & Gust, G. (2000) The influence of an extracellular polymeric substance (EPS) on cohesive sediment stability. Abstract. *INTERCOH 2000*, 4–8 September, Delft.

Trask, P.D. (1930) Mechanical analysis of sediments by centrifuge. *Economic Geology*, **25**, 581–599.

Underwood, G.J.C. & Smith, D.J. (1997) Predicting diatom exopolymer concentrations in intertidal sediments from sediment chlorophyll *a*. *Microbial Ecology*, **35**, 116–125.

Underwood, G.J.C. & Smith, D.J. (1998) *In situ* measurements of exopolymer production by intertidal epipelic diatom-dominated biofilms in the Humber estuary. In: *Sedimentary Processes in the Intertidal Zone*. Geological Society Special Publication 139 (eds K.S. Black, D.M. Paterson & A. Cramp), Geological Society of London, London, 125–134 pp.

Underwood, G.J.C., Paterson, D.M. & Parks, R.J. (1995) The measurement of microbial carbohydrate exopolymers from intertidal sediments. *Limnology and Oceanography*, **40**, 1243–1253.

Warwick, R.M., Goss-Custard, J.D., Kirby, R., George, C.L., Pope, N.D. & Rowden, A.A. (1991) Static and dynamic environmental factors determining the community structure of estuarine macrobenthos in SW Britain: why is the Severn Estuary different? *Journal of Applied Ecology*, **28**, 329–345.

Weliky, K., Suess, E., Ungerer, C.A., Muller, P.J. & Fischer, K. (1983) Problems with accurate carbon measurements in marine sediments and particulate matter in seawater: a new approach. *Limnology and Oceanography*, **28**, 1252–1259.

Wentworth, C.K. (1922) A scale of grade and class terms for clastic sediments. *Journal of Geology*, **30**, 377–392.

https://telegram.me/Geologybooks

Whitlatch, R.B. (1981) Animal-sediment relationships in inter-tidal marine benthic habitats: some determinants of deposit-feeding species diversity. *Journal of Experimental Marine Biology and Ecology*, **53**, 31–45.

Widdows, J., Brown, S., Brinsley, M.D., Salkeld, P.N. & Elliott, M. (2000) Temporal changes in intertidal sediment erodibility: influence of biological and climatic factors. *Continental Shelf Research*, **20**, 1275–1289.

Wirth, H. & Wiesner, M.G. (1988) Sedimentary facies in the North Sea. In: *Biogeochemistry and Distribution of Suspended Matter in the North Sea and Implications for Fisheries Biology* (eds S. Kemp, V. Dethlefse, G. Liebezeit & U. Harms), Mitt Geologisch-Paläontologischen Institutes der Universität, Hamburg. Heft 65, 269–287 pp.

Wright, S.W., Jeffrey, S.W. & Mantoura, R.F.C. (1997) Evaluation of methods and solvents for pigment extraction. In: *Phytoplankton Pigments in Oceanography* (eds S.W. Jeffrey, R.F.C. Mantoura and S.W. Wright), UNESCO, Paris, 261–282 pp.

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Chapter 3
Imaging Techniques

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Abstract

Imaging methods applied to benthos techniques allow the taking of *in situ,* non-destructive, representative and repeat identical samples. Methods are useful in surveys, mapping, observation, feature identification and enumeration, behaviour recording and location, evaluation and performance of samplers/platforms. Imaging methods can cover a wide range of scales and resolutions, from kilometres with acoustic imaging, to millimetres with optical methods. Typical acoustic methods include single beam, ground discrimination sonar, sidescan sonar and multibeam/swathe sonar. Many aspects related to the use of video are explained including sensors, format, transmission, media storage, power supply, illumination, calibration/measurement and still imaging. Reviews are made of carrier platforms (divers, drop frames, tow platforms, Remotely Operated Vehicles (ROV), Autonomous Underwater Vehicles (AUV), manned submersibles, vehicle navigation and data acquisition), special applications (sediment profile imagery, laser and medical technologies) and laboratory imaging and analysis.

Keywords imaging, mapping, observation, acoustic imaging, optical imaging, video, ROV, AUV, submersibles, SPI

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3.1 Introduction

Progress in our understanding of benthic ecology, especially in sub-littoral habitats, has in the past necessitated the use of traditional sampling methods where samples of sediment or fauna are removed for processing. These methodologies disturb, if not actually destroy, the objects under investigation, give no idea of the representativity of the sample and do not allow *sensu stricto* repeated sampling at the same spot.

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However, observational methods are non-destructive and allow information from one identical object to be gathered over time. This approach has long been common in rocky shore ecology where researchers can reach their study sites without getting wet and can document their observations directly *in situ*. While sample material is rarely removed, visual impressions are recorded. Other non-destructive methods include the observation of invertebrate behaviour in aquaria. Image recording in both these situations can be undertaken with non-specialist camera systems. However, when imaging devices are taken into an underwater environment, specialist systems and carriers must be deployed.

Imaging methodologies can be used in a variety of studies:

- Surveys
- Observation and documentation (non-destructive and non-selective)
- Recording of behaviour/activities
- Identification and enumeration of features
- Evaluation and performance of samplers
- Location of samplers/samples and platforms.

Common in-water imaging systems can be classified primarily into two types: acoustic and optical. Acoustic methods allow for wide-area (km range) low-resolution imaging (5 + m range), while optical methods allow smaller area (m range) high-resolution images (cm range). Table 3.1 shows the scales and resolutions of the most common imaging methods applied to benthic ecology. Additional information is also given in Table 3.1 in terms of comparative economics of capital cost and processing. Different methods have different uses and give different types of result. In an area survey, it may be beneficial to use more than one methodology with a nested sampling approach that covers different resolutions and scales. All methods are relatively expensive, requiring technical knowledge to operate

Table 3.1 Comparison of different imaging methods standardised to one hour's ship-time data gathering at 100 m depth. Cost of equipment is indicative as prices vary according to system complexity. Processing time is related to the one hour of data collected, more data may not require exact multiples of this.

Method	Area coverage (km^2)	Scale (m)	Resolution (m)	Equipment cost (Euro)	Processing time (h)
Multi-beam	1	200	1.0	>50,000	8
Sidescan sonar	1	200	0.2	30,000	8
Bottom discrimination sonar	0.1	10	10–100	22,000	2
Laser line scan system	0.05	7	0.01	>75,000	8
Towed underwater television	0.002	1	0.02	16,000	1–2
Remotely Operated Vehicles (ROV)	0.001	1–2	0.01	20,000–40,000	1–2
Still image camera	2*		0.01–0.001	10,000	2
Sediment profile imagery	2*	0.15	0.002	26,000	2

* This is the rough area that can be representatively covered in one hour.

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compared to a traditional grab or dredge, but all are relatively fast methods with minimal processing unless high levels of detail and analysis are required. Images can provide a great deal of information, besides giving direct measurements of visible parameters, they give a feel for an area, an idea whether it might be useful to take further samples and the ability to document over time. On this subject, we can only repeat that 'a picture is worth a thousand worms'.

Since our last review on imaging methods appeared (Smith & Rumohr, 2005), there has been a gradual evolution in digital technologies with two major technological innovations, the introduction and widespread use of HDTV (High-Definition TeleVision) and the introduction of LED (Light Emitting Diode) lighting systems. This chapter not only encompasses the older technologies as they are still being used but also gives details of primary methods for scientific imaging, from wide-area imaging systems to smaller area higher resolution systems, an overview of which is given in Table 3.1. The imaging methods are followed by sections on carrier platforms, special applications, laboratory imaging and analysis. An excellent review of scientific applications of imaging techniques to benthic sciences has been made by Solan *et al.* (2003). Typical examples of using imaging methods include the use of acoustic and optical techniques for habitat mapping (Brown *et al.*, 2002), the use of video for assessing patch dynamics (Parry *et al*., 2003) and invertebrate stocks (Smith & Papadopoulou, 2003), Sediment Profile Imagery (SPI) for anthropogenic impacts (Nilsson & Rosenberg, 2000; Smith *et al*., 2003), scaled imaging with underwater video (Pilgrim *et al*., 2000) and acoustic methods for habitat mapping (Greenstreet *et al*., 1997). With increasing importance and use of imaging material by scientists (particularly video), operational guidelines, methodologies and quality assurance issues have become more available, though mostly through the grey literature (MESH, 2005; Somerton & Glendhill, 2005; Coggan *et al*., 2008; CEN, 2011).

3.2 Acoustic imaging

Acoustic mapping techniques are an essential part of the imaging approach in recording physical attributes, habitat and community patterns of seafloor habitats at different spatial scales. Acoustic devices can be ranked according to their resolution capacity and the area covered. Historically, single beam echo-sounders developed for depth measurement have been used to depict bottom structure as well as some sedimentological properties depending on the reflecting properties of the seafloor. For all acoustic methods, the basic principles apply that the lower the frequency the longer the range, but the higher the frequency, the greater the resolution.

Acoustic ground discriminating systems

Single beam echo-sounders (30 kHz to 3.5 MHz) may be used to obtain a variety of information about the reflective characteristics of the seabed. Such systems reveal

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the contour and depth of the seabed and indicate the thickness and structure of the sediment layers. They send a pulse of sound at a particular frequency that reflects from the seabed and the echo is then picked up by a transducer. The time between the sending and the return of the signal is traditionally used to measure the water depth. There are presently a number of ground discriminating systems where details of the echo are electrically processed to obtain information on bottom type. While the RoxAnn™ and Echoplus use an electronically gated tail part of the first return echo (E1 – roughness) and the whole of the first multiple return echo (E2 – hardness), the QTC system (Quester Tangent Corporation) analyses the first echo by means of principal component analysis. The resulting 64 parameters are used for ground discrimination. It should be noted that the majority of Acoustic Ground Discriminating Systems (AGDSs) are hull-mounted and are limited to use in shallow waters (<200 m depth) either by frequency or increasing size of beam footprint and, therefore, resolution. Recent developments apply the analysis of echo-sounder returns to include multi-beam systems, therefore enabling wider swathes to be analysed.

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Sidescan sonar

Sidescan sonar is an acoustic imaging device (100–1000 kHz) used to provide wide-area, high-resolution pictures of the seabed. A geologists' tool for some time, it is now routinely used by benthic biologists. This acoustic method is used for charting seabed features (reefs, sand ripples, seagrass, gross changes in sediment type) and revealing special sediment structures of both biogenic and anthropogenic origin (e.g. feeding mounds or trawl tracks). The system typically consists of a towed underwater transducer (towfish/torpedo) connected via a fibre optic or coaxial cable to a shipboard control unit and recording device. The emitted narrow lobe of sonar energy has a beam geometry that ensonifies a wide swathe of the seabed. The returning echoes from the seafloor are received by the transducers and, amplified on a time varied gain curve, are transmitted to the recording unit. The recorder further processes these signals into either analogue or digital format, and then prints the echoes on paper or on a monitor screen, scan by scan over time, building up a waterfall image of the seabed.

Modern high-frequency sidescan sonar devices offer a high-resolution image of the seabed that can detect objects in the order of tens of centimetres at a range of up to 100 m on either side of the towfish. Sidescan sonar can produce, under optimal conditions, an almost photorealistic picture of the seafloor. Over several geo-referenced swathes, an overall resulting image, or mosaic, of the area can be built up forming an area map, where geological and sedimentological features, and even biological features (i.e. seagrass beds) are easily recognisable. A handbook for seafloor imagery analysis has been produced by Blondel and Murton (1997). Considerable increase in resolving capacity down to centimetres may be available in the future from the full use of Synthetic Aperture Sonar (SAS) in seafloor imaging.

Sidescan sonar may play a larger role in the future in seabed discrimination and sidescan processing software is becoming available that will automatically classify features on the seabed and, as in single beam AGDS, ground-truthing is necessary with higher resolving methods (sediment grabs, stills, video).

Swathe bathymetry

Swathe bathymetry through hull-mounted multi-beam or inferometric methodologies is a relatively new seabed mapping technology that produces high-density geo-located depth measurements through digital processing techniques, and can be used to create impressive shaded-relief or colour topographic maps. A major advantage of multi-beam systems over sidescan sonar is that they generate quantitative bathymetric data, which can also be used for classification. They may also utilise backscatter data to form images similar to those of sidescan data, but with lower resolution, partly due to the variable height above the seabed of the hull-mounted sensors. The beam width makes them less useful for object detection when the objects are less than 1 m square and they require very accurate information on navigation, roll, pitch and sway and also calibration of sound velocity.

For detailed presentation of these acoustic methods and their use in seabed characterisation, see Chapter 2.

3.3 Video

Underwater video camera systems

Two broad classes of camera system are used underwater: daylight cameras and low-light (intensified) cameras. The former are standard Charge Coupled Device (CCD) monochrome and colour cameras, while the latter comprise intensified cameras CCD (ICCD – Intensified CCD) cameras. The powerful monochrome Silicon Intensified Target (SIT) tube cameras are no longer produced, but are still used as pilot cameras and as standards when the light intensity is comparatively measured. Choice of camera is based principally on the sensitivity required by the user since underwater viewing range is related to light sensitivity of the camera (Table 3.2). The intensified systems are not only significantly more sensitive but also significantly more expensive. Most underwater cameras are CCD cameras, working in the daylight range with the addition of lights for work at night or at depth. Because of dissipation of light in water, the effective viewing range of a standard CCD camera will be a few metres, with better performance from a monochrome than from a colour camera. Intensified cameras are generally used for longer-range viewing and, with the same amount of light as a CCD camera, may have a viewing range of 10 m. They are also useful for research purposes where light may induce non-natural behaviour, for example in fish behaviour studies, or in turbid conditions

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Table 3.2 Typical sensor choices for optical imaging (type of image sensor, limiting sensitivity in lux, typical horizontal range, indicative cost in kilo-Euro (1000 m depth rating) and typical application).

Sensor	Limiting sensitivity (lux)	Limiting resolution (TVL)	Typical range (m)	Cost (KEuro)	Application
HD CMOS	2–4	720/1080	2–3	10	Inspection, monitoring and survey
Mono CCD	0.1	570	5	3.5	Near field inspection and monitoring
Colour CCD	0.5	470	2–3	7	Standard survey
SIT	5×10^{-4}	600	20	17	Navigation, all-round perception
ISIT	5×10^{-6}	525	40	30	Far field viewing and natural behaviour studies
Gen 1 ICCD	1×10^{-3}	480	23	14–17	Far field viewing and natural behaviour studies
Gen 2 ICCD	5×10^{-5}	450	23	19–22	Far field viewing and natural behaviour studies

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where additional lighting may make visibility worse through backscatter. Though the monochrome SIT camera is based on 45-year-old tube technology, in terms of price and resolution it was one of the best systems available, its major drawback being that the tube was very easily damaged by pinpoint light sources, especially the sun. While monochrome cameras tend to have a high degree of resolution with sufficient lighting, colour cameras do have the advantage of presenting natural colours and, therefore, more easily interpreted images. Sensitivity of cameras is also governed by spectral response, and SIT cameras were ideally suited for underwater applications as maximum sensitivity occurs in the blue/green region, coinciding with maximum transmission of water. CCD cameras have maximum response in the red light region where transmission in water is much poorer; however, they can be used with infrared lighting in low ambient light conditions over short ranges. This can be ideal for close range behavioural studies where the subjects are insensitive to infrared rays, both in the laboratory and the field. The highest quality of recording for CCD is seen in 3-CCD cameras (broadcast quality standard) where light entering the camera lens is split through a prism into red, green and blue light with a single CCD sensor for each colour. Because light is split into constituent wavelengths, 3-CCD cameras are highly sensitive to light and require a high level of ambient lighting.

High-definition television (HDTV) has become one of the new standard formats, firstly in digital broadcast and consumer movie releases, and latterly in lower-end consumer video products. Underwater HDTV cameras, although initially very expensive, are only now slightly more expensive than standard colour cameras. HDTV is a High-Definition (HD) format with more than twice the line resolution of standard cameras (HDTV: 1920×1080 pixels with 16:9 aspect ratio; SDTV: 640×480 pixels with 4:3 aspect ratio) and is the standard for documentary makers. The 16:9 wide screen format has become standard for consumer television screens and laptops, but at present has not replaced all computer screens and existing TV monitors (4:3, more square format). The High-Definition Serial Digital Interface

(HD-SDI) output from HD cameras or recording systems requires special cables (High-Definition Multimedia Interface (HDMI), HD component or fibre optic), but some may also output standard definition for viewing on non-HD equipment. HD cameras require more light than standard cameras and are normally coupled with wide-area flooding white lights (High-Intensity Discharge (HID), Hydrargyrum Medium-arc Iodide (HMI), LED; see the section entitled 'Illumination'). Unless high compression is used, digital recorders need to be in the order of hundreds of gigabytes. When longer profiles are recorded (as from remotely operated vehicles (ROVs)) the digital memory requirements can amount to terabytes of memory.

The use of stereo-video with parallel-mounted, matched cameras has been driven by the need for underwater inspection in oil field applications and where a three-dimensional (3D) view is required with regard to manipulation. Left and right cameras can be observed through head-mounted mini-monitors on the left and right eye, or through using polarising glasses and having two monitors overlapped by projection through a prism. A more recent development, the TV-Trackmeter system (Technomare, Italy), uses twin cameras and a computer processor. One single image is seen by the operator, but the operator can track objects or make 3D measurements in real time. At the time of writing, consumer 3D HDTV cameras are coming on the market. High-end consumer televisions have the ability to display 3D images with special glasses worn by the viewer (both polarising and alternate eye-blocking technologies). This will invariably come to the underwater world and will be reported in the next edition of this volume.

Lenses

Choice of lenses is another important feature of the camera depending on the application required. For general purposes, a wide-angle lens will cover more extensive areas at close range and have a good depth of field, but may have some distortion at the edges of view and pick up incidental light reflections near the surface. Optimum viewing angle for general underwater work is 60–70°. The use of zoom lenses underwater is becoming more common, especially in ROV systems and for close-up observation studies. At full zoom, they require a stable platform, better lighting and have a more limited minimum focal distance and depth of field than a conventional lens. In a self-contained system, the use of a zoom may not be necessary as this function can be undertaken by the carrier vehicle moving up to the subject.

Housings

Video cameras must be encased to protect them from seawater and pressure. Shallow-water self-contained systems can be constructed of plastic/epoxy or metal with irregular shapes, as the pressures exerted in diving depths are within the strength tolerances of modern light materials. Deep systems are encased in cylindrical housings with a clear port at one end and electrical/video connection

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at the other. Plastic/epoxy resin housings can be used to depths of no more than 300 m. Metals including anodised aluminium, stainless steel or titanium are used in construction of all deeper-water housings. Titanium, with the best strength to weight factor, is the most expensive material. Anodised aluminium is commonly used for small housings in the 600–1000 m range. The technology behind composite materials and ceramics continually improves, with the result that they may be used in deeper housings in the future.

The ported ends of the housings are simply sealed (commonly pop-on/pop-off, with a safety retainer) and rely on O-rings to prevent entry of water. Normally two O-rings are used for an endcap seal, an in-line seal and a faceplate seal. Most flooded housings are caused because of inattention to the O-ring, which is the least expensive part of the system.

Clear ports can be constructed of acrylic, glass or crystal. Acrylic is easily scratched, but because it has the same refractive index as water, light scratches are not seen underwater, but can be polished out of the face using fine abrasive cleaner. For deeper applications, glass or crystal ports are used. The shape of the port is very important, as a flat port will reduce the angle of view, make objects appear larger and produce some distortion at the edge of the picture. A domed port, although more difficult to produce, will restore the angle of view and real object size with little distortion.

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Data transmission

Video data can either be stored immediately, or transmitted and stored. To store the data on-board the carrier vehicle, a video recorder is necessary, which will require a low-volume, low-power recorder. Hi-8 format or digital video fit these criteria (see the section entitled ‘Storage media’).

Transmission to the surface is by umbilical cable, which will contain various wires to cover both power and control as well as data transmission. Data transmission can be either analogue through a simple coaxial cable (composite signal) or, for maximum quality, digital through a fibre optic cable. Fibre optics require special end members for connection and digitisation of the original analogue signal and therefore are more costly options. However, they have the benefit of a very thin cross-section, lightweight and broadband data transmission over long distances, with no signal attenuation over typical usage at sea (essential for HDTV and multi-beam). The maximum transmission distance without amplification for an analogue cable without significant loss of video signal is approximately 500 m, which can be increased up to 2000 m with amplification. The use of surface amplification may also amplify electrical ‘noise’ that has been picked up in the cable. Cables can have a diameter from a few millimetres up to several centimetres depending on their function and may be armoured with the inclusion of wire or kevlar sheaths. They may also be oil- or tar-filled to contain water ingress if the outer covering should be punctured. The use of armoured cables allows pulling

load to be put on the cable and instrumentation, including cameras, can be deployed directly from the cable without the need for a separate steel wire or rope. The armoured cable will allow the instrumentation to be retrieved manually or by winch.

Wireless through-water transmission of video is not currently possible at full frame rate in real time (25 frames per second in Europe), because of limitations in speed of transmission and bandwidth (limitations imposed by low frequencies required). Where applications do not require real time, it may be possible, while recording full video underwater, to transmit periodically to the surface compressed still images (low quality), with updates in the range of one per minute. Research is underway to increase wireless ability using compressed data.

Once at the surface, images can be wirelessly broadcast in a number of standard ways (radio transmitter or satellite) to show live images at any distance from the source. Live images have also been transmitted through the Internet for teaching purposes (low resolution) through cellular phone connection. Compression is required, but as technology improves (speed of computers and phone lines), the quality of transmitted images will continue to improve.

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Format

Across the world, there are three basic international colour TV standards for transmission, recording and playback:

(1) *PAL (Phase Alternating Line)*: Western European format (Europe, Australia, east coast of South America). 4.43-MHz colour subcarrier, 625 lines, 50 Hz, 25 frames per second.
(2) *SECAM (Supérieure Elégante Couleur et l'Affaire Magnifique)*: French format (France Eastern Europe, Middle East, many parts of Africa). 4.43-MHz colour subcarrier, 625 lines, 50 Hz, 25 frames per second.
(3) *NTSC (National Television Standards Committee)*: American format (America and territories, Canada, Japan, Korea, west coast of South America). 3.58-MHz colour subcarrier, 525 lines, 60 Hz. Modified NTSC with 4.43-MHz colour subcarrier, 30 frames per second.

The camera, monitor and recording system must be compatible (i.e. have the same format). Video recorders and high-quality monitors are often multi-format, and reasonably cheap systems can now be found to convert input to output across formats (e.g. Panasonic AG-W1-P recorder), although there will be some loss in original quality.

In addition, there are also six world regional formats for commercially purchased Digital Video Disk (DVD) (three regions with Blu-ray). A pre-recorded disk purchased in one region cannot be read by a player that has been purchased in another region. However, the player may allow several switches in region before it locks in to one region only.

Table 3.3 Typical recording medium choices for optical imaging (cassette recording type, resolution in TV lines, maximum tape length in minute, volume of cassette in relation to Digital Video (DV) cassette, Euro cost of high-quality cassette and typical available signal inputs to recorder).

Medium	Resolution (TVL)	Maximum tape length (min)	Cassette volume	Cassette cost (Euro)	Signal input
VHS	260	240	4	4	Composite
S-VHS	>400	240	4	12	Composite S-Video
Hi-8	>400	90	1.5	10	Composite S-Video
U-Matic HB	270 Colour 370 Mono	90	6	15	Composite
BetaCam SP	>600 Colour	90	8	20	Composite S-Video Component
HDTV tape	>600 Colour	90	8	100	HD-SDI
Digital tape	500	90	1	10	Digital Component Composite S-VHS
DVD	500	120*	1	1	Digital
Digital disk	500–1080	>600*	2–4	50	Digital

* Amount of time recorded on digital media is highly dependent on compression selected, which could allow extremely long recording, beyond regular scientific needs. Hard disk or flash memory media can be supplied in many sizes both in terms of data and size of the container.

Storage media

There are several standards for the recording of video images and comparative data are shown in Table 3.3. Tape systems (either analogue or digital) are still relevant for the scientist because of the need to make use of, or refer to, original or archive material. All new equipment is digital in hard disk, DVD or solid state media. For analogue tape media, VHS using composite input (single coaxial) was the most common standard to the end of the last century. S-VHS has higher resolution but requires a signal input with two separate lines for chrominance (colour information) and luminance (brightness information). This system was widely used although the input may be composite as underwater S-VHS cameras were rare. Betacam, the highest quality recording format on tape that replaced U-matic as the large format 'broadcast quality' recording system, was both bulky and expensive. The Hi-8 format was used where low volume and good quality are required, e.g. in diver-operated housings.

Digital recording is the current standard format in use, because of increasing resolution, constant quality (there is no loss of quality on copying), low power requirements, small size of cassette/disk and the ease of importing into a computer. Digital recorders use mounted discs, PC card storage devices and DVDs (the most economical widespread distribution disk). Digital recordings can be compressed on to disk with a standard DVD disk able to hold approximately two hours of

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video material. Digital disk media give the added advantage of being able to jump immediately to any part of the recording, and software is readily available for non-experts to edit videos on their desktop computers.

Power supply

Power supply is an important consideration for underwater video operations and particular attention should be paid to the stability of the voltage and frequency. Video cameras actually have low power requirements and work on 12 or fewer volts. An uninterruptible power supply (UPS – stabilised power supply) system is advisable where possible if only to power the surface recorder. For the underwater system, if the loading is too high for a UPS system, then a separate power supply is advisable. At sea, loading on a shared generator will vary with the ship's requirements and the mere act of turning on a cooker or heating system can cause on-screen and recording interference. Voltage irregularities may be smoothed in transformation, but frequency alterations have greater effects on recorders.

High voltages and currents are often encountered in underwater systems and extreme care should be taken to ensure that the system is correctly maintained, protected and earthed. Attention is drawn to the UK association of diving contractors code of practice for electricity underwater (AODC, 1985) or any other national security rules.

Video monitors

For a system operator, a high-quality 14- or 16-inch monitor will be optimal and it should be sited to reduce external light reflections, preferably in a darkened room or container. Control operations using video should not be over-crowded, and it is recommended that a separate monitor be placed elsewhere for viewing by large numbers of personnel, and at sea, especially for underway operations, a monitor should be placed on the bridge so that the captain may view operations directly. With HDTV cameras becoming more frequent, older 4:3 aspect ratio monitors may need to be replaced with 16:9 ratio monitors. Replacement may require less space, as new monitors are almost all flat screen.

Illumination

Full colour light penetration is limited underwater with absorption of higher wavelengths (red and orange) in a few metres' distance. Overall light penetration depends on local conditions and may be from metres in eutrophic sediment-rich waters to 100 + m in parts of the oligotrophic Mediterranean. Artificial light must be used at depth for optical imaging purposes and to bring out the 'natural' colours in colour images. Lighting systems normally consist of pressurised housings (at least around the bulb) and must be powered either from external sources (cable supply) or be

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self-contained with batteries. Self-contained systems are time-limited because of the high energy demand of the lights. Lights may have different types of reflectors and domes to produce different angles of diffusion.

Halogen lights (tungsten) are the most common type in use for general underwater lighting, having several advantages such as low-cost replaceable bulbs, instant on/off performance, capacity to change brightness levels; however, not only do they produce high heat but also a yellow/orange light with limited penetration and hence range, with a burn temperature of approximately 2500–3000 K, which gives less realistic colours. As filament lamps, they have a limited bulb life, are less robust and are sensitive to shock and vibration.

HID lights (high-intensity discharge gas arc lamps) came into more common use in the last decade. They require a more substantial housing for the electrical ballast system (either together with the light head or in a separate housing), take two to three minutes to reach full operational brightness, cannot immediately be restarted or dimmed, but have a longer bulb life (no delicate filament), although they are more expensive. HID lights produce a much more striking blue/white illumination, burning at 4000–6000 K, with a more realistic visualisation of colour and contrast. Similar wattage to halogen lights produces 4–6 times as much light; therefore, for the same brightness, much less power than halogen lights is necessary, an important issue in situations where batteries are used to power the light.

HMI lights (Hydrargyrum medium-arc iodide) are another type of gas discharge lamp. They are more expensive than HID lights, but have been the light source of choice for underwater movie film and documentary makers because of the very high-wattage heads available (up to 2400 W) for wide area coverage. Unlike HID lights, they are instantly restartable but still require two to three minutes to reach full power.

LED lights (light emitting diode) use the most up-to-date technology, which has become widely available in the last few years. Diodes size is very small and many can be grouped on one light source. They can be fitted into small housings, although they do require a small electronic board for power management. They are highly efficient (lumens per watt), have long life, are reliable, robust, have very low power consumption, can be switched on/off instantly and can be varied by switching on/off elements in the collective source. They can support very high light temperatures (5500–7000 K).

For still photographic systems, a dedicated flashgun synched to the camera has previously been a necessary accessory for precision illumination. However, modern digital camera systems can produce acceptable images on newer underwater lighting systems (HID/HMI/LED lights), although reflectors/diffusers may be needed to prevent hotspots.

Coverage and correct angle of illumination to the sensing head (photographic or video) are key features in producing good-quality images. Offsetting the lighting will produce greater shadow, which highlights parts of the image and gives an

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idea of 3D representation, although it should be borne in mind that large shadows will hide parts of the image. Large offsets will also reduce the amount of light backscatter to the lens in the case of high water turbidity.

Calibration and measurement

Live or recorded video can be calibrated for measurements either prior to use or continuously online. Prior to use, a fixed grid with measuring bars can be placed in front of the camera and the image recorded (underwater since the refraction index underwater is different from that in air). The calibration will hold as long as the view does not change. Thus, a sled-mounted camera with a fixed angle of view and fixed distance above the focus plane can be calibrated by filming a measured grid fixed to the seabed. The grid should be larger than the field of view so that any aberration towards the edge of the grid in the viewed image can be noted. In playback of recorded material the grid can be copied on to a transparent slide and fixed on the screen of the monitor. All objects in the plane of the calibrations can be measured fairly accurately, but there will be inaccuracies in objects above or below the measuring plane. This method can be used effectively for density measurements of particular features. For continual scaling, a number of lasers can be used. Lasers produce focused beams and if two lasers are parallel-mounted at a known distance apart, the viewed laser spots will remain parallel at the same distance apart if directed on to a perpendicular plane. This method gives the ability to make size measurements in the area of the projected laser beams. A third laser can be added to make size and distance measurements. This laser forms the third side of a right-angled triangle for the projected beams, but it is mounted at a very slight offset angle. The distance between the third beam and the other two varies with the distance of the projection and by using simple trigonometry, distance is calculated as a function of the offset angle and the distance between the third and either the first or second beam. Images can be calibrated with measurements made by means of computer software. Several commercial systems are available for simple measurements, e.g. Tritech Typhoon VMS system, C-Map Systems VideoRuler and Savante Laser Scanner.

3.4 Photography

Large-area satellite or aerial photography is generally applicable in coastal fringes where features can be detected on the shore, intertidal areas or coastal waters (phytal regions under ideal conditions with calm and clear water). Freely available Internet satellite photograph programmes (Google Earth, Bing Maps) can be very useful for coastal work with images having metre resolution and may be of use in mapping underwater habitats (e.g. seagrass or reefs) to water depths of 10 m or so in very clear waters. Higher resolution satellite images or aerial photographs

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with <1 m resolution may be available for purchase from specialist companies. Both types of image are prone to atmospheric shading (clouds) and for both cases there is an absolute need for 'ground truth' information with data gained using traditional methods. Hyperspectral or mono-spectral (e.g. infrared) images can be used to discern various patterns, such as vegetation, that may not be visible in normal visual images.

Underwater photography has been used since just after the invention of the camera, but came into prominence with SCUBA diving and the proliferation of, first, the Nikonos series of cameras, housed land cameras and then smaller, cheaper compact cameras adapted for underwater use. In the past, analogue film cameras had a 35 mm frame size standard; modern digital cameras have kept the same aspect ratio and most images are stored in the same file type, *jpeg* (a standard compression type, read by almost all computer programmes that can handle images). The technology of digital cameras is constantly developing with miniaturisation, greater sensitivity, better image compression and storage capacity. Sensors resolution grows continuously and at the time of printing is around 10–12 megapixels. Cameras can routinely store 200–1000 images on normal media. This is particularly useful in deep-sea specially housed cameras that may be deployed in excess of six months or autonomous underwater vehicles (AUV)/ROV systems on continuous monitoring transects. Underwater photography was, and is, much used in deep-sea investigations. In particular, the famous Edgerton cameras (BENTHOS) opened the door to a new field of research (Kidd & Huggett, 1980; Foell & Pawson, 1986). Images can be downloaded directly to a computer on the surface through special connector ports. Digital images can be stored with time data or even position data in an associated file or even under the same file name. An external light source is normally needed with a battery pack for a flash to continuously charge the flash for the required deployment duration. With sensitive CCD sensors, separate flashguns are not always necessary and good-quality underwater light (HMI, HID, LED) can be used over short ranges. The light source is best placed to one side of the camera forming an angle of approximately 45° between flash, subject area and camera. This provides shadow, which not only is very useful for understanding structures seen in the image but also reduces backscatter reflections.

Still photographs are a necessary complement to any video recordings since photographic resolution is still better than most video documentation (video loses resolution with increased processing). Underwater photography can supplement the semi-quantitative data from divers and, whether deployed by diver or remotely, allows meso-scale areas or transects to be recorded accurately. Furthermore, it permits life-like reproduction of fine structures and live positions of invertebrate fauna, which are normally destroyed during sampling. It should be noted that the relationship between digital video and digital still cameras has become blurred with both systems providing access to recording both video and still images, although a single instrument cannot replace the quality of the individual cameras.

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3.5 Carrier platforms

Imaging sensors can be carried on a wide variety of carrier platforms, involving both simple and complex, mobile and static, depending on the work to be carried out. Fig. 3.1 shows a cost duration profile for a number of different types of platform. Some overlap exists with other chapters from the handbook and readers should also see Chapters 4 and 7.

Diving

Divers were used as carrier platforms even before the introduction of SCUBA equipment. Because of their relative freedom of movement underwater, small self-contained units are the most popular, especially camcorders and photo cameras. With the advent of digital cameras, divers are able to take a very large number of photographs and are no longer constrained by the 36-shot limit of film cameras, which allows for a greater degree of flexibility. Divers can also see, to a limited extent, the quality of the photos that are being taken. With recent advances in mixed gas technology and decompression issues, divers can now routinely go deeper and penetrate harsher environments. The relevance to imaging is that pressure housings must take this into consideration and while diver camera systems were once proofed to 30 m depth, they must now be routinely proofed to 100 m depth. With a self-contained camera, the diver has direct control to select what to record and how best to carry this out, but should be aware of the special limitations and techniques that need to be applied underwater including lighting techniques, stability, positioning,

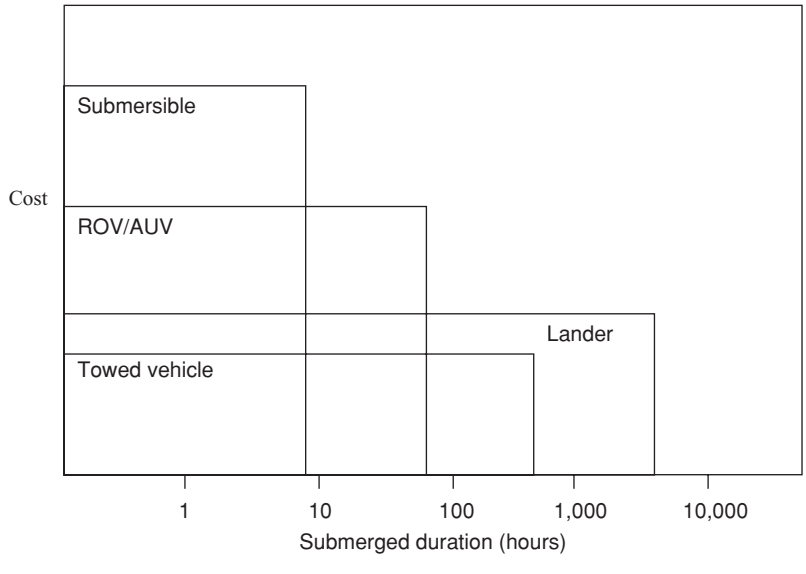

Fig. 3.1 Relative cost against duration of recording of different imaging platforms.

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patience and maintaining water clarity. It should be noted that with a low-light camera, more features may be seen on video than are visible to the diver in the water.

Drop frames

Camera systems can be mounted on remote frames, either self-contained or using a cable. Where a frame is suspended above the seabed, it will move with the motion of the surface vessel, making viewing the image unpleasant in rough weather conditions. The camera can be in a fixed mount or mounted on a pan and tilting motor allowing the camera view to be changed. Underwater lights may be fixed to the pan and tilt so that they track the camera view. To prevent a suspended frame from rotating, a tail fin can be attached to keep the frame oriented into the general direction of movement or into the current. Cameras can also be mounted on to seabed sampling equipment both for directed sampling (the final drop of equipment is controlled over a particular area) and to assess the area from which the sample comes (e.g. information on representativity). Self-contained cameras are also deployed on landers where a system is free-deployed, and later retrieved by using an acoustic release (or time release), releasable ballast weights and sub-surface floatation. These systems can have deployment times ranging from hours to months and consequently require low-volume, low-power systems and for long-term deployments will usually work on time lapse, with photographs or video taken at timed intervals.

Specialised towed platforms

Towed camera sleds are in contact with the seabed so that the camera system can be maintained with a fixed view of the seabed. Therefore, the camera view can be easily calibrated for quantitative viewing. A vertical view gives good imaging over a horizontal plane. However, very low speeds (<1 knot) must be maintained for acceptable resolution and there is little information to be gained concerning relief. Therefore, sled cameras are often mounted to look obliquely forward, giving images with an expanding forward view, which are more difficult to quantify. The advantage of this view is that it gives some warning of obstacles in front of the camera and that there is better visual information in the third dimension of relief and height of an object above the seabed. Towed sleds are commonly used to 500 m depth with umbilicals and live viewing of images. Management of the tow cable/umbilical is very important as it can easily drag in front of the sled and, therefore, should be buoyed at intervals close to the sled with submersible buoys. Maintenance of the correct speed is also very important for a clear camera view. Too fast a speed will blur video pictures, while too slow a speed may allow the cable to drop in front of the sled, or the towing vessel to lose steerage way and may stir up the sediment. Shand and Priestley (1999) describe a sled system whose basic design is used by a variety of institutions around Europe (Fig. 3.2).

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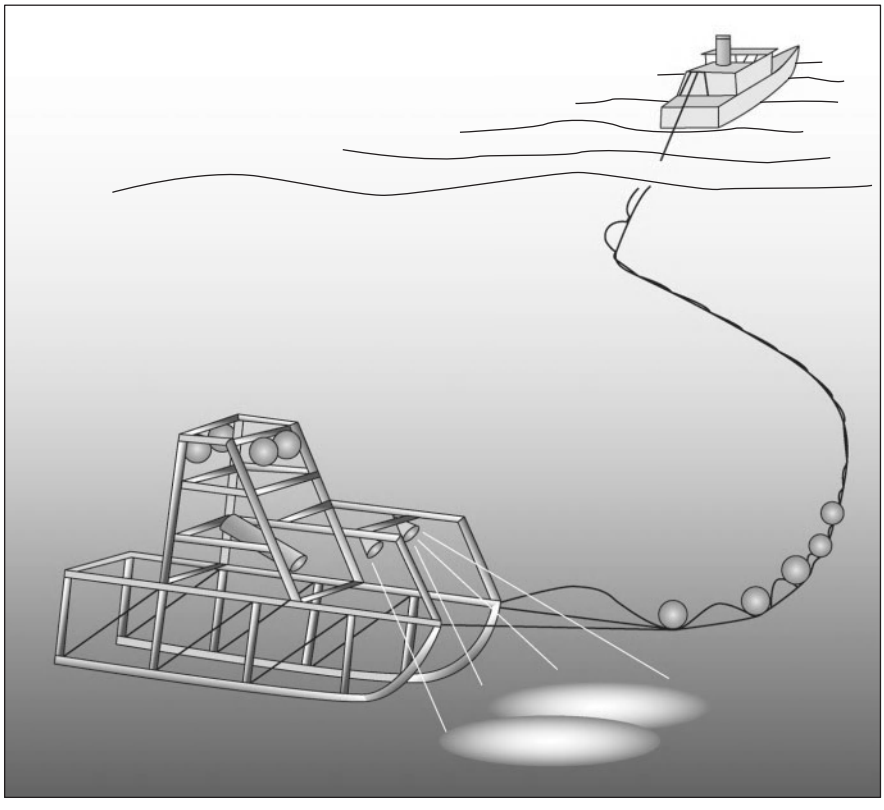

Fig. 3.2 Schematic of 'Aberdeen-type' towed video sled.

A number of specialised towed or drifting vehicles have been developed in-house by large research institutions and private companies, principally for deep-water research, e.g. TOBI and SHRIMP (National Oceanography Centre, United Kingdom), SCAMPI (IFREMER, France), Camper & TowCam (WHOI, United States) and CAMPOD (DFO, Canada). A brief review of these platforms is given in Wernli (2000). These vehicles may contain camera systems for recording the seabed features and may be independent, have umbilical transmission systems or be acoustically or thin-umbilically controlled. Cameras are positioned to look downwards and can be switched on and off by altitude switches. The altitude can be printed on or between photographic frames on still systems so as to quantify the camera view. The main difficulty arising from using these systems is to maintain the vehicle within a particular altitude range of the bottom, which must be done by constant alteration of the length of paid-out cable. The sensors systems must be well protected within the camera frame to reduce the damage caused by inevitable impact with the bottom (frame-polishing events).

Some specialised towed vehicles with controllable surfaces can allow the vehicle to offset from the tow path in a vertical or lateral direction. The remote controlled

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television (RCTV) has four rotating cylinders that utilise the Magnus effect to provide lift in the vertical or lateral plane, whereas the remotely operated television (ROTV) has a box kit structure with small wings on the leading edge to provide the same lift. A disadvantage of this type of vehicle is the requirement for forward movement; it cannot be stopped in mid-use to investigate a target item further. The vehicles can be loaded with a variety of sensors (cameras, lights, sonars and other sensors) and are used commercially to undertake pipeline, cable and route surveys. The main scientific role of these vehicles is in fisheries research, either in trawl behaviour or fish behaviour with respect to trawling and measuring their individual echo target strength. The vehicle is towed adjacent to a trawl and can manoeuvre all around the trawl or even inside, for visual or acoustic inspection.

Remotely operated vehicles

Remotely operated vehicles (ROVs) are tether-controlled and powered vehicles with independent motors and payloads comprising sensor (navigation and imaging) and optional equipment (manipulators, tool packages, scientific samplers, etc.). Throughout the world there are some 200 commercially available models ranging from simple eyeball ROVs in the water (1–2 kW power, 30–600 m depth) to workhorse ROVs used in the oil industry (50–100 kW power, 600–3000 m depth) and bottom crawlers for cable and pipeline burial. There are also a number of specifically developed or adapted ROVs for scientific research, some of the larger of which are shown in Table 3.4 with further details in Wernli (2000). Fig. 3.3 shows several of these large systems. The sensor load that an ROV can carry depends on its size, power availability and the specification of the umbilical cable for transmission purposes. For small ROVs, cameras may be mounted within a general pressure housing, but most cameras are externally mounted in their own pressure housings on pan and tilt motors. The pilot will have at least one camera with a wide-angle lens, and additional cameras can be mounted to cover a variety of views including: intensified tube and CCD cameras for viewing in far field or turbid waters, zoom lens cameras or mini-CCD cameras that can be mounted in almost any place on the ROV (e.g. the end of a manipulator). The importance accorded to obtaining high-quality video imaging is recognised by the use of a centrally placed HDTV camera on all large science ROVs. Larger scientific ROVs have specialised tool skids and adaptations, which may incorporate CTDs and other physico-chemical sensors, suction samplers, water bottles, core baskets, specialised cameras and other specialised sensors (Auster & Tusting, 1997).

Autonomous underwater vehicles

Autonomous underwater vehicles (AUVs) are unmanned, untethered, battery-powered with autonomous navigation capabilities (Fig. 3.4). There are some hybrid vehicles that are tethered by extremely thin umbilicals for data transfer (track control, status and sensing information) normally used not only for mine countermeasures but also in science (Fletcher *et al.*, 2009). Some other vehicles

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Table 3.4 Large science-orientated Remotely Operated Vehicle (ROV) systems in service.

ROV name	Depth rating (m)	Weight (kg)	Payload (kg)	Horse power	Operator	Country
ABISMO*	11,000	3397		13	JAMSTEC	Japan
Aglantha	2,000	740	100	26	Argus/University of Bergen/IMR	Norway
Bathysaurus	5,000	850	110	14	Argus/University of Bergen/IMR	Norway
Doc Ricketts	4,000	4760	275	75	MBARI	USA
Dolphin	3,300	3800	150	67	JAMSTEC	Japan
Holland I	3,000	3240	312	100	Marine Institute	Ireland
ISIS	6,500	3000	190	30	National Oceanography Centre	UK
Jason 2	6,500	3000	150	30	WHOI	USA
Kaiko 7000†	7,000	5600	150	47	JAMSTEC	Japan
Kiel 6000	6,000	3700	100	80	IFM-GEOMAR	Germany
Kraken	1,000	635	80	13	University of Connecticut	USA
Luso	6,000	2200	200	60	EMEPC	Portugal
Nereus‡	11,000	2800	25	7	WHOI	USA
Phoca	3,000	1500	100	37	IFM-GEOMAR	Germany
Quest 5	4,000	3300	250	80	Marum	Germany
ROPOS	5,000	2700	200	40	CSSF	Canada
Ventana	2,300	2570	400	40	MBARI	USA
Victor	6,000	4600	150	80	IFREMER	France

*ABISMO is an extreme deep-water ROV/crawler hybrid vehicle deployed from an above-bottom launcher vehicle (Yoshida *et al.,* 2009).
†The original Kaiko made several trench dives and reached and sampled the seabed at 10,924 m depth but was lost on the surface in 2003. Kaiko 7000 is its 2004 more moderate replacement.
‡Nereus is a hybrid vehicle and can act as an Autonomous Underwater Vehicles (AUV) independently or ROV controlled through an expendable fibre optic tether (Fletcher *et al.*, 2009).

may be partially controlled through a slow acoustic link. AUVs have been at a high state of development in the last five years with a small number of models commercially available. The technology was originally driven by the military in the development of 'smart' torpedoes, but is currently being driven by exploration companies for large-area mapping. They can carry a small suite of scientific sensing instrumentation, for example CTDs and sonar mapping systems. Surface vessels do not necessarily need to be specialised and the AUV can undertake a mission while the support vessel is doing other work. Vehicle navigation normally consists of an inertial navigation system using a mixture of internal compass, Doppler speed logs, 3D motion reference units and dead reckoning software. Some systems can surface periodically to take GPS location updates. Navigation can also be pre-programmed within a long baseline sonar navigation system. Camera systems can be mounted on the AUV, but the AUV must have the ability to provide a stable platform and travel safely close to the bottom within imaging range. Sensors, lighting and recording systems are limited in terms of size and power availability. A few AUVs have been specially developed for near-bottom work including ABE (WHOI, United States) and IAUV (MBARI, United States).

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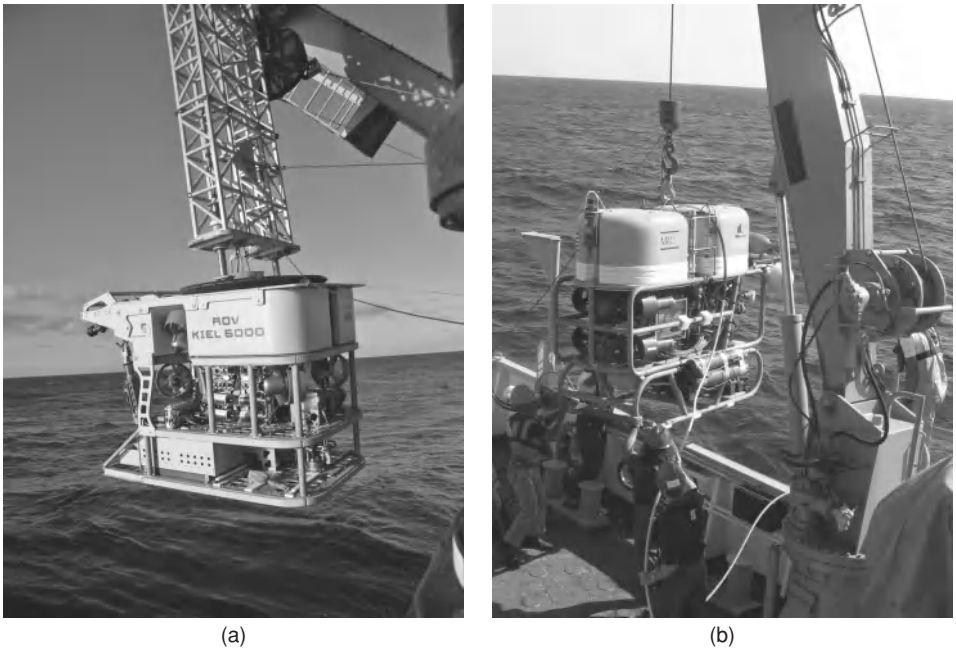

(a) (b)

Fig. 3.3 Science Remotely Operated Vehicles (ROVs) (a) Kiel 6000 (credit: GEOMAR) (b) HCMR 2000 m Max Rover. (For a colour version of this figure, see Plate 3.1.)

Manned submersibles

There are a number of manned submersibles available to the scientific community with a variety of depth ratings from 300 to 6000 m. A listing of the deeper-rated research submersibles is shown in Table 3.5. The submersible has certain

(a) (b)

Fig. 3.4 Autonomous underwater vehicles (AUV) (a) Gavia coastal AUV system with primarily imaging sensors (courtesy of Teledyne Gavia) (b) Remus 6000 large oceanographic AUV system with near-bottom imaging capabilities (credit: Hydroid). (For a colour version of this figure, see Plate 3.2.)

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Table 3.5 Major characteristics of deep-diving manned submersibles.

Submersible name	Maximum depth (m)	Personnel (crew + observer)	Seabed time	Speed (km/h)	Country of operation
Alvin*	4500	1 + 2	4–5 h	2.5	USA
Jiaolong†	7000	3	12 h	–	China
Mir I and II	6000	1 + 2	10–15 h	9.3	Russia
Nautile	6000	2 + 1	4–5 h	4.6	France
Pisces V	2000	1 + 2	6 h	3.7	USA
Shinkai 2000	2000	2 + 1	4 h	5.6	Japan
Shinkai 6500	6500	2 + 1	4 h	4.6	Japan
SeaLink	914	2 + 2	4 h	2	USA
NR1‡	724	11 + 3	30 d	4.5	USA

*Alvin is in overhaul with a rating after 2012 for 6500 m depth and bottom time six to seven hours.
†At time of press, Jialong is in tests building up to 7000 m capability.
‡The NR1, a military submarine with primary objective to allow naval oceanographic research, was deactivated in 2008.
Source: Adapted from Rona (2000).

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advantages over an ROV of being untethered and being able to put a scientist onto the seabed to intervene through remote manipulation or to use the most sophisticated imaging system of all, human stereoscopic vision. Almost all submersible systems are fitted with video and still photographic systems, allowing a permanent record to be kept. Cameras are mounted externally, although some may be hand-operated through viewing ports. Deep submersibles have small viewing ports and external cameras may give a less restricted external view. Requirements are for small size and low power consumption. Hand-held camera systems can be run off their own batteries, but larger units (recorders and hull-mounted cameras) will be limited by having to be powered from the submarine battery system. Most research submersibles are equipped with a variety of external pelagic and benthic sampling and sensing equipment. Bottom time is generally limited to approximately four hours with a possibility of one to two dives per day.

Navigation and positioning of the carrier platform

For many investigations, it is necessary to know the position of the carrier vehicle for tracking to a particular location. It should not be assumed that a towed carrier is positioned at an exact distance behind the towing vessel. Although expensive, acoustic beacon systems are the best solution for high-accuracy positioning, where transponders are positioned on the seabed covering the work area. The carrier vehicle is equipped with a pinger and the support vessel with another transponder. By communicating with the transponders, the control system can locate the position of the pinger. Long baseline systems can work over a range of kilometres with metre or even sub-metre accuracy. With an ultra-short baseline system, the transponders are all located in one acoustic head deployed from the surface vessel. This is a less expensive but less accurate system. It can be used over a range of approximately

1 km with accuracies in the range of 5–20 m. Accuracy and range for both systems are dependent on a number of factors including water layers, depth and bottom topography. Conditions will be less than optimal in shallow waters with an irregular bottom because of scattering and reflection of the acoustic signal. Acoustic beacon systems by themselves give a relative positioning in relation to the transponder head. For absolute positioning the system can be linked to a GPS or DGPS satellite positioning system, giving the geographic coordinates of the carrier platform.

Data acquisition and processing

With regard to multiple data acquisition processing, there are several software solutions for tying the data acquired into one system, enabling video and positioning data to be merged into a single data system, together with a variety of other environmental data and annotations. Complete surveys can be set up, overlaid onto existing maps, charts and bathymetry, and played back at a later date. This allows for better survey management, faster post-processing and analysis of video. Examples of this include Ocean Floor Observation Protocol (OFOP – www.ofop.texel.com; accessed 27 October 2012) and ADELIE from IFREMER (www.ifremer.fr/fleet/systemes_sm/adelie/; accessed 27 October 2012). Some specialist software programmes (e.g. MATISSE, IFREMER, France) allow for mosaicking of images, a very useful tool for building up images larger than the field of camera video, and others may allow for faster recording and enumerating features. Recording and enumerating is becoming more important in replacing traditional sampling procedures, especially for habitat analysis or survey/monitoring and new methods are continually being applied (e.g. Barry & Coggan, 2011, with visual counting methods).

3.6 Special applications

Sediment profile imagery

Sediment profile imagery (SPI) or REMOTS (Remote Ecological Monitoring Of The Seafloor) utilises an imaging device in an inverted periscope (optical prism) that penetrates the sediment and facilitates the imaging of the sediment water interface and upper sedimentary layers (approximately 15 cm $\times$ 20 cm in area), allowing fine-scale analysis of physical (see also Chapter 2), chemical and biological features (Rhoads & Germano, 1986). Fig. 3.5 shows a typical SPI system. The camera system (photographic or digital) is mounted in a pressure housing, while the distilled water-filled prism is pressure-compensated. The flash head is normally mounted in the prism and great care must be taken to ensure overall even illumination over the faceplate. Direct measurements can be made from the images and Rhoads and Germano (1986) developed a computer-aided system for analysis of such

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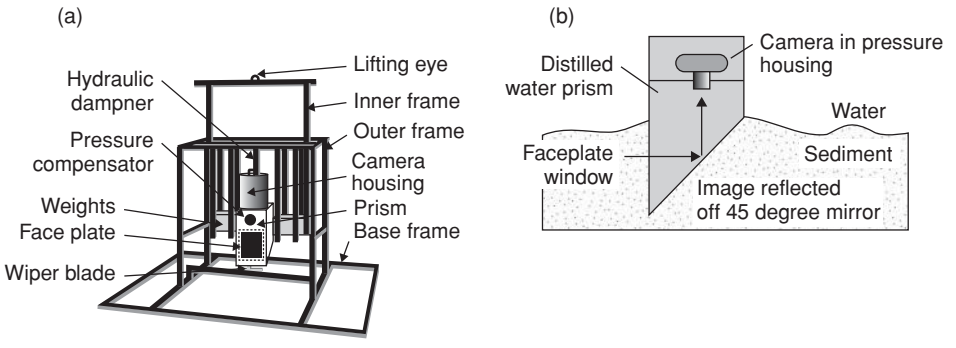

Fig. 3.5 Sediment profile imagery system (a) remote frame (b) detail of the prism/photo head.

parameters as grain size, surface boundary roughness, mean (apparent) redox depth, methane gas pockets, thickness of overlying (dredged/disposed) material, visible epifauna, tube density and type, faecal pellet layer, microbial aggregations, feeding voids, faunal dominants, successional stages. This development made the retrieval of a variety of abiotic and biotic measures much easier and quicker compared with earlier methods, i.e. by cores. One of its primary uses is in large-area surveys where local hotspots need to be identified and sampled in more detail. Systems are frame-mounted and wire-deployed for remote use or can be diver-operated for directed sampling in shallow waters and under fish farms where boats cannot operate. Germano *et al*. (2011) have comprehensively reviewed all aspects of SPI technology.

Laser technologies

Laser Line Scan Systems (LLSS) work in ways similar to sidescan sonar with a swathe of seafloor being illuminated by a crossing laser beam and the reflected light gathered. As the towfish moves, individual lines build up a waterfall image of a seabed swathe. Unlike sonar, the system is more affected by water turbidity. Height of the towfish above the seabed determines the swathe width and desired resolution. Typically, at 5 m height a swathe of 7 m can be covered with resolution of 1 cm. The system works at intermediate scales and resolution between sidescan and video. However, there are very few systems available and the cost is very high. First-generation LLSS produced monochrome two-dimensional images, while second-generation systems include three-colour and 3D systems (Carey *et al.*, 2003).

Hobson *et al*. (2000) have developed the use of laser holography for *in situ* pelagic research (imaging and distribution). With limited use in benthic applications, the camera can be used for imaging of plankton and particulate matter and may have applications to the sediment water interface. The system images a volume of water (up to 0.1 m^3) recording true, full-field 3D optical images with resolution up to 5 μm. Cameras systems are still developmental as is the software needed for

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microscale spatial recording and analysis. New applications in the benthos may open in the future for this type of methodology.

Application of medical technologies

X-ray imaging is mainly used in the laboratory to investigate the internal structure of sediment cores for both sedimentological and biogenic structures. In the same way, although with safer operation, ultrasound has been used in the laboratory for investigation of sediment structure in aquaria, and also ongoing activity in sediments and behavioural/physiological observation of organisms. In both cases, commercial equipment from medical applications has been used, although it is not necessarily suited to the marine environment. One further step has been in the use of Computer Axial Tomography (CAT-scans), that allows a better estimation of sediment structures (step-by-step X-rays along a single axis allowing the build-up of 3D images). Its use in sediment stability studies has been shown by Michaud *et al.* (2003). This type of equipment is only available in hospitals and medical clinics and, therefore, access to the technology is very limited.

3.7 Laboratory imaging

Video recordings are often used for teaching and demonstration purposes and most institutions have a stereoscope or microscope linked to a video recorder for this purpose. The use of video allows a number of observers to share the view of an object at the same time and it may be more economic than investing in a second viewing head for the stereo/microscope. It is also a useful technique for recording behaviour of small organisms or recording living organisms for identification purposes where preservation techniques may make them unrecognisable (e.g. turbellarians or platyhelminthes). The video camera will be a small sensor head without integral lens that can be attached through an optional C-mount connector on the microscope. Lighting is extremely important and the system will require adjustable high-power spotlighting (100+ W fibre optic light source). Positioning of the illumination source is very important with stereoscopy for either overall illumination, or highlighting with the use of shadow by side illumination. Circular fibre optic sources or in-line light sources (light source shining down the optical path) are sometimes used, but they may be inadequate in spot power under high magnifications and if the object is immersed in a fluid, there may be unwanted reflections from the fluid surface. Biological samples are best observed under water, again to prevent unwanted reflections from wet surfaces.

Standard video cameras can also be used in the laboratory for observational and behavioural purposes, e.g. looking down over tanks or mesocosms. Care should be taken to ensure that there is adequate protection from electrical short-circuits and that the equipment should be protected from corrosion in humid environments. Recording equipment is best situated in a remote dry area.

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Endoscopy and Boroscope relying on fibre optic principles have been used in tank aquarium systems to view the inside of closed structures, for example burrow systems. However, these systems are not widely used and suffer from corrosion. It is often better to arrange a view by using glass-walled tanks and external camera systems, where an animal may burrow against the glass wall allowing non-intrusive/non-disturbed recording from the other side of the wall.

Video cameras are increasingly used to illustrate research work for permanent record or public relations. It is always useful for the scientist to have a video camera on hand and he/she should have some familiarity with general video techniques and practice (including framing, lighting, stability, holding on shots and not zooming in and out while recording unless absolutely necessary). For video production it is probably necessary to use professional services with detailed planning, rehearsal, taking and retaking of shots. Filming at this level cannot be done during a normal scientific working day and time should be set aside for this.

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3.8 Image analysis

A large amount of data can be recovered from an image, ranging from anecdotal (description of a process) to semi-quantitative data (e.g. degree of coverage of an organism on a rock: a, none; b, low; c, medium; d, high; e, total) and finally quantitative. Quantitative data can be abstracted manually from a calibrated image by direct measurement and transformation. Computer software programmes are readily available to undertake this automatically. Computer image analysis systems can be extensive, covering the entire operation from image collection to output of analysis. These systems tend to be expensive and it is more common for the scientists involved in image analysis to have their own image input and storage system and to utilise readily available software ranging from professional analysis software to simpler image processing software (e.g. Adobe PhotoShop) and shareware (e.g. NIH Image). Most software allows on-screen measurement or, in more detailed systems, the filtering of images, automatic abstraction of shapes or parts and their automatic measurement.

Consideration should be given to the intended application of the final images, whether they are for internal use, scientific publication or public domain publication. For high-quality output, high-quality original images are needed as well as access to good printers. A grabbed video image may be acceptable on-screen, but it will not print in sufficient detail for publication. Storage and archiving are also important as different storage formats have different shelf lives. Thermal printouts from sidescan sonar records will be affected by temperature, digital media by external voltage changes or magnetic fields. CDs quoted as having an indefinite shelf life are in fact limited to some 25 years. Important images should be kept at least in duplicate in separate locations in fireproof conditions. The quality of the material should be periodically checked and recopied if necessary onto newer standardised

archiving formats. A comprehensive review of principles on this subject can be found in Glasbey and Horgan (1995).

3.9 Afternote

Finally, we would like to add, that with our increasing use of the digital world, almost every image is now manipulated in some way or the other, whether it is a change in the storage resolution, enhance contrast or change the colouration. Ethically speaking, the scientist should always note when publishing that original images have been manipulated (if only to say that they have been enhanced).

References

AODC (1985) *Code of Practice for the Safe Use of Electricity Underwater*. AODC 035. International Marine Contractors Association, London. www.imca-int.com (accessed 27 October 2012).

Auster, P.J. & Tusting, R.F. (1997) Scientific sampling systems for submersible vehicles (guest editors). *Marine Technology Society Journal*, **31** (3), 83.

Barry, J. & Coggan, R. (2011) The visual fast count method: critical examination and development for underwater video sampling. *Aquatic Biology*, **11**, 101–112.

Blondel, P. & Murton, B.J. (1997) *Handbook of Seafloor Sonar Imagery. Wiley Praxis Series in Remote Sensing*, John Wiley & Sons, Chichester, 314 pp.

Brown, C.J., Cooper, K.M., Meadows, W.J., Limpenny,D.S. & Rees,H.L. (2002) Small-scale mapping of sea-bed assemblages in the eastern English Channel using sidescan sonar and remote sampling techniques. *Estuarine, Coastal and Shelf Science*, **54**, 263–278.

Carey, D.A., Rhoads, D.C. & Hecker, B. (2003) Use of laser line scan for assessment of response of benthic habitats and demersal fish to seafloor disturbance. *Journal of Experimental Marine Biology and Ecology*, **285–286**, 435–452.

CEN (2011) Water quality – visual seabed surveys using remotely operated and towed observation gear for collection of environmental data. European Committee for Standardisation, prEN 16260:2011, 21 pp.

Coggan, R., Mitchell, A., White, J. & Golding, N. (2008) Recommended operating guidelines (ROG) for underwater video and photographic imaging techniques. EU MESH (Mapping European Seabed Habitats) project, 32 pp. http://www.searchmesh.net/ (accessed 27 October 2012).

Foell, E.J. & Pawson, D.L. (1986) Photographs of invertebrate megafauna from abyssal depths of the north-eastern equatorial Pacific Ocean. *Ohio Journal of Science*, **86**, 61–68.

Fletcher, B., Bowen, A., Yoerger, D.R. & Whitcomb, L.L. (2009) Journey to the Challenger Deep: 50 years later with nereus hybrid remotely operated vehicle. *Marine Technology Society Journal*, **43** (5), 65–76.

Germano, J.D., Rhoads, D.C., Valente, R.M., Carey, D.A. & Solan, M. (2011) The use of sediment profile imaging (SPI) for environmental impact assessments and monitoring studies: lessons learned. *Oceanography and Marine Biology: An Annual Review*, **49**, 235–298.

https://telegram.me/Geologybooks

Glasbey, C.A. & Horgan, G.W. (1995) *Image Analysis for the Biological Sciences*. John Wiley & Sons, Chichester.

Greenstreet, S.P.R., Tuck, I.D., Grewar, G.N., Armstrong, E., Reid, D.G. & Wright, P.J. (1997) An assessment of the acoustic survey technique, RoxAnn, as a means of mapping seabed habitat. *ICES Journal of Marine Science*, **54**, 939–959.

Hobson, P.R., Lampitt, R.S., Rogerson, A., Watson, J., Fang, X. & Krantz, E.P. (2000) Three-dimensional spatial coordinates of individual plankton determined using underwater hologrammetry. *Journal of Limnology and Oceanography*, **45**, 1167–1174.

Kidd, R.B. & Huggett, Q.J. (1980) Rock debris on abyssal plains in the northeast Atlantic - a comparison of epibenthic sledge hauls and photographic surveys. *Oceanologica Acta*, **4**, 99–104.

MESH (2005) Review of standards and protocols for seabed habitat mapping. Rapport, EU MESH (Mapping European Seabed Habitats) project, 192 pp. http://www.searchmesh.net/ (accessed 27 October 2012).

Michaud, E., Desrosiers, G., Long, B., de Moutety, L., Crémer, J.-F., Pelletier, E., Locat, J., Gilbert, F. & Stora, G. (2003) Use of axial tomography to follow temporal changes of benthic communities in an unstable sedimentary environment (Baie des Ha! Ha!, Saguenay Fjord). *Journal of Experimental Marine Biology and Ecology*, **285–286**, 265–282.

Nilsson, H.C. & Rosenberg, R. (2000) Succession in marine benthic habitats and fauna in response to oxygen deficiency: analysed by sediment profile-imaging and by grab samples. *Marine Ecology Progress Series*, **197**, 139–149.

Parry, D.M., Kendall, M.A., Pilgrim, D.A. & Jones, M.B. (2003) Identification of patch structure within marine benthic landscapes using a remotely operated vehicle. *Journal of Experimental Marine Biology and Ecology*, **285–286**, 497–511.

Pilgrim, D.A., Parry, D.M., Jones, M.B. & Kendall, M.A. (2000) ROV image scaling with laser spot patterns. *Underwater Technology*, **24**, 93–103.

Rhoads, C. & Germano, D. (1986) Interpreting long-term changes in benthic community structure: a new protocol. *Hydrobiologia*, **142**, 291–308.

Rona, P.A. (2000) Deep diving manned research submersibles. *Sea Technology*, **41** (12), 15–26.

Shand, C.W. & Priestley, R. (1999) A towed sledge for benthic surveys. Scottish Fisheries Information Pamphlet, 8 pp.

Smith, C.J. & Papadopoulou, K.-N. (2003) Burrow density and stock size fluctuations of *Nephrops norvegicus* in a semi-enclosed bay. *ICES Journal of Marine Science*, **60**, 798–805.

Smith, C.J. & Rumohr, H. (2005) Imaging techniques. In: *Methods for the Study of Marine Benthos* (eds A. Eleftheriou & A. McIntyre), 3rd edn, Blackwell Publishing Ltd., Oxford, 87–111 pp.

Smith, C.J., Rumohr, H., Karakassis, I. & Papadopoulou, K.-N. (2003) Analysing the impact of bottom trawls on sedimentary seabeds with sediment profile imagery. *Journal of Experimental Marine Biology and Ecology*, **285–286**, 479–496.

Solan, M., Germano, J.D., Rhoads, D.C., Smith, C.J., Michaud, E., Parry, D., Wenzhöfer, F., Kennedy, R., Henriques, C., Battle, E., Carey, D., Iocco, L., Valente, R., Watson, J. & Rosenberg, R. (2003) Towards a greater understanding of pattern, scale and process in marine benthic systems: a picture is worth a thousand worms. *Journal of Experimental Marine Biology and Ecology*, **285–286**, 313–338.

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Somerton, D.A. & Glendhill, C.T. (eds) (2005) Report of the National Marine Fisheries Service Workshop on Underwater Video Analysis. US Department of Commerce, NOAA Tech. Memo. NMFS-F/SPO-68, 68 pp. http://spo.nmfs.noaa.gov/tm/ (accessed 27 October 2012).

Wernli, R. (2000) The present and future capabilities of Deep ROVs. *Marine Technology Society Journal*, **33** (4), 26–40.

Yoshida, W., Ishibashi, S., Watanabe, Y., Inoue, T., Tahara, J., Sawa, T. & Osawa, H. (2009) The ABISMO mud and water sampling ROV for surveys at 11,000 m depth. *Marine Technology Society Journal*, **43** (5), 87–96.

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Chapter 4
Diving

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Abstract

Scientific diving incorporates almost all current diving techniques. Although the vast majority is still conducted using open-circuit SCUBA breathing compressed air, it includes other gas mixtures such as nitrox, heliox and trimix and other systems such as surface-supply and semi- and fully closed circuit rebreathers (CCRs). In recent years, mixed gas rebreathers have been used for several deep reef investigations. The scientific application of techniques more commonly considered to be the realm of oil and gas industry divers, saturation diving and undersea habitats, is also considered here.

Data collection and recording techniques reviewed here include core sampling, resin casting, yabby pumps, manta boards, simple writing slates, voice communication systems, video and stills cameras. For monitoring programmes involving divers, the methods employed for site relocation and marking are considered, including the use of sub-surface marker buoys, snag-lines, transponders and the use of pneumatic drills, pitons and underwater setting resin to create long-term markers. Standard reef and fish survey techniques such as line intercept transects (LITs), Marine Nature Conservation Review semi-quantitative survey methods and random swim survey protocols are also described.

Keywords diving systems, SCUBA, open-circuit SCUBA, rebreathers, saturation diving, undersea habitats, reef surveys, line intercept transects, voice communication systems, yabby boards, manta boards, sub-surface marker buoys, transponders, random swim survey protocols

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4.1 Diving systems

All diving depends on a supply of breathing gas. Gas supply systems can be broadly classified into self-contained or SCUBA systems (properly known as self-contained breathing apparatus) and remotely supplied systems, where gas is piped to the diver through an umbilical.

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SCUBA

The majority of scientific diving is undertaken using SCUBA systems. This system allows the diver greater freedom of movement than most remotely supplied systems. It is also the system taught by all recreational diving training agencies, the route by which most scientists initially learn to dive. SCUBA equipment is generally relatively lightweight, and low cost. Crucially, for many scientific studies, the diver does not have to overcome the drag exerted by currents on a breathing gas umbilical (which can be considerable when the diver is operating a significant distance away from the umbilical tender). Therefore, it is much easier for the free-swimming diver to hover just above the seabed without being swept rapidly down-tide, or to maintain position on the seabed without being heavily weighted. These are crucial requirements for many scientific studies. SCUBA has the disadvantage that the dive profile is limited by the quantity of breathing gas a diver can carry.

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Remotely supplied systems

Remotely supplied systems, where the divers' breathing gas is supplied via an umbilical either directly from a low-pressure compressor or from storage cylinders, comprise the most commonly used system for most civil engineering and offshore oil industry divers. The remote supply is most commonly surface-supply, e.g. a compressor or air bank located on a boat or quayside, but may also be a supply from a diving bell or habitat to a tethered diver. Remote supply systems normally use positive pressure full-face band-masks or lightweight diving helmets fitted with hard-wire communication systems (see the section entitled 'Voice communication systems'). Such systems are also widely used by archaeological divers, who are often working within a small, well-defined area, frequently in very low visibility. However, they are rarely used by biological divers, largely due to the considerable bulk and weight of the equipment and the limited freedom of movement the diver has (being tethered by the umbilical). Situations where surface-supply systems are used by biological divers include when working in very low visibility or polluted waters, such as sampling close to effluent outfalls, or where regulations require it (e.g. if conducting scientific diving around offshore oil or gas establishments).

Hookah systems

Hookah systems (Fig. 4.1) are lightweight surface-supply systems, whereby the diver is supplied with air from a small, portable compressor through a light air hose. These are mainly low-pressure systems, where the compressor output is around 6 bar (88 psi). Hookah systems are in common use in Australia, Asia and the Americas by recreational divers, with several systems aimed primarily at the recreational market being widely available (e.g. *Brownie Third Lung*, *Air-line* and *Air-Dive* systems). However, they are most frequently used by commercial marine

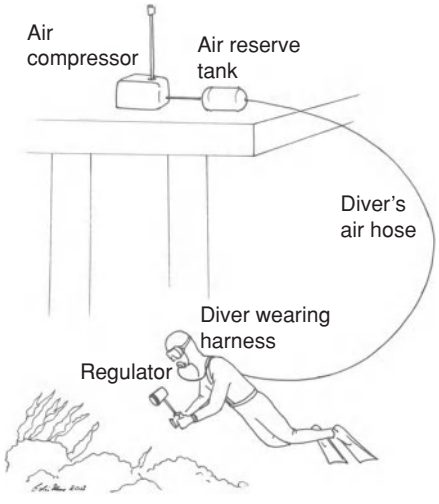

Fig. 4.1 Diver using hookah system. © Colin Munro

harvesters, e.g. abalone, sea urchin and sea cucumber divers. Hookah systems offer the benefits of unlimited air supply without the excessive weight and considerable cost of conventional surface-supply equipment. Although used relatively rarely by biological divers, hookah systems are used at times to supply divers working out of the *Aquarius* underwater habitat (see Section 4.2). As with conventional surface-supply equipment, hookah systems are widely used by archaeological divers.

Breathing gas and supply systems

Air and nitrox

Most SCUBA systems use air as the breathing gas. However, the use of oxygen-enriched air (commonly known as nitrox) is growing in popularity for scientific studies (e.g. Dinsmore, 1989a, 1989b; Stanton, 1991; Holt, 2001; Joiner, 2001; Munro, L., 2001). In 2002, approximately half of the research dives undertaken at Dunstaffnage Marine Laboratory, Scotland, were conducted using nitrox mixes (Simon Thurston, pers. comm., 2002). Nitrox contains higher than air percentages of oxygen (i.e. >21%). This reduces the partial pressure of nitrogen in the breathing gas, and so the amount of inert gas that will dissolve into the body at any given pressure. Consequently, the decompression penalty incurred is reduced. An additional potential benefit accrued relates to the narcotic effect of nitrogen when breathed at elevated partial pressures. It is well documented that the cognitive abilities of divers breathing compressed air at depths of 30 m or more are significantly impaired (e.g. Baddeley *et al.*, 1968; Fowler *et al*., 1985). Behnke *et al.* (1935) first identified increased partial pressures of nitrogen as the prime narcotic agent when breathed in compressed form. Thus, reduction of the partial pressure of nitrogen

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breathed at depth may be expected to reduce the narcotic effect of that gas. Whilst rigorous scientific studies are lacking, anecdotal evidence suggests that symptoms of narcosis may be reduced when breathing nitrox mixes as opposed to air within the 25–40 m depth range (the range most suited for use of nitrox). A cost-benefit comparison of nitrox and air SCUBA systems in scientific diving operations is given in Holt (2001).

Heliox and trimix

Heliox, the combination of helium and oxygen, was initially proposed by Professor Elihu Thompson, an American scientist better known for his work on high-voltage generators and X-rays, as a means of reducing the narcosis divers experienced when breathing compressed air at depth. Unfortunately, helium was prohibitively expensive at that time. However, the discovery of vast reserves of helium in Texas natural gas wells in the early 1920s dramatically changed this. As America then controlled virtually all the world's helium reserves, the early work was conducted solely by the US Navy. Helium is approximately four times less narcotic than nitrogen, and so breathing heliox virtually eliminates the problem of inert gas narcosis for the diver operating at depth. Saturation and desaturation speeds of inert gases are inversely proportional to the square root of their atomic mass. Thus, body tissues will saturate or desaturate with helium some 2.7 times faster with nitrogen. Consequently, no-stop times will be shorter for heliox than air (one reason why heliox is an inappropriate gas for shallow dives). This is offset on deeper dives by shorter decompression stops, especially if nitrox is breathed during decompression. Helium conducts heat approximately six times faster than air. Thus, helium mixes should not be used for dry suit inflation. A common misconception is that breathing heliox cools the diver at a significantly greater rate than breathing air. Although it conducts heat faster, heliox has a lower heat capacity than air as helium is a less dense gas than nitrogen. Heliox is generally considered most appropriate for use as a breathing gas at depths of 60 m (200 ft) or greater.

Trimix, a mixture of oxygen, nitrogen and helium, is frequently used in place of heliox for deep diving. By replacing some of the helium in the inert gas component of the mix with nitrogen, the costs of breathing gas is significantly reduced. The trade-off is that a degree of nitrogen narcosis is introduced. High pressure nervous syndrome (HPNS) is a condition causing tremors, dizziness and nausea and impairment of cognitive function that can occur at depths greater than 160 m (525 ft), believed to be due to hyper-excitation of nerve cells within the brain (Hunger & Bennett, 1974). There is some suggestion that the nitrogen in trimix mixtures may help ameliorate the effects of HPNS (Bennett *et al.,* 1981).

In 2001, America's National Oceanic and Atmospheric Administration (NOAA) Undersea Research Program ran a trimix and technical diving programme for diving scientists working on deep-water species, at the Caribbean Marine Research Centre in the Bahamas. Following on from this course, trimix was used for investigations

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of invertebrate and algal biodiversity, and chemical ecology, at working depths of 300–400 ft (91–122 m) on the Bahamian outer reef walls (Dr Marc Slattery, University of Mississippi, and Dr Michael Lesser, University of New Hampshire, pers. comm., 2002). Pyle (2000) identified the deep regions (60–150 m) of coral reefs as a zone where fish biodiversity is still largely unrecorded due to the expense of deploying submersibles and their inherent limitations when operating in complex habitats. By conducting dives using trimix as the working depth breathing gas, he and collaborators have collected and identified 50 fish species new to science (e.g. Pyle, 1992, 1996, 1998, 2000; Pyle & Randall, 1992; Gill *et al*., 1996; Earle & Pyle, 1997; Randall & Pyle, 2001a, 2001b).

Open-circuit SCUBA

Since the invention of the aqualung in the early 1940s, most self-contained diving has been conducted using open-circuit diving systems, i.e. where breathing gas is delivered to the diver at close to ambient pressure, and exhaled gas is then vented into the surrounding water through a one-way valve. This is the principle of the early twin hose, single-stage demand valves, and is the way current single hose, twin-stage regulators still operate. For the diving scientist, it has many advantages. The system is widely available, relatively cheap and robust. The operation of open-circuit SCUBA is also simple and requires little training (although competence in differing conditions, and with different gas mixes may require further training). Perhaps its greatest advantage for someone conducting work underwater that requires a high level of concentration is that it is a very reliable and almost totally automatic system, requiring only a modest level of monitoring during the dive. Given these advantages, it is likely that open-circuit SCUBA, containing air or nitrox gas mixes, will remain the system of choice for most scientific diving conducted in waters less than 45 m depth, for the foreseeable future. However, open-circuit SCUBA systems have a number of disadvantages. Air bubbles vented into the water are noisy, with open-circuit systems producing significantly higher levels of noise than closed or Semi-Closed Rebreathers (SCRs) (Radford *et al*., 2005), and some studies indicate that this can influence the behaviour of many fish species and vagile invertebrates (e.g. Chapman *et al.*, 1974; Sayer *et al.*, 1996) making observation of natural behaviour difficult. Lobel (2001) notes that the near-field vibrations produced by exhaled bubbles are probably similar to the hydrodynamic disturbance caused by fast-moving predators. However, there is some conflicting evidence; Cole *et al*. (2007) found no significant differences in fish counts whether open-circuit or closed-circuit systems were used, with higher counts recorded for some fish species when open-circuit systems were used. Open-circuit systems are also quite wasteful of breathing gas, as the exhaled oxygen and diluent gas (e.g. nitrogen in air) are lost from the system once exhaled by the diver. Thus, for work requiring extended dives or deep dives, the diver must carry large amounts of compressed breathing gas. Additional gas cylinders worn by the diver not only

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make entry and exit from the water very physically demanding, but also restrict the diver's ability to manoeuvre freely over a study site without disturbing it, and to carry equipment necessary for the study (e.g. cameras and sampling devices).

Closed and semi-closed systems

The alternative to open-circuit diving systems is some form of rebreather. Rebreathers differ from open-circuit systems in that they are able to capture and re-use all or part of the diver's exhaled gas. The inspired and expired gas is maintained within a breathing loop, thus using more efficiently the total volume of gas carried by the diver. This consists of an inhalant and exhalent tube attached to the mouthpiece. A counterlung within the loop provides flexibility, allowing the loop volume to change as the diver inhales and exhales. Most systems incorporate two counterlungs, an inhalation and an exhalation bag, balancing breathing resistance. Exhaled gas passes through a CO_2 'scrubber' before passing to the inhalant side of the loop. As a consequence, rebreathers greatly extend the potential dive duration of a given amount of breathing gas. They also dramatically reduce exhalent bubbles and, therefore, bubble noise.

Rebreathers fall into three categories: (i) oxygen rebreathers, (ii) SCRs and (iii) closed-circuit rebreathers (CCRs).

Oxygen rebreathers

In oxygen rebreathers, 100% oxygen is the only compressed gas carried by the diver. It is generally the simplest rebreather in construction, and its development occurred around the same time as open-circuit SCUBA. While oxygen rebreathers are the most gas efficient (in terms of the amount of gas carried that is available for metabolic use), they suffer from the severe limitation that they cannot be used deeper than around 6 m due to the risks of acute oxygen toxicity symptoms at partial pressures (pp) of O_2 greater than 1.6 bar (though the pressure at which such symptoms will occur is known to be highly variable, 1.4 bar is currently considered the maximum safe partial pressure for working dives, 1.6 bar where the diver is at rest). Oxygen rebreathers have little use in current scientific diving procedures.

Semi-closed rebreathers

Semi-closed rebreathers (SCRs) consist of a breathing loop, to which breathing gas is supplied periodically from a compressed gas cylinder in order to compensate for oxygen consumed metabolically and gas lost from the system (e.g. through mouthpiece leakages). They allow a small amount of gas to vent periodically from the breathing loop, in order to prevent build-up of the inert component (normally nitrogen or helium) of the gas being breathed. Two principal types of SCR are manufactured: (i) active-addition and (ii) passive-addition. Active-addition is the simpler form, adding gas to the breathing loop at a constant, preset rate; passive-addition SCRs link the rate of addition to the diver's breathing rate, a more complex but also more efficient design. Several SCR units have entered the recreational dive

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market in recent years. Shallow water systems primarily designed for nitrox diving are now comparable in price to conventional open-circuit SCUBA.

Closed-circuit rebreathers

Closed-circuit rebreathers (CCRs) differ from SCRs in having two separate gas cylinders: (i) an oxygen cylinder and (ii) a diluent gas cylinder (generally air, nitrox, heliox or trimix). The oxygen partial pressure (ppO_2) is maintained at a relatively constant level, generally through the action of electronic sensors and servos. As oxygen is depleted from within the breathing loop, it is replaced from the oxygen cylinder. The diluent gas cylinder tops up the breathing loop as the gas in the breathing loop compresses with depth, and in some designs also acts as an emergency open-circuit bail-out gas. Thus in normal usage gas is only lost from the breathing loop due to expansion as the diver ascends. CCRs are of more complex design than SCRs, and generally cost significantly more. The training requirement is also normally greater.

Rebreather use in biological studies

Rebreathers have been used increasingly in scientific studies. During studies of reef fish acoustic behaviour, Lobel (2001) found that he was able to approach fish more closely, and that he observed a greater number of fish and fish species, while using an SCR. Sayer and Thurston found that the number of fish observed during transect counts was significantly higher when using SCRs than when using open-circuit systems, although the number of fish species was not (Martin Sayer, pers. comm., unpublished data). Pyle (1999) used CCR systems to study and identify reef fish species living at depths between 60 and 90 m; Pence and Pyle (2002) also used CCR systems to conduct deep reef fish surveys around Fiji. Parrish and Pyle (2002) compared costs and efficiency of open-circuit SCUBA systems with those of CCR systems, while surveying commercial black coral beds off Hawaii (Fig. 4.2). They found that, for deep dives, the set-up time, costs of consumables and decompression penalties were significantly greater for open-circuit than for closed-circuit systems. However, these operational costs must be weighed against the higher initial purchase costs and training times.

4.2 Saturation diving and underwater habitats

Saturation diving works on the principle that once the diver's body tissues have become saturated with dissolved gas (for the pressure experienced at that depth), then extending the dive time creates no additional decompression penalty. By making excursions from a pressurised habitat, the diver's time on the seabed and the frequency of repeat excursions is limited only by cold and the capacity of his self-contained breathing equipment.

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Fig. 4.2 Diver using closed-circuit rebreather while surveying black coral off Hawaii. © Richard Pyle, University of Hawaii. Reproduced with permission.

On 14 September 1962, Albert Falco and Claude Wesly began a week's immersion, living at a depth of 10 m (33 ft) off the coast of Marseilles in a large cylindrical habitat called *Diogenes*. This was Jacques Cousteau's *Conshelf I* project. Eight days earlier, and approximately 100 miles away, Robert Stenuit spent 24 hours living at 60 m (200 ft), breathing oxy-helium in a habitat designed by American inventor Ed Link. These parallel trials were the first real attempts by scientists to live and work under pressure on the seabed for extended periods. *Conshelf I* and Link's '*Man-in-the-Sea'* programmes were followed by a series of more ambitious projects through the early and mid 1960s: *Conshelf II* and *III*; '*Man-in-the-Sea II*' and the US Navy's *Sealab* habitats extended both the depth and duration for working saturation divers. *Tektite*, a cooperative effort between the US Navy, NASA and the US Department of the Interior, was the first subsea habitat from which significant marine biological studies were undertaken. This was followed by *Hydrolab*, which operated from 1966 to 1985 in the Bahamas and Caribbean. Over 600 researchers worked out of *Hydrolab* during this time.

Aquarius (Fig. 4.3), an undersea laboratory run by NOAA's National Undersea Research Program (NURP) and the University of North Carolina, was built in 1986. Originally deployed in the US Virgin Island, it was moved to the Florida Keys, off Key Largo, in 1993. Between 1993 and 2011, some 90 research missions, using the *Aquarius* undersea laboratory, have been undertaken. *Aquarius* is stationed at approximately 16 m depth, 4.5 km offshore. A 10 m diameter Life Support Buoy moored directly above provides breathing gas, power and communication links to the habitat. The bottom times available to *Aquarius* divers are much greater than those of surface-orientated divers; NOAA has calculated that 60–70 days of surface-orientated diving would be required to provide the equivalent bottom time

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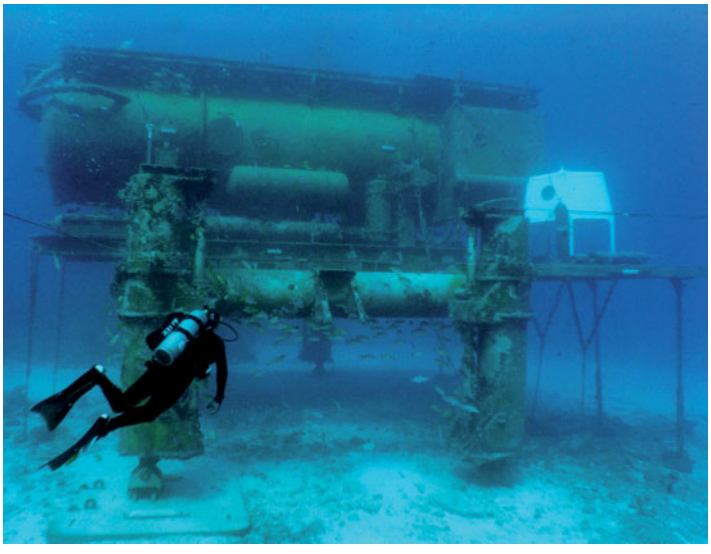

Fig. 4.3 The undersea laboratory *Aquarius*, a diving habitat off the coast of Florida. © University of North Carolina at Wilmington. Reproduced with permission. Aquarius is owned by NOAA and is operated by the University of North Carolina at Wilmington. (For a colour version of this figure, see Plate 4.1.)

to a 10-day mission (Miller & Cooper, 2000). Diving excursions from the laboratory are conducted using protocols similar to those employed by cave divers, as the extended decompression required creates a physiological 'ceiling' preventing the divers from ascending. Researchers swim to and from their study sites, which may be up to 200 m distant from the laboratory, along radiating travel lines (Mark Hulsbeck, NURC research diver, pers. comm., 2002). Saturation diving is particularly suited to studies requiring large amounts of in-water time over a period of days or weeks.

4.3 Data collection and recording

Since the ability of diving surveyors to recall observations made during a dive is known to be worse than the recall of similar observations made in the terrestrial situation (Godden & Baddeley, 1975), it is normally wise for surveyors to equip themselves with some means of directly recording *in situ* observations. A range of widely used methods is described below.

Slate or notepad and pencil

Often the simplest methods are best. Plastic or laminate board slates are a cheap, low-tech option; they are easy to construct, and to replace, when they inevitably

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are lost or damaged. Waterproof notepads are widely available from nautical and surveying supplies companies. Though these will greatly increase the amount of data a diver can record during a dive, they are not as robust as slates, and pages may sometimes be lost underwater. In temperate and polar waters, cold quickly reduces manual dexterity, with the result that handwriting can become difficult to read when transcribing later. Where practical, a pre-prepared layout to the slate can greatly improve the dive efficiency (e.g. a species checklist with space for recording tally, abundance or density). A point to bear in mind is that pencils float, and so should be tied to the slate or the diver, otherwise a pencil momentarily released is a pencil lost.

Voice recording

The surveyor's spoken observations underwater can be recorded either by direct recording, or by transmission to the surface. Use of communications equipment to record data enables surveyors to dive without the encumbrance of hand-held slates or photography equipment. Audio data can be easily stored on tape, CD, etc., but is very time-consuming to analyse. The current market in underwater communications equipment is expanding rapidly, so communications equipment is likely to be increasingly used as a tool for marine survey recording.

Direct recording

Direct recording usually consists of a waterproof housing strapped to the diver's cylinder, with a microphone mounted in a specialised mouthpiece. Gamble (1984) describes a bone conduction system in which the microphone is held against the diver' skull by the suit hood. Direct recording systems are relatively cheap and are ideal for use in surveys where divers are free-swimming on SCUBA.

Voice communication systems

Voice communications have long been standard requirements for commercial diving operations, but are still relatively infrequently used in scientific diving programmes. This is partly due to the fact that until quite recently, most voice communication systems were 'hard wire'; i.e. they required a physical connecting line from the diver to the surface transceiver. The SCUBA diver then became a tethered diver, with all the limitations this involves. However, situations where tethered diving is required, or at least preferred, e.g. under ice or low-visibility conditions, are also ones where the added safety of direct communication with the surface may be preferred. Hard-wire voice communications can be either two wire (simplex) or four wire (duplex or 'round robin') systems. Simplex systems allow the diver's speech and breathing to be continuously monitored by the surface team while there is no surface to diver communication. However, when the press-to-talk button on

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the surface set is depressed, the diver cannot be heard. Duplex systems are more expensive, but allow continuous communication both ways. At the time of writing, hard-wire communication systems still provide the clearest and most reliable diver-to-surface voice communication.

Through-water communications have been around for many years, but their reliability has not always been 100%. Most work by transmitting sound on a sideband carrier frequency. One problem is that the sound quality will deteriorate rapidly over distance (sound intensity will decrease with the square of distance due to spherical spreading), although many systems currently claim ranges of up to 1 km in ideal conditions. Quality of the sound may also deteriorate through diffraction or reflection by seabed objects or thermoclines, or through interference from engine noise. That being said, through-water communications offer the great advantage of maintaining the freedom that SCUBA equipment provides.

Some through-water communication systems are available that can be used with normal SCUBA half-masks. These generally incorporate modifications to the diver's regulator mouthpiece in order to facilitate speech. The majority of both through-water and hard-wire systems are designed to work with positive pressure full-face masks, such as the *AGA Divator* or *Kirby Morgan EXO 26* (Fig. 4.4). Most systems have the facility for all dive communication to be continuously recorded on audio tape. Skomer Marine Nature Reserve (MNR) dive team currently use a modified through-water communication system whereby the surface supervisor can speak to the survey diver(s), allowing instructions and safety checks to be delivered easily; divers can respond by taps, acknowledging receipt and comprehension of the message (Phil Newman, Skomer MNR, pers. comm.).

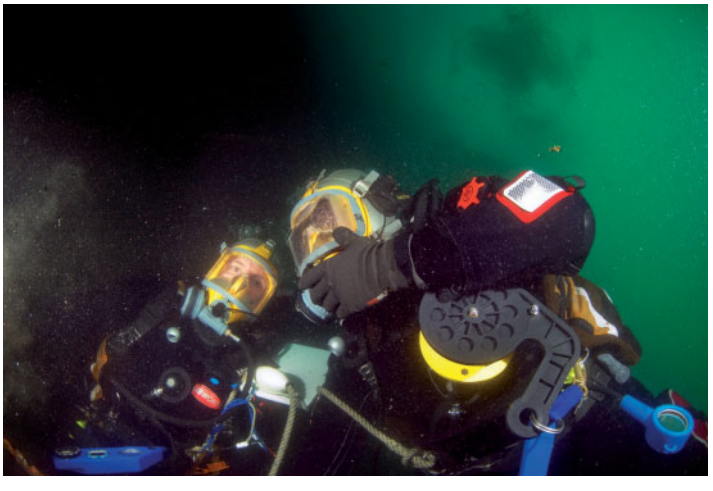

Fig. 4.4 Plymouth University Diving & Marine Centre professional diving students using positive pressure full-face masks. Diver on right can be seen operating press-to-talk through-water *Buddy Phone* voice communications. © Colin Munro. (For a colour version of this figure, see Plate 4.2.)

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Image recording

Stills photography

As underwater photography is a vast subject on its own, this section can touch on only a few relevant points. It is assumed that the reader is familiar with basic photographic principles, as there is not scope here to discuss systems and methodologies from first principles. For more detailed information on taking underwater photographs and the systems available, *The Underwater Photographer, 4th edition* (Edge, 2010) is a very comprehensive text.

The camera is an extremely useful tool for the underwater biologist. It allows large amounts of data to be collected very rapidly, an important factor given that time underwater is normally quite limited. Images collected from the same area over time can be used to identify and track changes in habitat or species assemblage, species growth or behavioural patterns. Photographic images can be viewed at high magnification to count or identify specimens that are difficult to see clearly underwater. They can also be used to record the appearance of species with which the researcher is unfamiliar. Digital cameras have almost completely replaced film-based cameras in the past few years; this has brought many advantages to the scientific diver. One major advantage is the immediacy of digital imaging, where pictures can be checked during the dive rather than needing to wait for film to be developed on land. As repeating dives is often time-consuming and expensive, the ability to ensure that data has been captured successfully is extremely useful. As film cannot be changed underwater, only 36 images (the capacity of one 35 mm film roll) could be taken during a dive; large capacity data storage cards have now removed this severe restriction, allowing several hundred images to be taken during the dive. The more forgiving nature of digital cameras (compared to film cameras) for inexperienced photographers, coupled with the greater level of detail that can be extracted from good digital images and the high number of images that can be taken on a single dive, allows many surveys to be conducted by scientists with limited identification skills for the taxa under investigation as images can be analysed after the dive. Most digital SLR cameras (DSLR) allow the user to save images in either RAW or JPEG file format (or both simultaneously). RAW files are uncompressed files largely unaltered and containing all data recorded by the camera's image sensor. They are not directly usable as images and must be converted to another format (e.g. JPEG or TIFF) before significant editing can take place. In this sense they are often referred to as 'digital negatives', being analogous to film negatives in the image development process. In essence, a RAW file is one where the sensor data has yet to be demosaiced, i.e. the image data within the file consists only of values for red, green and blue at each pixel location. Moreover, RAW files come in a number of proprietary forms specific to major camera manufacturers (e.g. NEF, Nikon; CR2, Canon). JPEG (Joint Photographic Experts Group) is a file format that compresses the image to reduce file size. It is a 'lossy' compression

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format, so a certain amount of data will be discarded. For a given memory card capacity, considerably more JPEG than RAW images can be stored. From a data extraction viewpoint, the most important difference is that RAW files have 12 or 14 bit intensity information (in essence, brightness levels) compared to JPEG files that have only 8 bit. These additional intensity levels in RAW files allow much greater detail to be extracted from shadow areas. Thus, when deciding whether to save images as JPEG or RAW in camera one needs to consider what the images will be used for, how much detailed information it is desired to extract and the number of images to be captured during each dive. A further consideration is that, where images are taken at incorrect (markedly over- or under-exposed) settings, a not uncommon occurrence in underwater photography, RAW files will allow markedly better recovery of the image to acceptable levels. A general rule of thumb is that, unless the additional storage space and processing time is a significant factor, the images should be captured in RAW format.

The current camera choices available to the diving scientist are compact cameras, system compact cameras (compact cameras with interchangeable lenses) and DSLR (digital single lens reflex cameras). Underwater housings are available for most popular models. Compact cameras offer the advantage of being small, light and generally relatively inexpensive. In size (when inside housings) they are comparable to *Nikonos* film cameras. This can be useful where they are replacing film cameras in long-term monitoring programmes and must fit into framers originally designed for *Nikonos* cameras. A good example of this is the stereo-photography system employed as part of Skomer MNR long-term monitoring programme (described in the section entitled 'Photo-monitoring techniques'). Most available housings for compact cameras are made from clear plastic materials, although aluminium housings are available for some models. Compact cameras have the disadvantage that the widest field of view (FOV) of the integral zoom lens is generally less than optimal for underwater habitat recording, commonly around 75°, and minimum focussing distances of around 0.3 m. A shorter camera-to-subject distance may be desirable for recording smaller species and for producing clear images in turbid waters. Both these disadvantages can be overcome by the use of supplementary lenses or lens adapters that widen the FOV, reduce the minimum focussing distance or both.

The bulk of camera housing (and ease with which dome ports may be damaged) also tends to mean that they cannot simply be clipped on to the diver's buoyancy jacket as an ancillary tool, while other parts of a study are conducted. Camera housing is mostly constructed from aluminium alloy or clear plastic. A very few are constructed using stainless steel. Aluminium housings are undoubtedly more robust than those constructed from plastic; however, they are heavier (on the surface) and significantly more expensive. Plastic (polycarbonate, acrylic, ABS, etc.) housings are much cheaper and are available for a far wider range of DSLR and compact camera models. As they are transparent, any leaks that occur can be spotted much more quickly than on metal housings.

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Photo-monitoring techniques

Photo-monitoring of individual colonies or species' assemblages using fixed location quadrats or transects is a widely employed technique for gathering data on temporal change within assemblages and linear growth of organisms (e.g. Green, 1980; Bullimore, 1983; Hiscock, 1984; Lundälv, 1985; Munro, 1998; Bell *et al*., 2006). For monitoring of epibenthic communities, the area covered by each photograph will be selected in part by the size and density of the species forming the community, and the degree of spatial heterogeneity observed.

Even with a wide-angle lens, the maximum area covered will be constrained by water turbidity. English *et al.* (1997), considering photo-monitoring on coral reefs, recommend using a *Nikonos* camera with 15 mm lens (FOV 92°), located on a tetrapod where the ends of the legs form a 1 m square (camera-to-subject distance of 800 mm). The current Australian Institute of Marine Science (AIMS) Benthic Reef Communities photo-monitoring programme employs a 14.7 megapixel compact digital camera. This does not have the same FOV as the *Nikonos* system and so a camera-to-subject distance of 50 cm is employed, giving a 25 cm × 34 cm image. In more turbid temperate waters, smaller frame sizes tend to be used, facilitating shorter camera-to-subject working distances. Photo-monitoring quadrat sizes of 666 mm × 500 mm have been used successfully for monitoring sessile epilithic fauna around Lundy Island, Bristol Channel, United Kingdom (described in Hiscock, 1986); while a frame size of 520 mm × 400 mm (camera-to-subject distance of 450 mm) was used for the more turbid, estuarine waters of Milford Haven estuary, South Wales (described in Munro, 1999; Moore, 2000). At Skomer Marine Reserve, also in South Wales, a 500 mm × 400 mm framer is used for photographing species within low hydroid/bryozoan dominated epilithic turfs (Fig. 4.5). Larger

Fig. 4.5 A photo-monitoring framer being used to photograph *Alcyonium glomeratum* soft coral colonies. Orange floats are to compensate for weight of the framer. © Skomer Marine Nature Reserve, Countryside Council for Wales. (For a colour version of this figure, see Plate 4.3.)

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framer sizes (up to 1000 mm × 700 mm) are used for recording the presence of bigger colonies, e.g. massive bryozoans such as *Pentapora fascialis* (Blaise Bullimore, pers. comm.). Originally designed to work with *Nikonos* film cameras, these systems have now been adapted to use either DSLR or compact digital cameras. This can cause some issues with continuous data sets, especially when recording small or difficult to distinguish specimens. In the Skomer MNR sponge assemblage monitoring programme it was noted that the number of sponges recorded increased in the 2009–2010 recording year. This was attributed to the increase in image resolution resulting from the change from stereo-film cameras to DSLR for image recording (Burton *et al*., 2011).

Accurate comparison of images from the same station taken at different times is highly dependent on the precision of camera positioning. Photo-transects can be quickly and simply constructed by stretching a tape or marked line between fixed points. However, this method rarely provides sufficient accuracy of positioning to ensure a good correlation of images between monitoring periods. As rock or coral surfaces are rarely perfectly flat, the transect will tend to pass above or below protrusions as it is being set up. Thus, on some occasions, parts of the transect line will be higher than true horizontal, at other times lower. Thus, the precise location of the line may vary between successive sampling times. The longer the transect and more widely spaced the fixing points, the more this problem is exacerbated.

An additional consideration is that wide-angle lenses, in particular, suffer significantly from peripheral distortion and very steep perspective (i.e. subjects closer to the camera appear much larger than those more distant). While this effect makes little difference to the aesthetic appeal of a photograph, it can cause significant problems when trying to make size comparisons. Incorrect placement of the camera, causing a shift in location of a colony within the image frame, can cause apparent growth or shrinkage where none has in fact occurred (Fig. 4.6). Steepened perspective also makes accurate growth measurements of three-dimensional colonies technically complex to achieve, even when the camera is precisely repositioned every time.

When photographing species growing on rock faces, a framer is frequently used to ensure the camera focal plane is parallel to the rock face, and camera-to-subject distance is constant. The frame can also provide a scale bar and act as a moveable quadrat. Permanent markers (e.g. pins fixed into drilled holes or pitons hammered into crevices) help ensure precise positioning of the framer. These can be used to mark two corners of the photo-monitoring quadrat. For photographing sequential quadrats along a transect, a bar or rail firmly fixed to the rock, with locating marks along its length, works well. A 25 mm diameter pipe secured by pitons is one method employed at Skomer MNR, South West Wales (B. Bullimore, Countryside Council for Wales, pers. comm., 2002). The camera framer is hung on the rail by hooks, then aligned with the distance marks. This process is repeated along the rail. Methods for fixing attachment points or marks onto the seabed are discussed in more detail in Section 4.4.

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Fig. 4.6 Apparent growth of an erect sponge due to peripheral distortion and steepened perspective. © Colin Munro. (For a colour version of this figure, see Plate 4.4.)

When extracting measurements of species from photographs, either by direct measurement from prints or slides, or using image analysis software on digital images, a calibrating scale needs to be placed alongside the species of interest. Stainless steel or aluminium rules are convenient as they do not float or rust. However, polished metal is highly reflective and care needs to be taken when placing the rule to ensure that light from the flash does not bounce directly off the rule back into the camera lens, 'burning out' the calibrating scale on the photograph. Positioning of the scale as close to the subject as possible is critical, given the peripheral and perspective distortion that occur with wide-angle lenses. Additionally, scale will change from the centre to the periphery of the image (Fig. 4.7), thus measurements taken near the edge of the image should be treated as approximations only.

For photographing large, erect, planar species such as gorgonians and some branching sponges, a matt, opaque scaled background (Fig. 4.7) provides a calibration scale. Placing the scaled background directly behind the target colony isolates it from other colonies close behind. This helps prevent confusion as to whether a particular branch is part of the target colony or one growing directly behind it, a confusion that can easily occur when analysing photographs taken where colonies occur in dense aggregations.

The requirements for precise station marking in monitoring programmes, the analysis problems created by camera misalignment and methods to solve them are discussed in greater detail in Bullimore and Hiscock (2001), Munro (2000) and Grist (2000).

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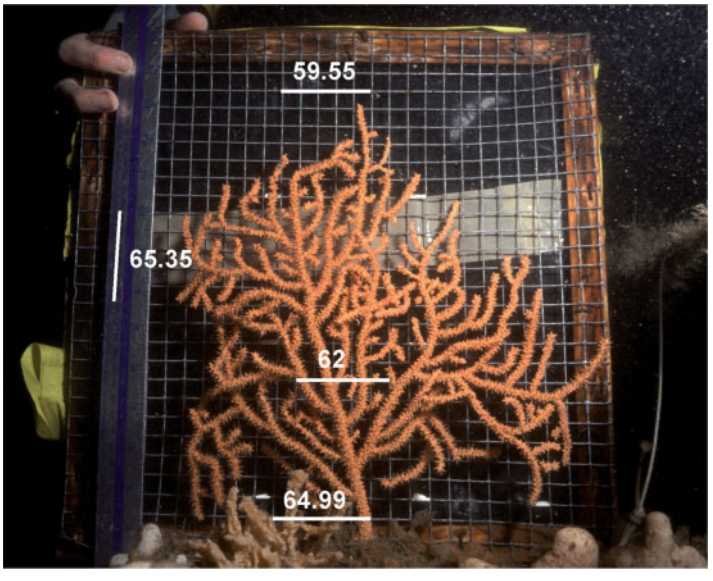

Fig. 4.7 Distortion inherent in a wide-angle lens causing changes across a photo-monitoring image. © Colin Munro. (For a colour version of this figure, see Plate 4.5.)

Except in shallow and clear waters, artificial lighting is essential to expose images correctly, ensure a good depth of field and provide colour in the image. A flash with a beam angle that covers at least the entire angle of view of the lens used is needed (e.g. around 100° for a 15 mm lens). It should also have sufficient flash energy output (normally at least 80 J for coverage of a wide-angle lens) to allow a small aperture to be used, providing good depth of field. Dual flash set-ups help eliminate shadowing, which can often mask areas of the picture, especially where the rock surface has noticeable mounds and depressions.

Stereo-photography

Twin camera stereo-photographic systems using film cameras have been used to measure growth and monitor change in benthic assemblages (e.g. Lundälv, 1971; Green, 1980; Bullimore, 1983, 1985, 1986). Chong and Stratford (2002) describe a system for stereo-photogrammetric measurement of red hydrocoral growth, which they found to have an accuracy of ± 1.2 mm (95% confidence). This system utilised two *Nikonos* cameras mounted on a framer. An important consideration was that the necessity for the use of a custom-built device to ensure simultaneous triggering of the camera shutters and both standard and underwater calibration. The system used at Skomer MNR also uses a reference frame, here constructed from 25 mm box-section aluminium, filled with expanded polyurethane to prevent water ingress and add buoyancy. It was originally used with two *Nikonos* (subsequently replaced with digital compact cameras) mounted on the frame in such a way that

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they share the same focal plane and focal paths. The original system designed for the reserve's monitoring programme is fully described in Bullimore (1983), with additional modifications described in Bullimore (1986).

The distance between cameras, camera-to-subject distance and lens choice is determined to ensure a high degree of overlap between each pair of photographs (necessary for three-dimensional viewing) and that the reference frame lies completely within the FOV of each camera. Choice of camera-to-subject distance will be determined by the prevailing underwater visibility. Bullimore and Hiscock (2001) suggest that a minimum visibility of four times the camera-to-subject plane distance is required in order to gain useable images. Further details of system design and minimum monitoring areas for adequate community description are given in Bullimore and Hiscock (2001). To view stereo-photography paired images a stereo-comparator is used. A system developed for Skomer MNR monitoring programme, using an adapted stereo-microscope, is described by Bullimore (1987). To extract measurements from stereo-images and thus calculate branch length change in red hydrocorals, Chong and Stratford (2002) employed an off-the-shelf digital stereo-plotting workstation.

Video systems

The following comments reflect the situation at the time of writing. However, video technology and electronic data storage and analysis systems are evolving rapidly, and future improvements will inevitably address some of the current limitations of underwater hand-held video.

Video is extremely useful for recording overall pictures of habitat appearance. By recording along a set path (e.g. a belt transect) a permanent record of an extensive site can be recorded. Video will provide seamless images covering extensive areas, with greater similarity to the diver's viewpoint of such areas than the discrete snapshots provided by a photo-montage. At the time of writing, high definition (HD) video recording has become widely available on consumer camcorders suitable for use in diver-operated underwater housings. In addition to this, increasing numbers of DSLRs are capable of recording HD video. HD video currently means either 720p, 1080i or 1080p video; 720 refers to frame sizes of 1280×720 pixels, 1080 is 1920×1280, the P refers to progressive scan, I to interlaced frames. Video has frequently been used for recording larger species and life forms along transects. Increasingly this is being replaced by digital stills; for example, Australian Institute of Marine Science (AIMS) have in recent years changed to sequential stills taken along their reef monitoring transects (Australian Institute of Marine Science, 2008), due to the markedly higher resolution obtainable from digital stills. Film cameras were generally limited to 36 frames per roll or film (and thus dive) and required a greater degree of training or experience to produce good images. As digital stills no longer have these limitations it is likely that stills will replace video in many monitoring programmes. Several current or recent video protocols are described

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later, as described in the AIMS monitoring (Australian Institute of Marine Science, 2008). It should be quite possible to replace video with stills while keeping other aspects of the methodology similar.

Video belt transect surveys have been adopted by many statutory bodies and NGOs as part of their survey and monitoring protocol (e.g. the Australian Institute of Marine Science; the Joint Nature Conservation Committee of the UK; Hawaii's Coral Reef Assessment and Monitoring Programme; NOAA's National Undersea Research Programme). A widely adopted protocol for use on tropical waters with good visibility and high ambient light levels is described by Osborne and Oxley (1997). They suggest a discrete point sampling method. The surveyor swims at a constant speed along the transect, holding the camcorder at a set height (25 cm recommended) above the reef and recording directly downwards. A 50 m long transect is recommended. The recorded images are analysed by playing back the tape and viewing on a suitable monitor with five points marked on the screen. The tape is stopped at set intervals and the species located underneath each of the five points are identified and logged. They suggest 40–80 pauses (giving 200–400 data points) along the transect to provide good estimates of total hard coral cover. For each life form/species category, percentage cover is estimated by dividing the number of points at which that group was recorded by the total number of data points, and multiplying by 100. A similar protocol developed by field biologists based at the Virgin Island Field Station is described in Rogers *et al*. (2000).

Video footage of permanent transects can be used for quantitative monitoring of life forms or larger, conspicuous species. It is also particularly useful for positively identifying subtle or qualitative changes in habitats that are difficult to confirm purely from diver's descriptions, e.g. slight increases in sediment veneer overlying reefs. If using video transects for comparative monitoring of benthic species and habitats, it is important that the video path starts from the same end of the transect every time, as massive or branching species can appear very different when viewed from different angles. A differing viewpoint may also change which low-lying colonies are visible and which are obscured. Use of autofocus is not generally recommended as this is an unnecessary drain on the camcorder's battery and may cause the lens to focus on drift weed or other objects floating past the camcorder. Wide-angle fixed focus, with focus set to around 0.3 m, appears to work well for habitat recording with most systems in most conditions. Depth of field is a function of lens angle of acceptance, aperture size and shutter speed. On most auto-exposure systems, it is recommended that the camcorder be preset to shutter priority (if available on the system) and to a relatively slow shutter speed, e.g. around 1/50th of a second. This should maintain optimal exposure and depth of field in most conditions. Recording should be started a few seconds before recording the subject of interest; this allows for any slight mechanical delay and provides excess frames for any editing that may be required later. In low-light conditions, the use of lights will not only bring back colour to the image but also sharpen the image by (i) reducing the aperture size and thus increasing the depth

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Fig. 4.8 Video system being used to record behaviour in the sea hare *Aplysia punctata*. © Colin Munro. (For a colour version of this figure, see Plate 4.6.)

of field and (ii) reducing 'noise' on the image from excessive signal amplification. However, by automatically reducing the aperture size, the area outside the lights' beam will become appreciably darker in the image, thus the total viewable area will be dramatically reduced. Lights should ideally have a very wide beam and be fitted with diffusers to prevent 'hotspots' occurring in the picture. The use of two lights, with beams angled in slightly different directions, helps spread light more evenly and further reduce areas of image burnout. Video belt transect surveys have also been widely adopted for fish surveys (see the section entitled 'Fish survey techniques', 'Belt transect').

Video is useful for helping to locate sites, by recording a swim-path to the study site (see Section 4.4). This is especially helpful for orientating workers who have not previously visited a site. It also has obvious applications in recording behaviour, either as part of a behavioural study (Fig. 4.8) or as an aid to identification (e.g. where the movement of a particular species is distinctive).

4.4 Underwater site marking and relocation

General considerations

Accurate relocation of specific sites on the seabed is a requirement of many benthic studies, but is one of the biggest problems for the diving marine biologist. The problem is not so great in clear oceanic waters or when working on man-made structures such piers or breakwaters, or very close to shore, where a well-defined

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swim-path can be followed from the surface. However, in poor visibility, or offshore where the diver must descend from a boat, searching for a specific site frequently wastes valuable bottom time. Loss of data through failure to relocate a site is also something experienced by most diving scientists at some stage in their career.

When working offshore in any depth of water, it is always advisable to descend a shot or buoy line marking the site if the water is not sufficiently clear to allow the target to be seen from the surface. Modern position fixing allows very accurate positioning of the support vessel above the selected site. However, a free-descending diver may drift horizontally some distance during the descent, even in quite slight currents. They may also likely drift down-tide while on the surface performing final equipment checks before submerging. Thus, for example, in making a descent to around 20 m depth, a diver may easily reach the seabed off-station by 15 m or more. In poor visibility this can easily result in failure to locate the study site.

Standard Global Position System (GPS) receivers are now capable of ± 10 m accuracy for around 95% of the time. However, this does not necessarily always translate into vessel positioning and shot line deployment within 20 m of the study site. Human response time and vessel manoeuvrability will impose additional errors. Thus, where low visibility is expected, locating aids fixed to the seabed may be required to guide the diver to the study site. Snag-lines fixed close to the seabed, running either side across the tidal axis or (on a sloping seabed) along a depth contour, greatly increase the diver's chances of quickly locating the study site. Ideally, these should indicate on which side of the site the diver is located (e.g. by using different colour or thicknesses of line, or tagged lines, either side). Snag-lines may be augmented by high-visibility sub-surface buoys attached along their length. Sub-surface buoys, fixed to rocks or heavy weights, rising 0.5–2 m above the seabed, dramatically increase the diver's chances of spotting study sites in poor visibility. Rigid 6 inch diameter fishing floats used by commercial fishermen for trawl nets are ideal. Fig. 4.9 illustrates such a system, with fishing floats attached by floating polypropylene line to concrete paving slabs, used for relocating monitoring stations on subtidal reefs several miles from shore (Munro & Baldock, 2012). The mid-water air space with such floats is also acoustically highly reflective. As a consequence they show up as a well on a vessel's echo-sounder, providing useful confirmation of correct positioning. Metal structures may be constructed to help define and relocate the study area. Metal grids are commonly used in archaeological excavations, and the construction of a raised 4 m $\times$ 4 m tubular galvanised steel grid to map sea fan colonies is described in Munro and Munro (2002). Large metal tripods have also been used to mark site and attach locator beacons at monitoring sites (e.g. Holt & Sanderson, 2001). Where steel structures are constructed to mark sites, it is recommended that a sacrificial zinc anode be attached to the structure in order to reduce corrosion.

A more expensive method but one very useful in low-visibility conditions is the use of relocation transponders. The Countryside Council for Wales (CCW) have employed some produced by Sonardyne Ltd. at some sites in their Across-Wales

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Fig. 4.9 Six inch diameter plastic fishing floats attached to a concrete paving slab by floating polypropylene line, used to mark a monitoring station. © Colin Munro. (For a colour version of this figure, see Plate 4.7.)

Diving Monitoring Project (Whittington *et al*., 2006). An important consideration with these is that they require an annual maintenance visit to replace the batteries in the transponder; however, this should not be a problem if annual (or more frequent) monitoring is conducted. Sonardyne also produces Lightweight Release Transponders (LRTs), which are both located and released acoustically by a surface commander unit. These have also been employed in the Across-Wales Diving Monitoring Project, attached to site marking pyramids. They are the first means of site relocation, providing a visual surface marker enabling divers to be deployed directly above the site (Whittington *et al*., 2006).

Surface sightings are less used now that GPS is relatively inexpensive. However, transits (a position line established by noting the alignment of two conspicuous landmarks) and bearings taken using a sighting compass provide good backups to electronic navigational aids. In areas where land is close and features conspicuous, sightings may be used as the main means of locating the vessel above the study site. It is recommended that photographs are taken of all landmarks forming transits or from which compass bearings have been taken.

In low-visibility conditions, or where the seabed lacks distinctive features, it is advisable to employ as many complementary aids to site location as possible. Where underwater markers have been fixed, wide-angle photographs should be taken to show their appearance and location relative to seabed features. If visibility does not allow this then detailed maps can be drawn illustrating distance and direction of the study site from other conspicuous features. Waterproof copies of diagrams and photographs may be made by encapsulating these in plastic, allowing the divers

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Fig. 4.10 A diver-operated air drill being used to mark a sub-littoral monitoring station in Milford Haven. © Colin Munro. (For a colour version of this figure, see Plate 4.8.)

to consult them underwater on subsequent monitoring visits. Video footage of the location and pathway between markers is also highly effective in aiding divers to form a mental map of the area and to recognise features along the pathway to the site.

Air drills and underwater fasteners

Diver-operated air drills may be used to create attachment points on rock or coral for lines, buoys or quadrats. Small, pistol grip pneumatic drills constructed from stainless steel and powered by diving cylinders have been used to mark sub-littoral monitoring sites around Lundy Marine Nature Reserve and Milford Haven estuary (Fig. 4.10). Suitable drills are manufactured by *Ingersoll Rand*, with a maximum chuck size of 13 mm. Using masonry drill bits, these can be used to drill holes several centimetres deep in limestone. On granite they make little impression on solid rock, but can be used to enlarge small crevices (albeit with considerable effort and numerous drill bits). Once holes have been drilled, these can be filled with a suitable underwater setting resin. Polyester resins formulated for bonding with stone and masonry work well, with those supplied in cartridges that can be applied using a mastic gun being easiest to use underwater, e.g. *Fischer* UN No 1866 or a similar type. Locating pins or eyebolts are then pushed into the resin and allowed to set. This provides a secure fitting that should last for several years provided it is not placed under significant load. Stainless steel, aluminium or brass fittings are recommended. The duration for which a full cylinder will power a drill obviously decreases with depth. At around 18 m, a 15 litre (120 cu ft) cylinder will empty in 8–10 minutes.

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Acoustic pingers and receivers

Where visibility is very poor, the use of acoustic transponders may be necessary. Acoustic pingers that can be attached to the monitoring station are available from a number of manufacturers (e.g. *Benthos Inc*., Massachusetts; *Sonardyne*, Blackbushe, UK; *Vemco Ltd.*, Nova Scotia; *InterOcean Systems*, San Diego). Acoustic pingers will emit pulses for between a few days and several years. Many systems allow the signal power and frequency of pulses to be varied, and so the active lifespan of the pinger to be varied. Some models are activated by immersion, while others are user-activated. Delayed-activation pingers are also available, effectively extending their working life if initial relocation is not planned until some time after deployment. Relocation is achieved by a boat-mounted receiver (fixed beneath the water's surface), or a hand-held receiver operated by the diver. The display on such receivers varies, typical systems having an LED display indicating direction to the acoustic beacon and signal strength. Many systems allow for transponders to have different 'address' codes, allowing the diver to locate and unambiguously identify a number of close-together stations. Typical stated locator ranges vary from 60 to 750 m, depending on system and conditions. Acoustic beacons have been successfully deployed on monitoring stations on Sarn Badrig Reef, North Wales (Holt & Sanderson, 2001) and Milford Haven Waterway.

By setting up a network of transponder beacons on the seabed, such systems can be used to map the location of features accurately across an extensive site. This is achieved by the diver moving to different seabed features, and recording the distance (displayed on the hand-held receiver) to each beacon. Alternatively, a beacon may be attached to the diver, allowing its location to be dynamically tracked as they travel across the site. Such systems have been developed primarily for the oil and gas industry, but have been adopted by underwater archaeologists for mapping wreck sites (e.g. the wreck of the *Coronation*, Cornwall, England, and the *Resurgam* submarine in Colwyn Bay, Wales).

For open coast sites, it is generally recommended that surface buoys should not be used as a key method of relocation if this can be avoided. Unless attachment points and buoy lines are of very strong construction and secured to very heavy objects on the seabed, they are likely to disappear during storms. Additionally, constant movement through waves and tide will chafe through lines, and fouling growth may eventually cause the buoy to sink. Surface buoys are also attractive items for passing fishermen or others in small boats. If a surface buoy is used, it should not be attached directly to the study structure. Unless the structure weighs more than one tonne, strong wave action on the buoy can drag the structure off-station.

4.5 Sampling methods

Corers

Core sampling is a cheap and simple means of collecting quantitative samples from soft sediment. In their simplest form, these are lengths of plastic or metal tubes

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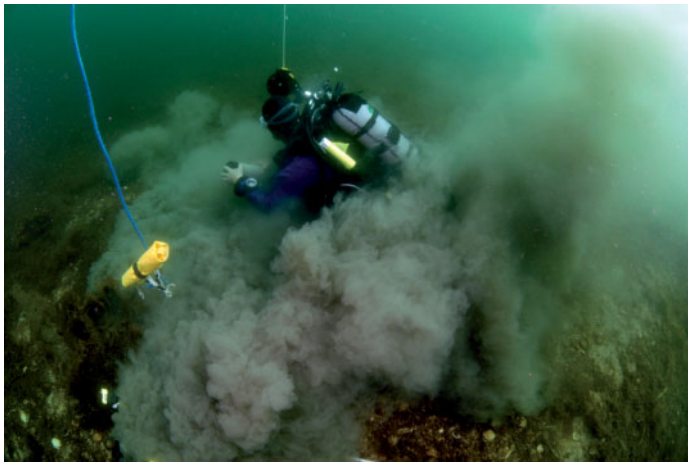

Fig. 4.11 A diver collecting a core sample in soft sediment. Coring can create large plumes of suspended sediment, temporarily reducing visibility. © Colin Munro. (For a colour version of this figure, see Plate 4.9.)

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of known diameter, which are pushed into the sediment by the diver. Where the sediment is coarse or consolidated, some workers suggest cores may be hammered into the sediment (e.g. Angel & Angel, 1967; Gage, 1975). However, extraction of an open-ended core will normally result in partial loss of the sample. To prevent this, it is usually necessary to dig around the core and seal the end (either with a bung or by hand) before attempting to withdraw it. Digging by hand in material that requires the cores to be hammered in can be difficult, especially if it has to be repeated several times during a dive.

It is normally recommended that the cores be fitted with rubber bungs or caps to retain the sample. In practice, these are often difficult to fit underwater, the cores being full of water, which is incompressible. An alternative is to slip plastic bags over either end and retain with rubber bands. This is usually a two-person task (especially as the act of coring will often have severely reduced visibility), but can be accomplished quickly. It should also be borne in mind that coring may mobilise large amounts of sediment in to the water column, reducing visibility to nil (Fig. 4.11). Where cores can be capped very securely underwater, a mesh bag or similar can be used to carry them. A more secure method is to place them within a crate. For larger number of cores, or large volume cores, this can be tied to a surface buoy (Fig. 4.12). The divers can then ascend this line at the end of the dive, and the crate hauled up by the boat crew. Cores must, of course, be securely held in (e.g. by elasticated cord across the top of the crate) and the crate should be weighed to reduce the risk of it tipping while being hauled.

Suction samplers

Diver-operated suction samplers are useful for excavating relatively large sediment samples. Airlifts have been used by underwater archaeologists since the 1950s (Du

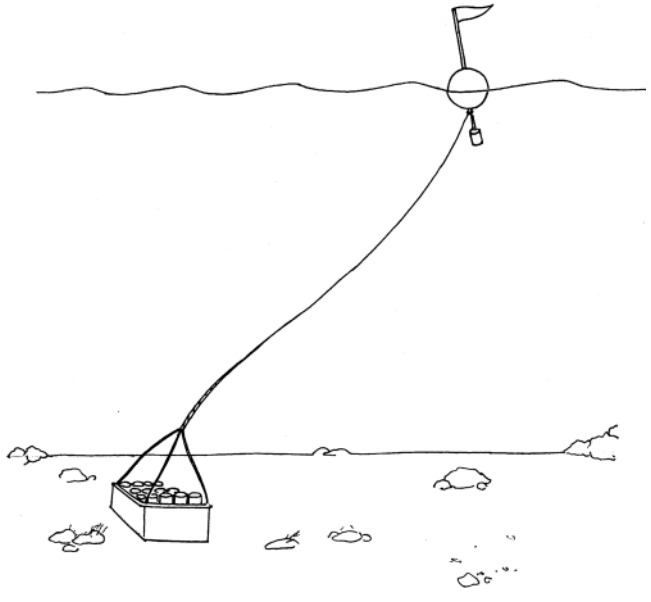

Fig. 4.12 A crate attached to a surface buoy being used to hold cores. © Colin Munro

Plat Taylor, 1965) to clear over-burden from wreck sites and to uncover artifacts. Airlifts work on the Venturi principle. Compressed air is fed into the lower end of a long rigid pipe, which is normally guyed at an angle of 45° from horizontal. Air rises up the tube before escaping out of the far end. The entrained air causes a drop in pressure around the mouth of the pipe and so 'lifts' water and sediment up into the pipe (Fig. 4.13).

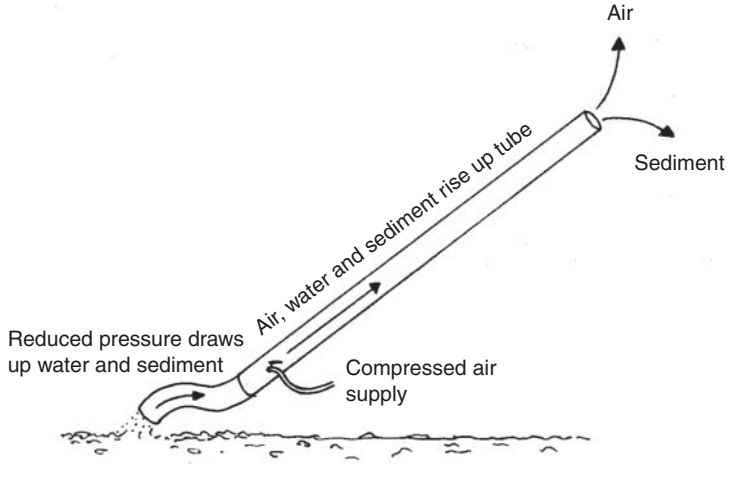

Fig. 4.13 Diagram illustrating the principle of an air lift or suction sampler. © Colin Munro

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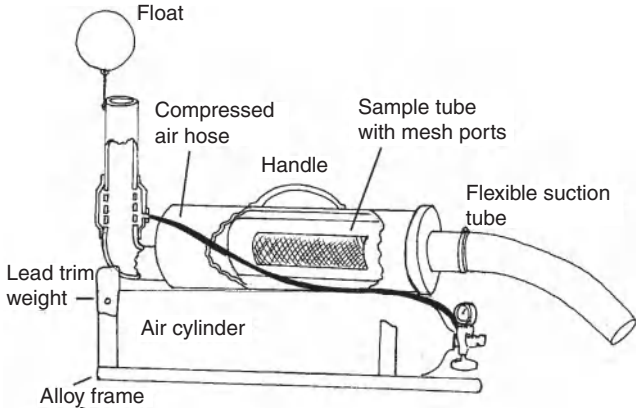

Fig. 4.14 The Hiscock and Hoare sampler. (Adapted from Rostron (2001) after Hiscock and Hoare (1973).) Available from: http://www.jncc.gov.uk/marine/mmh/Pg%203-10.pdf

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However, archaeologists' airlifts tend to be large, designed to move much larger amounts of sediment than biologists normally work with. They also need to be anchored in place to counteract their buoyancy while in operation. Consequently, lighter weight systems were designed that were powered by diving air cylinders and easily moved into position and operated by two divers. Barnett and Hardy (1967) described a modified archaeological airlift, designed for sampling soft sediment infauna. This design incorporated a large diameter cylinder on the seabed end of the pipe, and a sieve and sampling bag on the far end. The sampling cylinder was pushed a few centimetres into the seabed, and the sediment was drawn up through the sieve, retained material then falling into the sampling bag. This system was further modified by Hiscock and Hoare (1973). They designed a smaller, portable system incorporating a flexible tube attached to the entry end of the sampling cylinder (Fig. 4.14). The Hiscock and Hoare sampler was designed primarily for sampling epilithic fauna, the sample chamber capacity being too small to be effective on most sediments. Quantitative sampling is achieved by sampling within a quadrat, using a paint scraper to dislodge attached species. More recent systems such as the bucket sampler and the JW Miniature sampler (designed by John Woolford of Pembrokeshire, Wales) have been adopted by many of the Government Agencies within the United Kingdom and several marine survey consultancies. These designs (Figs. 4.15 and 4.16) have sample-retaining bags of differing mesh sizes, depending on the sediment type and sampling protocol. Bags can be changed underwater, allowing divers to take numerous samples. By use of a reference cylinder attached to the end of the flexible pipe, or by sampling within a quadrat to a given depth, quantitative samples can be processed.

Suction samplers are particularly useful in mixed or coarse sediments, where cores or grabs may have difficulty penetrating (e.g. Robinson & Tully, 2000) or where large deep samples are required, such as collecting deep-burrowing

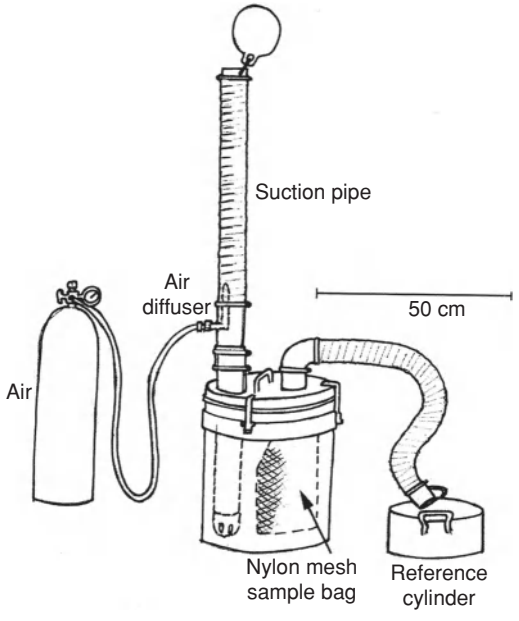

Fig. 4.15 A bucket suction sampler design. (Adapted from Rostron (2001) after Hiscock (1987).)

megafauna. The main disadvantages of suction samplers are that they can draw in animals from surrounding substrates, thus inflating abundance estimates within the quadrat, and they may abrade animals as they are drawn in with the sediments (Simenstad *et al*., 1991). Much of the above information of sampler design is adapted from Rostron (2001), where additional details on system design and operation can be found.

Fig. 4.16 Divers using a suction sampler. © Dale Rostron, Subsea Surveys. (For a colour version of this figure, see Plate 4.10.)

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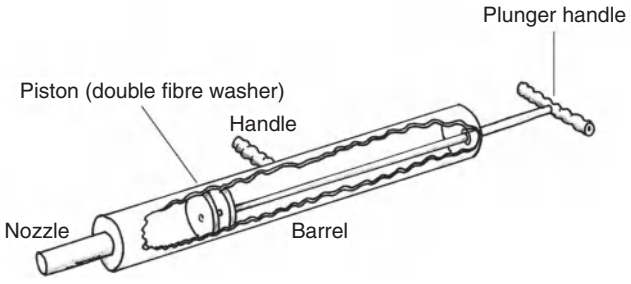

Fig. 4.17 Slurp gun design. © Colin Munro

Yabby pumps and slurp guns

Yabby pumps are long, narrow steel or plastic tubes containing a central plunger. They are used extensively in Australia to capture 'yabbies' (fresh water crayfish) by placing the end of the tube over the animal's burrow entrance and sharply raising the plunger. Yabby pumps have been adopted by numerous workers (e.g. Dworsschak & Jorg, 1993; Abed-Navandi & Dworschak, 1997; Abed-Navandi, 2000) to capture burrowing crustaceans underwater, especially thalassinid shrimps that excavate deep or complex burrows. Slurp guns operate on the same principle as yabby pumps, but are generally of larger diameter (Fig. 4.17). They are used for the collection of crevice fauna or fast-moving animals such as fish or shrimps (e.g. Lincoln & Sheals, 1979; Squires *et al*., 1999). Slurp guns have also been used for the collection of sea-ice algae. They have the advantage (compared to techniques working from the ice surface such as coring or cutting down through with an auger) of being able to sample several nearby sites rapidly. However, some material may be lost through leakage from the cylinder (Poulin, 2001).

Scrapers

Paint scrapers are useful tools for collecting low-turf species such as hydroids or bryozoans, and encrusting sponges, colonial tunicates and barnacles. For very small animals, a pipette may be helpful for catching and transferring the detached specimens to plastic bags.

4.6 Other study techniques

Resin casting

The structure of burrows in sediment can be investigated by resin casting. This technique was first developed by the marine geologist E.A. Schinn (1968), and has since been widely used by marine biologists studying burrowing megafauna (e.g. Atkinson & Nash, 1985, 1990; Dworschak & Jorg, 1993; Nickell & Atkinson, 1995;

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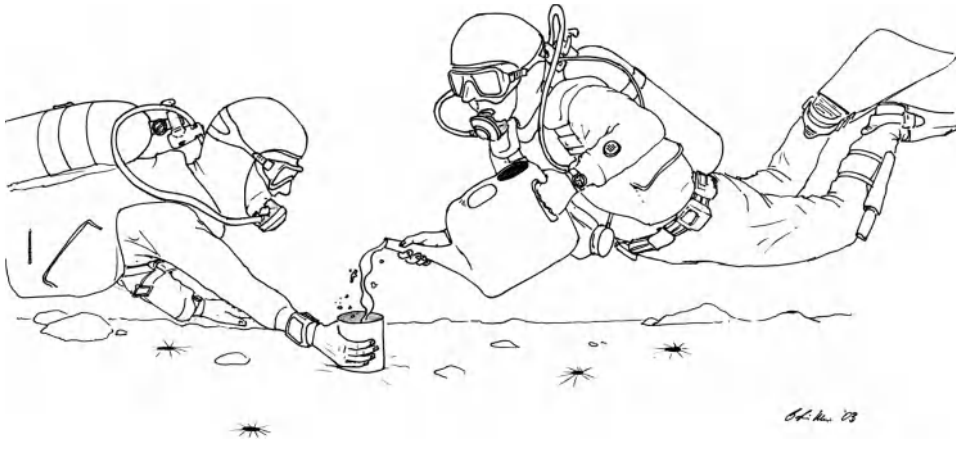

Fig. 4.18 Divers pouring resin into a burrow. © Colin Munro

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Nickell *et al*., 1995a; Coelho *et al.,* 2000). The methodology is fully described by Atkinson and Chapman (1984). Two main types of resin are used for this technique: (i) polyester resins that use a small amount of catalyst and set relatively quickly (a few minutes to several hours) and (ii) epoxy resins that require larger amounts of catalyst and set more slowly (a few hours to several days). Polyester resins are used predominantly in temperate and polar regions. Epoxy resins are more durable, but considerably more expensive than polyester resins. Most epoxy resins will not set at low temperatures, and so are used chiefly in the tropics (R.J.A. Atkinson, pers. comm.).

The resin is carried in a watering can or similar container, with catalyst normally being added just prior to diving. The resin, being slightly denser than water, can be poured underwater into burrows (Fig. 4.18). A cylinder or box framing the burrow mouth helps direct the resin and also aids relocation. Inevitably, some small droplets of resin will be suspended in the water column during the pouring process and will adhere to the diver's equipment. For this reason, cameras and similar equipment should be kept well clear during the pouring process. Once the resin has set (it is normal to leave overnight) the cast can be dug out of the sediment.

4.7 Survey methods

Manta tows

Manta boards are essentially large boards, generally constructed from wood or Glass-Reinforced Plastic (GRP), that act as hydrofoils. They are designed to be towed behind small boats, attached using a water ski tow rope or similar. A diver or snorkeller hangs on to the board and is pulled through the water, either at the

surface looking down or up to a couple of metres below the surface. Different designs will have handles or hand-holds for the diver, and some will allow the diver to angle the board in such a way that it rises or descends. An attached writing slate on which the diver can record data is incorporated in many designs.

Manta boards are used to survey larger areas than divers could cover in a reasonable time unaided. The level of detail recorded is limited, because the diver passes fairly rapidly over areas, and higher above the seabed than their normal free-swimming height. This is a low-cost, low-tech method useful for broad-scale survey, mapping habitat type, assessing extent of widespread, highly visible impacts or identifying areas for more detailed survey. A protocol developed by the Australian Institute of Marine Science (AIMS) for surveying coral reef features is described by English *et al*. (1997).

Tows should be conducted at a constant speed. This should be slow enough that the diver does not have to put undue effort into holding on rather than recording (normally 1.5 knots or less). Speed should also take into account the horizontal visibility. While good design will help minimise unbalanced pressure on upper and lower surfaces, greater speeds may also cause the manta board to tend to lift towards the surface, or worse, dive towards the seabed. Resisting this tendency can be extremely tiring and distracting for the diver. When the diver is being towed below the surface, a means of communication between the diver and the surface team on the boat must be developed. Care should be taken to ensure that octopus valves are tucked out of way, as these can free-flow due to the rate of water flowing past them rapidly depleting the diver's air supply.

Manta tows have been used extensively to estimate coral cover and assess numbers of and damage (feeding scars) caused by crown-of-thorns starfish, *Acanthaster planci* (e.g. Kenchington & Morton, 1976; Fernandes *et al*., 1993; CRC Reef Research Centre, 2001). Recording sheets with pre-printed species, habitat or condition categories are recommended by English *et al*. (1997) as this greatly speeds up recording. Manta board tows have also been used successfully in good visibility in temperate waters, e.g. for surveying habitats within Port Erin Bay (Riley & Holford, 1965) and for identifying the location of maerl beds within the Firth of Clyde (pers. obs.). More complex towed vehicles have been developed for specific purposes, such as the semi-enclosed two-man vehicle used by Main and Sangster (1978) for observing mobile fishing gear.

Transect and quadrat surveys

By quantifying the survey area, transect or quadrat surveys greatly facilitate gathering of data on species percentage cover and density. By setting more or less precise (depending on protocol used) limits on the area considered, they also help make comparisons between study areas more valid. The more commonly used types of quadrat and transect survey are described here.

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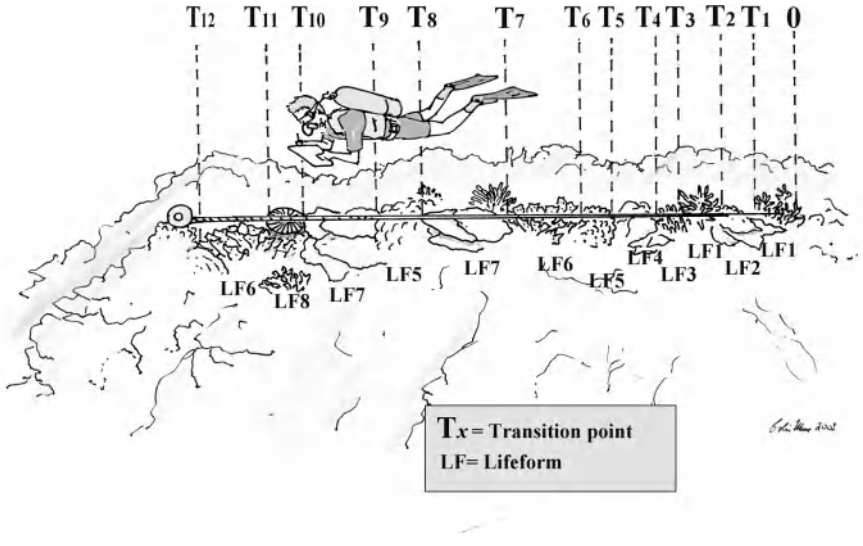

Fig. 4.19 Line intercept transect survey. © Colin Munro

Line intercept transect

The Line Intercept Transect (LIT) is a widely used method for assessing percentage cover within the benthic community of coral reefs (e.g. Marsh *et al*., 1984; Bradbury *et al.*, 1986). The majority of workers using this technique have used a 20 m tape, marked in distance increments, simply stretched out along the reef following a depth contour. Survey divers then record the distance along the transect at which each species or life form transition occurs (Fig. 4.19). This process is usually repeated at different depths, with five replicate transects at each depth. Percentage cover of each species or life form is calculated by dividing the cumulative lengths of the target species or life form by the length of the transect and multiplying by 100. A formal protocol for use of this method for recording coral reef life forms data has been developed by the Australian Institute of Marine Science (AIMS), and is described in English *et al*. (1997). This is a simple and inexpensive method, suitable for use by non-specialists when classifying species at life form level. However, some life forms are difficult to standardise and the method is considered inappropriate for monitoring small changes in community structure (English *et al*., 1997).

Line point transect

The line point transect is similar in design to the LIT, but with marked points along the length of the transect line. All benthic species lying directly underneath points are recorded. Unlike the LIT method, size of colonies is not recorded. Liddell and Ohlhorst (1987) used transects marked at 200 mm intervals. They

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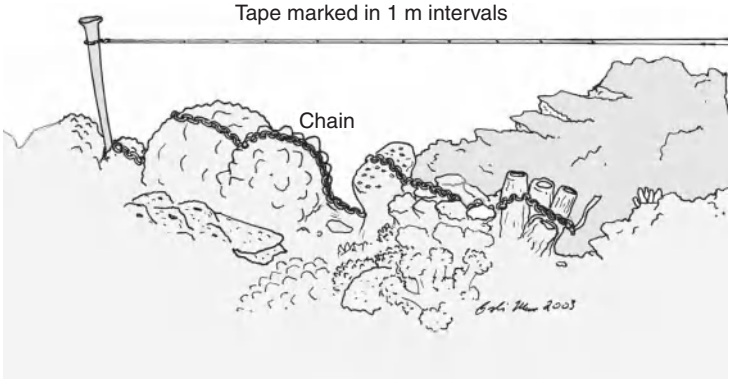

Fig. 4.20 Chain transect. © Colin Munro

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recommended a minimum of five 20 m long transects per site. They considered the results generated by this method to be comparable with those of the LIT, but requiring less survey time. However, because of the limited number of discrete survey points along the transect, uncommon small species are likely to be missed. Because of its simplicity, the line point transect survey method is frequently used by volunteer diver programmes (Pinca *et al*., 2002).

Chain transect

The chain transect is used to gather data on species presence and abundance, and to provide a measure of seabed relief. A line transect, marked in 1 m increments, is tensioned between two stakes set so that the transect runs parallel to the reef crest or shore. Starting at one end of the transect, a lightweight chain (1.5–2 m long) is then draped along the reef, below the line, following the contours of reef (Fig. 4.20). The length of chain covering each species or life form is then measured by counting links. The number of links under each metre of line transect is also recorded. Where the reef is multi-layered, e.g. where tabular coral forms a tier directly over encrusting forms, the total length of all surfaces under the chain should be measured. By calculating the ratio of total chain length to transect length, an indication of reef rugosity is obtained.

This method provides a very detailed picture of reef composition and, unlike the LIT, takes the three-dimensional nature of reef colonies into account. Against this, chain transects are slow to deploy and to survey. They are most suited for surveying areas dominated by massive or encrusting life forms, and are inappropriate for use on areas supporting large numbers of highly branched species of otherwise structurally complex species (e.g. *Acropora* spp., gorgonians or branching sponges). They may also be inappropriate in areas supporting many fragile species. Chain surveys have been widely used for surveying and monitoring coral reefs in the Caribbean and Florida Keys (e.g. Meier & Porter, 1992; Rogers *et al.*, 1997;

Rogers & Miller, 2001). A detailed protocol for use of the chain survey method on coral reefs is given is described in Rogers *et al*. (1994).

Belt transects

Belt transects are essentially long quadrats laid, or visualised, on the seabed. The length and width of the transect used will depend on the size and density of the species being recorded, the underwater visibility and to a certain extent the depth (limiting time available for deployment and recording). Belt transects are useful for recording sessile species that occur in low densities or are patchily distributed. They are also extensively used for counting coral reef fish (see the section entitled 'Fish survey techniques').

In its simplest form, a belt transect consists of a diver or dive pair swimming along a compass bearing for a set number of fin-beats (or set time), recording target species within a set width either side of the diver's course (e.g. Bunker, 1985). This method allows a large number of transect surveys to be conducted quickly, with very little set-up time required. However, such transects are poorly defined: the diver's course may be influenced by currents and individual divers may cover differing distances and estimate transect width differently. Therefore, it is most useful where collecting large amounts of data in a short period of time is more important to the study than a high degree of precision in defining the study area.

A more precise area can be covered by laying a measured line along the seabed. This is most simply achieved by attaching the line on a diver's reel to a fixed weight. The line, on which distance increments have been marked, is then run out a prescribed distance. A second small diving weight attached to the reel helps keep the line taut. The diver or diver pair can then return to the beginning of the line and survey back towards the reel. The width surveyed either side of the line can be better controlled by the divers carrying a metre rule to verify the boundaries. By dropping a shot line within the survey area, then randomly selecting a number of compass bearings along which the transect should be laid, several unbiased transects can be surveyed in rapid succession. Where the seabed is uneven, a taut transect line will be raised some distance off the bottom in places. Additionally, where the transect is long, it may not be practical to try and get the line taut. In these situations, a weighted line is more appropriate. This can be made by crimping small lead weights at intervals along the line. For very long transects that are to remain in place for some time, leaded rope (with lead wire woven in) can be bought from commercial fishing outlets.

Belt transects with fully defined boundaries are more time-consuming to lay but are useful where very accurate counts of colonies or individuals per unit area are required. A simple, effective method uses two lengths of tubular steel (scaffold pole sections being ideal) as boundaries at either end of the corridor. Polypropylene line, with weights crimped on at intervals, forms the sides of the corridor. The steel ends provide rigidity and weight to keep the side lines taut once the transect is laid

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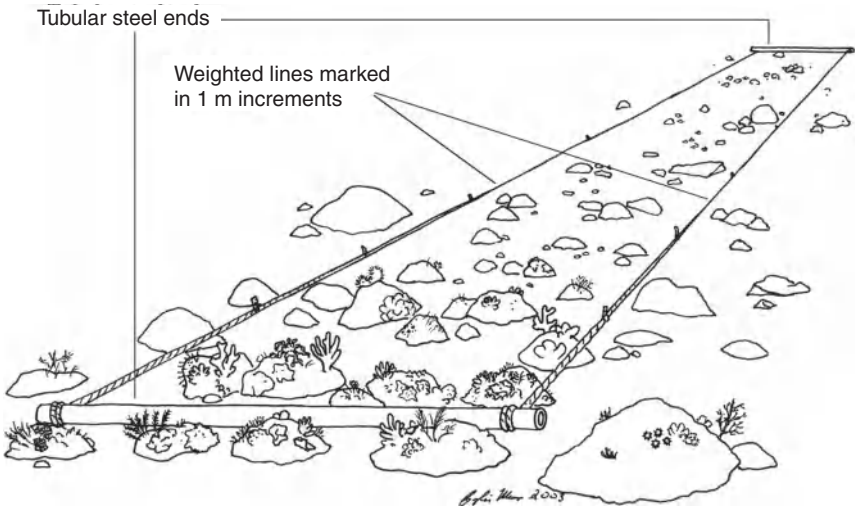

Fig. 4.21 Belt transect. © Colin Munro

(Fig. 4.21). Repeatedly folding the lines back upon themselves, and securing with rubber bands, makes the transect more manageable for carrying from the surface to the survey site and quick to lay once in place. On completion of the survey the divers attach a buoy line to one end, and the transect may be hauled from the surface. In relatively low visibility, a 1 m wide belt transect has proved most efficient (e.g. Munro, 1992; Munro & Baldock, 2012). A 2 m wide belt, with a central line dividing it into two 1 m corridors allows larger areas to be surveyed, while still requiring little deployment time. By replacing the steel ends with weighted plastic tubes, the transect can be hung from pitons or bolts fixed on a vertical face. This can act as a very large quadrat for surveying or monitoring low-density species on vertical rock habitats. Fully defined belt transects are particularly suitable for video surveys, if the lines marking the lateral edges to the transect are marked in distance increments and kept within the camcorder's FOV at all times.

Quadrats

Quadrats may be random or fixed. Randomly placed quadrats allow more powerful statistical analysis to be applied to data collected, and are often most appropriate for surveying habitats that are fairly uniform, such as seagrass beds, maerl beds or mud habitats (e.g. counting burrow densities). Reef habitats are generally too heterogeneous for random quadrats to describe their species composition adequately without an enormous investment of time. On a purely practical level, the placement of quadrats is also usually partly determined by the nature of the rock or coral surface. Areas containing large crevices or high ridges will be unsuitable as (i) it will not be possible to place the quadrat flush against the rock surface and (ii) the

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crevice or ridge may significantly increase the surface area beneath the quadrat. Thus, placement of fixed quadrats on reefs is generally a compromise between unbiased representation of the reef community and practical constraints.

For surveying or monitoring reef-building corals, 1 m^2 quadrats are recommended by most workers (e.g. Rogers *et al*., 1994; English *et al*., 1997), with smaller or larger quadrats used depending on size and spatial distribution of target organisms. On temperate reef systems, 0.25 m^2 (50 cm × 50 cm) are often preferred: (i) because generally lower visibility makes surveying or photography of larger areas difficult; (ii) because most temperate rocky reef species are quite small and often cryptic, thus systematic counting within a larger area would be extremely time-consuming and prone to error and (iii) because larger-size quadrats would prove difficult to position flush against most rocky reefs.

Moore *et al*. (1999) concluded that for sub-littoral rock habitats, less than ten 0.1 m^2 (32 cm × 32 cm) quadrats were required to provide a good, overall description of the epilithic community, and to give reasonable estimates of mean abundance for a few dominant species (i.e. in terms of numbers per unit area or percentage cover). However, they also found that a much larger number of quadrats would be required to give reasonable estimates of mean abundance for most epilithic species present. Bohnsack (1979), studying marine canal wall biota in the Florida Keys, considered that ten 0.6 m^2 quadrats were sufficient to describe the community present.

Differing growth forms (e.g. foliose algae, encrusting sponges, octocorals with interconnected stolons) and dramatic differences in size and density between juveniles and adults (e.g. barnacles or mussels) can present considerable difficulties for the surveyor attempting to record accurate and statistically comparable data for community monitoring. Strung quadrats, whereby the area within the quadrat is subdivided into a grid of squares, can be used to provide better estimates of percentage cover or for frequency counts (Fig. 4.22). An advantage of frequency counts is that they provide a single measure of abundance that is applicable to different growth forms. Moore *et al.* (1999) found that it took up to twice as long to survey a set unit area using frequency counts as it did recording percentage cover.

Quadrats have the advantage in that they are particularly suitable for photo-monitoring. Using GIS or image analysis software, much of the analysis can be conducted post-dive. This has the advantage that difficult species can be checked against relevant texts or with specialists in that group. Conversely, the ability to check the surface texture, three-dimensional shape and to move aside shading species is lost (although stereo-photography allows a degree of three-dimensional viewing). For monitoring programmes, a combination of *in situ* counts or percentage cover estimates complemented by photo-monitoring is recommended (e.g. English *et al.*, 1997; Munro, 1999). Comparisons between 0.1 m^2 quadrats and belt transect records suggest that quadrats are much more time-consuming to survey, but are better at recording common but inconspicuous species. Conversely, they tend to miss or under-record larger, low-density species (Moore *et al*., 1999).

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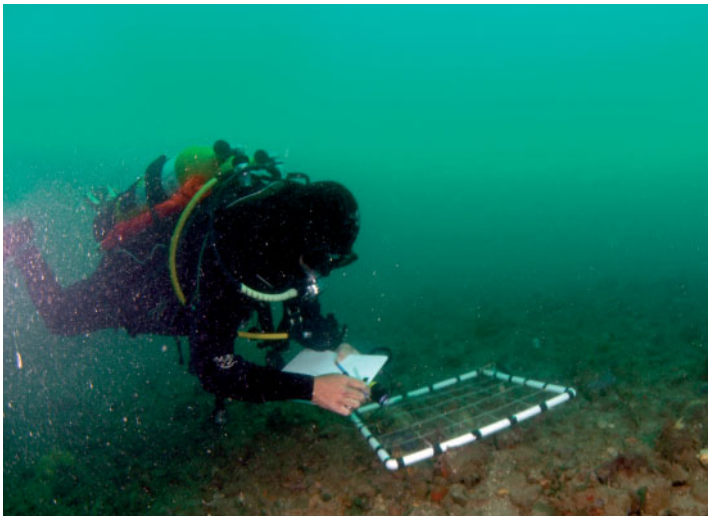

Fig. 4.22 A diver counting individuals within a strung quadrat. © Colin Munro. (For a colour version of this figure, see Plate 4.11.)

Plotless and rapid survey techniques

Several rapid methodologies, and methodologies where no defined survey area is required, have been developed for surveying or assessing benthic habitats. Many of these protocols are country- or region-specific, having been developed specifically for local habitats or conditions, or the requirements of particular organisations. Some of the more widely used methods are described below.

Rapid assessment protocol

The rapid assessment protocol (RAP) is a methodology developed specifically for assessment of degradation of reefs in the Western Atlantic and Gulf or Mexico. The protocol focuses on the principal reef-building corals, algae (macroalgae and encrusting corallines) and *Diadema* urchins (considered the key grazers on reefs in this region). The core method is to estimate the percentage of living, dead, bleached and diseased coral on the reef, by recording along a 10 m transect. Algal cover is estimated by recording percentage cover within a 25 cm × 25 cm quadrat at 2 m intervals along the transect. Canopy height of the algae is also recorded, and used to estimate algal biomass. *Diadema* numbers are estimated by using the laid 10 m line as a 1 m wide belt transect (using a metre rule for guidance) and counting all specimens within that belt. Transects are placed randomly, with a minimum of six per site considered necessary for proper assessment. Fish are recorded by using either a 30 m long, 2 m wide belt transect or a random swim whereby all fish species are recorded and relative abundance estimated. The above is merely an outline of the protocol. Full details of the methodology are given in Steneck

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et al. (2000) and AGGRA methodology, version 3.1 (Atlantic and Gulf Rapid Reef Assessment, 2000).

MNCR methodology

In the United Kingdom, the Joint Nature Conservation Committee's Marine Nature Conservation Review (MNCR) team has developed a standard, semi-quantitative, methodology for surveying both intertidal and subtidal benthic flora and fauna. This is essentially a roving diver method, designed to be used by experienced biologists only. Divers, working in pairs, initially record the substrate, depth and relief of the habitat. They then swim randomly across the seabed, recording species observed and biogenic features (e.g. burrows or mounds in sediment) as they go. Abundances of species and physical features are recorded separately for each habitat the divers pass across. The methodology is based around the SACFOR scale. Using this, species are recorded, either in terms of percentage cover or density (depending on growth form) in six logarithmic steps (designated **S**uperabundant; **A**bundant; **C**ommon; **F**requent; **O**ccasional; **R**are). The actual density or percentage ranges cover assigned to these codes depends on the size range of the species being recorded. For example, large solitary ascidians would most likely fall into the 3–15 cm high category. For such a species a density of 1–9 per 100 m^2 would be classed as Occasional, while species over 15 cm high (such as a gorgonian) occurring at this density would be classed as Frequent. The MNCR SACFOR scale is reproduced in Table 4.1.

This system is not sufficiently quantitative to be used for monitoring purposes. Lack of temporal or spatial boundaries tend to make abundances too subjective. This is exacerbated by limitations of the SACFOR scale, inevitable in any compromise between simplicity and encompassing all species. For example, the lowest abundance category 'Rare' equates to 1–5% cover; for species <1 cm tall; however, on most temperate reef systems many small epilithic species that appear relatively common to the diver will actually cover far less than 1% of the rock surface (e.g. 400 *Dendrodoa grossularia* tunicates per m^2 would occupy approximately 1% of the surface area). However, this methodology does offer considerable advantages. It is suitable for use in all benthic habitats. It requires no pre-survey set-up time and provides detailed species lists and descriptive data that can be used to categorise and compare sites. Survey and recording methods are described in Hiscock (1996).

Fish survey techniques

Although fish survey techniques include many of those described earlier, there are also a number of methods used specifically for recording fish. The particular method selected will, in part, depend on the nature of the fish species being recorded (e.g. schooling and cryptic). The majority of fish survey techniques using divers have been developed for use on coral reefs. This is largely because the generally

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Table 4.1 The MNCR SACFOR scale.

Growth form			Size of individuals/colonies					
% cover	Crust/ meadow	Massive/ turf	<1 cm	1–3 cm	3–15 cm	>15 cm	Density	
>80%	S		S				>1/0.001 m^2 (1 cm × 1 cm)	>10,000/m^2
40–79%	A	S	A	S			1–9/0.001 m^2	1000–9999 m^2
20–39%	C	A	C	A	S		1–9/0.01 m^2 (10 cm × 10 cm)	100–999 m^2
10–19%	F	C	F	C	A	S	1–9/0.1 m^2	10–99 m^2
5–9%	O	F	O	F	C	A	1–9/m^2	
1–5% or density	R	O	R	O	F	C	1–9/10 m^2 (3.16 m × 3.16 m)	
<1% or density		R		R	O	F	1–9/100 m^2 (10 m × 10 m)	
					R	O	1–9/1000 m^2 (31.6 m × 31.6 m)	
						R	<1/1000 m^2	

Use of the MNCR SACFOR abundance scales

The MNCR cover/density scales adopted from 1990 provide a unified system for recording the abundance of marine benthic flora and fauna in biological surveys. The following notes should be read before their use.

(1)	Whenever an attached species covers the substratum and percentage cover can be estimated, that scale should be used in preference to the density scale.
(2)	Use the massive/turf percentage cover scale for all species, excepting those given under crust/meadow.
(3)	Where two or more layers exist, for instance foliose algae overgrowing crustose algae, total percentage cover can be over 100% and abundance grade will reflect this.
(4)	Percentage cover of littoral species, particularly the fucoid algae, must be estimated when the tide is out.
(5)	Use quadrats as reference frames for counting, particularly when density is borderline between two of the scale.
(6)	Some extrapolation of the scales may be necessary to estimate abundance for restricted habitats such as rockpools.
(7)	The species (as listed above) take precedence over their actual size in deciding which scale to use.
(8)	When species (such as those associated with algae, hydroid and bryozoan turf or on rocks and shells) are incidentally collected (i.e. collected with other species that were superficially collected for identification) and no meaningful abundance can be assigned to them, they should be noted as present (P).

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good visibility there allows divers to identify fish at a reasonable distance, plus the habitat complexity makes divers better suited than most remote survey techniques.

The most widely used methods fall into three categories: (i) belt transects, (ii) stationary counts and (iii) random swims. Some of the more commonly used variations of these methods are described here.

Belt transect

As with sessile benthic organisms, belt transect surveys are suited to recording species occurring in low densities or those that are patchily distributed. The method was first described by Brock, 1954, and remains a commonly used technique

(e.g. Craik, 1981; Russ, 1984a, 1984b; Pattengill-Semmens & Semmens, 2003). For recording fish, the belt transect is visualised as a box-section corridor, the width, and 'ceiling' height determined by target species and visibility. Rogers *et al.* (1994) recommend a 2 m wide transect for smaller and cryptic species, and up to 5 m for larger species such as groupers and snappers, with a transect length of between 50 and 100 m. They emphasise that swimming speeds should be constant and standardised in order to ensure good comparison between surveys. A similar protocol for conducting coral reef fish census using belt transects has been developed by the Great Barrier Reef Marine Park Authority (GBRMPA, 1978, 1979). A 5 m wide, 5 m high and 50 m long corridor within which all target species are recorded has been adopted for the Long-Term Monitoring of the Great Barrier Reef (LTMGBR) by the AIMS, with a 1 m wide transect being used to record Pomacentrids. Both methods are fully described in English *et al*. (1997) and are also described in the AIMS Standard Operating Procedure No. 3 (Halford & Thompson, 1994) available online on the AIMS website.

In temperate water, fish species diversity tends to be much lower than on coral reefs, but underwater visibility tends to be much lower also. In shallow, rocky areas the presence of kelp stands adds to the difficulty of conducting visual censuses. On complex rocky habitats off West Scotland, Sayer and Thurston (unpublished data) used 10 m long and 2 m wide transects to survey active fish. A standardised speed along the transect of 2 m min^{-1} was maintained by the divers. Comparing results with other techniques, and with activity profiles for fish over 24-hour periods gained from underwater television, they concluded that diver surveys do not yield absolute numbers for fish present. However, relative comparisons between years and seasons can be made if a standardised technique is adopted.

Video belt transects are increasingly being used to replace or complement diver observation belt transects (e.g. Parker *et al*., 1994; Sinclair Knight Merz, 1999; Booth & Beretta, 2002; Thrush *et al*., 2002). Video offers the obvious benefits that footage can be replayed and played at slow speed. This means that identification does not need to be made instantaneously, and estimates of numbers can be rechecked.

Random swim survey protocols

The basis of these methods is that a diver, or divers, swim(s) over the survey site in randomly chosen directions, either for a set period of time or within a given area. Several variations on this principle have been developed by different workers. Widely used in the Western Atlantic and Caribbean, the Random Swim Technique (also known as the Rapid Visual technique) uses survey duration as the limiting parameter. The method does not record information on species density, only the number of species recorded during the dive. Once a species is recorded, it is then ignored and the surveyor actively looks for unrecorded species. The survey duration is subdivided into discrete units: Rogers *et al*. (1994) recommend a 50-minute dive

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divided into five 10-minute intervals. A measure of relative abundance is gained by scoring species according to the time interval in which they are first recorded (e.g. species recorded between 0–10 minutes will score 5, species recorded between 41–50 minutes will score 1). This method has the advantage that it can be applied over reefs where the relief would make deployment of a transect difficult. A disadvantage is that it provides little quantitative data. It is considered to provide better data on total species richness than either stationary or belt transect techniques. Variations of this technique are described in Jones and Thompson (1978), Kimmel (1985) and Rogers *et al.* (1994).

The Roving Diver Technique is a protocol widely used for collecting data on coral reef fish using volunteer divers (e.g. Jeffrey *et al*., 2001). The method involves divers swimming around within 100 m radius of the dive start point. All fish species observed are logged and abundance assessed on a four-category logarithmic scale (1; 2–10; 11–100; >100). Comparing roving diver and transect survey methods, Schmitt *et al.* (2002) found that a higher number of rare species was recorded with the roving diver method, but that both methods provided a more complete overall species assessment than either method was able to provide in isolation. Detailed methodology for the roving diver technique, and a comparison with the stationary survey method, are given in Bohnsack (1996).

Stationary technique

This method, developed by Bohnsack and Bannerot (1986), was designed to provide standardised quantitative data on relative abundance and frequency of occurrence of all fish species recorded at a site. A brief description of the method is given below, with full details provided in Rogers *et al.* (1994). Sites are randomly selected and pertinent features such as depth, structural complexity, substratum and underwater visibility recorded. A 15 m line (ideally at tape measure) is then laid taut on the seabed. This forms the diameter of a visualised cylinder, extending from the seabed to the surface. The surveyor moves to the mid-point of the line and begins to scan the water within the imaginary cylinder. During the initial five minutes of the survey, only species presence is recorded. It is assumed that by the end of this period, most of the species that will be recorded during the survey will have already been logged. The remaining survey time is spent working through the species list generated, recording numbers of each species and estimating their length. The total survey time should not exceed 15 minutes. A variation of this method proposed by Kimmel (1993) records all species during the entire 15-minute period (as opposed to only those noted in the first five minutes). The argument for this is that some cryptic and sedentary species will adapt to the diver's presence over time, and thus may only be observed in the later stages of the survey. The stationary technique relies on divers being able to identify species quickly. While it reduces bias due to disturbance from moving divers, cryptic species will tend to be under-recorded. It also has limited use in poor visibility.

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References

Abed-Navandi, D. (2000) Thalassinideans new to the fauna of Bermuda and the Cape Verde Islands. *Annalen des Naturhistorischen Museums in Wien*, **102B**, 291–299.

Abed-Navandi, D. & Dworschak, P.C. (1997) First record of the thalassinid *Callianassa truncata*, Giard & Bonnier, 1890 in the Adriatic Sea (Crustacea: Decapoda:Callianassidae). *Annalen des Naturhistorischen Museums in Wien*, **99B**, 565–570.

Angel, H.H. & Angel, M.V. (1967) Distribution pattern analysis of a benthic community. *Helgoländer Wissenshaftliche Meeresuntersuchungen*, **29**, 439–459.

Atkinson, R.J.A. & Chapman, C.J. (1984) Resin casting: a technique for investigating burrows in sublittoral sediments. *Progress in Underwater Science*, **9**, 15–25.

Atkinson, R.J.A. & Nash, R.D.M. (1985) Burrows and their inhabitants. *Progress in Underwater Science*, **10**, 109–115.

Atkinson, R.J.A. & Nash, R.D.M. (1990) Some preliminary observations on the burrows of *Callianassa subterranea* (Montagu) (Decapoda: Thalassinidea) from the west coast of Scotland. *Journal of Natural History*, **24**, 403–413.

Atlantic and Gulf Rapid Reef Assessment (2000) AGGRA methodology, version 3.1. Atlantic and Gulf Rapid Reef Assessment, MGG-RSMAS, University of Miami, FL. www.agrra.org/method/methodhome.html (accessed 18 November 2012).

Australian Institute of Marine Science (AIMS) (2008) Program design and sampling methods: reef monitoring. http://www.aims.gov.au/docs/research/climate-change/position-paper.html (accessed 15 November 2012).

Baddeley, A.D., Defigueredo, J.W., Curtis, J.W.M. & Williams, A.N. (1968) Nitrogen narcosis and performance underwater. *Ergonomics*, **11**, 157–164.

Barnett, P.R.O. & Hardy, B.L.S. (1967) A diver operated quantitative sampler for sand macrofaunas. *Helgolander Wissenschaftliche Meeresuntersuchungen*, **15**, 390–398.

Behnke, A.R., Thomson, R.M. & Motley, E.P. (1935) The psychological effects from breathing air at 4 atmospheres pressure. *American Journal of Physiology*, **112**, 554–558.

Bell, J.J., Burton, M., Bullimore, B., Newman, P. & Lock, K. (2006) Morphological monitoring of subtidal sponge assemblages. *Marine Ecology in Progress Series*, **311**, 205–212.

Bennett, P.B., Coggin, R. & Roby, J. (1981) Control of HPNS in humans during rapid compression with trimix to 650 m (2131 ft). *Undersea Biomedical Research*, **8** (2), 85–100.

Bohnsack, J.A. (1979) Photographic quantitative sampling of hard-bottom benthic communities. *Bulletin of Marine Science*, **29** (2), 242–252.

Bohnsack, J.A. (ed.) (1996) Two visually based methods for monitoring coral reef fishes. In: *A Coral Reef Symposium on Practical, Reliable, Low Cost Monitoring Methods for Assessing the Biota and Habitat Conditions of Coral Reefs* (eds M.P. Crosby, G.R. Gibson & K.W. Potts), 26–27 January 1995. Office of Ocean and Coastal Resource Management, NOAA, Silver Spring, MD.

Bohnsack, J.A. & Bannerot, S.P. (1986) A stationary visual census technique for quantitatively assessing community structure of coral reef fishes. NOAA NMFS Technical Report 41, Department of Commerce.

Booth, D.J. & Beretta, G.A. (2002) Changes in a fish assemblage after a coral bleaching event. *Marine Ecology in Progress Series*, **245**, 79–91.

https://telegram.me/Geologybooks

Bradbury, R.H., Loya, Y., Reichelt, R.E. & Williams, W.T. (1986) Patterns in the structural topology of benthic communities on two corals reefs of the central Great Barrier Reef. *Coral Reefs*, **4**, 161–167.

Brock, V.E. (1954) A preliminary report on a method of estimating reef fish populations. *Journal of Wildlife Management*, **18**, 297–308.

Bullimore, B. (1983) Skomer Marine Nature Reserve subtidal monitoring project: development and implementation of low-budget stereo-photographic monitoring of rocky subtidal epibenthic communities. Unpublished Report to the Nature Conservancy Council, Peterborough.

Bullimore, B. (1986) Skomer Marine Reserve subtidal monitoring project: photographic monitoring of subtidal epibenthic communities, August 1984–November 1985. SMRSMP Report No. 5. Report to the Nature Conservancy Council.

Bullimore, B. (1987) Photographic monitoring of subtidal epibenthic communities on Skomer Marine Reserve, 1986. SMRSMP Report No 6. Report to the Nature Conservancy Council.

Bullimore, B. & Hiscock, K. (2001) Procedural guideline No. 3-12. Quantitative surveillance of sublittoral rock biotopes and species using photographs. In: *Marine Monitoring Handbook* (eds J. Davies, J. Baxter, M. Bradley, D. Connor, J. Khan, E. Murray, W. Sanderson, C. Turnbull & M. Vincent). Joint Nature Conservation Committee, London.

Bunker, F. (1985) A brief review of the ecology of the sea fan *Eunicella verrucosa* and a survey of the Skomer population in 1985. Unpublished Report to the Nature Conservancy Council, Peterborough.

Burton, M., Lock, K., Newman, P. & Gibbs, R. (2011) Skomer Marine Nature Reserve project status report 2010. CCW Regional Report, CCW/WW/10/08, Countryside Council for Wales.

Chapman, C., Johnstone, A., Dunn, J. & Creasey, D. (1974) Reactions of fish to sound generated by diver's open-circuit underwater breathing apparatus. *Marine Biology*, **27**, 357–366.

Chong, A.K. & Stratford, P. (2002) Underwater digital stereo-observations technique for red hydrocoral study. *Photogrammetric Engineering and Remote Sensing*, **68**, 745–751.

Coelho, V.R., Cooper, R.A. & Rodrigues, A.S. (2000) Burrow morphology and behaviour of the mud shrimp *Upogebia omissa* (Decapoda: Thalassinidea: Upogebiidae). *Marine Ecology Progress Series*, **200**, 229–240.

Cole, R.G., Syms, C., Davey, N.K., Gust, N., Notman, P., Stewart, R., Radford, C.A., Carbines, C., Carr, M.H. & Jeffs, A.J. (2007) Does breathing apparatus affect fish counts and observations? A comparison at three New Zealand fished and protected areas. *Marine Biology*, **150** (6), 1379–1395.

Craik, G.J.S. (1981) Underwater survey of coral trout *Plectropomus leopardus* (Serranidae) populations in the Capricornia Section of the Great Barrier Reef Park. *Proceedings of the Fourth International Coral Reef Symposium*, **1**, 53–58.

CRC Reef Research Centre (2001) *Crown of Thorns starfish on the Great Barrier Reef. Current State of Knowledge, April 2001*. Cooperative Research Centre of the Great Barrier Reef World Heritage Area, James Cook University, Townsville.

Dinsmore, D.A. (1989a) The operational advantages of enriched air nitrox versus air for research diving. *Marine Technology Society Journal*, **23** (4), 42–46.

https://telegram.me/Geologybooks

Dinsmore, D.A. (1989b). NURC/UNCW nitrox diving program. In: *Workshop on Enriched Air Nitrox Diving*, *National Undersea Research Program Research Report*, Vol. 89, Issue 1 (NURC/UNCW R-89-20) (eds R.W. Hamilton, D. Crosson & A.W. Hulbert), pp. 75–84.

Du Plat Taylor, J. (ed.) (1965) *Marine Archaeology*. Hutchinson, London.

Dworschak, P.C. & Jorg, A.O. (1993) Decapod burrows in mangrove-channel and back-reef environments at the Atlantic Barrier reef, Belize. *Ichnos*, **2**, 277–290.

Earle, J.L. & Pyle, R.L. (1997) *Hoplolatilus pohlei*, a new species of sand tilefish (Perciformes: Malacanthidae) from the deep reefs of the D'Entrecasteaux Islands, Papua New Guinea. *Copeia*, **2**, 383–387.

Edge, M. (2010) *The Underwater Photographer*, 4th edn. Focal Press, Oxford.

English, S., Wilkinson, C. & Baker, V. (1997) *Survey Manual for Tropical Marine Resources*, 2nd edn. Australian Institute of Marine Science, Townsville.

Fernandes, L., Moran, P.J. & Marsh, H. (1993) A system for classifying outbreaks of crown-of-thorns starfish as a basis for management. Abstract. *Proceedings of the Seventh International Coral Reef Symposium, Guam*, **2**, 803–804.

Fowler, B., Ackles, K.N. & Porlier, G. (1985) Effects of inert gas narcosis on behaviour – a critical review. *Undersea Biomedical Research*, **12**, 369–402.

Gage, J.D. (1975) A comparison of the deep sea epibenthic sledge and anchor-box dredge samplers with the van Veen grab and hand coring by divers. *Deep-Sea Research*, **22**, 693–702.

Gamble, J.C. (1984) Diving. In: *Methods for the Study of Marine Benthos* (eds N.A. Holme & A.D. McIntyre), 2nd edn, Blackwell Publishing Ltd., Oxford, pp 99–139.

GBRMPA (1978) Workshop on reef fish assessment and monitoring. GBRMPA Workshop Series No. 2, Great Barrier Reef Marine Park Authority, Townsville.

GBRMPA (1979) Workshop on coral trout assessment techniques. GBRMPA Workshop Series No. 3, Great Barrier Reef Marine Park Authority, Townsville.

Gill, A.C., Pyle, R.L. & Earle, J.L. (1996) *Pseudochromis ephippiatus*, new species of dottyback from southeastern Paupa New Guinea (Teleostei: Perciformes: Pseudochromidae). *Revue Francais Aquariologie*, **23** (4), 97–100.

Godden, D. & Baddeley, A.D. (1975) Context dependent memory in two natural environments on land and underwater. *British Journal of Psychology*, **66**, 325–331.

Green, N. (1980) *Underwater Stereophotography Applied in Ecological Monitoring. Report 1: Methods and Preliminary Evaluation*. Norwegian Institute for Water Research, Oslo.

Grist, N. (2000) A review of aspects of the use of electronic methods for the analysis of monitoring photographs. Unpublished Report by Unicomarine Ltd. to English Nature (Devon, Cornwall & Isles of Scilly), Truro.

Halford, A.R. & Thompson, A.A. (1994) *Visual Census Surveys of Reef Fish. Standard Operational Procedure Number 3. Long-Term Monitoring of the Great Barrier Reef*, Australian Institute of Marine Science, Townsville, 22 pp.

Hiscock, K. (1984) Sublittoral monitoring at Lundy: July 28th to August 4th, 1984. Unpublished Report by the Field Studies Council Oil Pollution Research Unit to the Nature Conservancy Council, Peterborough.

Hiscock, K. (1986) Marine biological monitoring at Lundy: March 26th to 29th, July 26th to August 2nd. Unpublished Report by the Field Studies Council Oil Pollution Research Unit to the Nature Conservancy Council, Peterborough.

https://telegram.me/Geologybooks

Hiscock, K (ed.) (1996) Marine nature conservation review: rationale and methods. Unpublished Report by the Joint Nature Conservation Committee, Peterborough.

Hiscock, K. & Hoare, R. (1973) The ecology of sublittoral communities at Abereiddy Quarry, Pembrokeshire. *Journal of the Marine Biological Association of the United Kingdom,* **55**, 833–864.

Holt, R.H.F. (2001) Monitoring and technological developments in scientific scuba diving. In: *The Establishment of A Programme of Surveillance and Monitoring for Judging the Condition of the Features of Pen Llyn a'r Sarnau cSAC: Progress to March 2001*, Vol. 1, Countryside Council for Wales Contract Science Report, No. 380 (eds W.G. Sanderson, R.H.F. Holt & L. Kay). Countryside Council for Wales.

Holt, R.H.F. & Sanderson, B. (2001) Proceedural Guideline No. 6-2. Relocation of intertidal and subtidal sites. In: *The Establishment of a Programme of Surveillance and Monitoring for Judging the Condition of the Features of Pen Llyn a'r Sarnau cSAC: Progress to March 2001*, Vol. 1, Countryside Council for Wales Contract Science Report, 380 (eds W.G. Sanderson, R.H.F. Holt & L. Kay). Countryside Council for Wales.

Hunger, W.L., Jr. & Bennett, P.B. (1974) The causes, mechanisms and prevention of the high pressure nervous syndrome. *Undersea Biomedical Research*, **1** (1), 1–28.

Jeffrey, C.F.G., Patten-Semmens, C., Gittings, S. & Monaco, M.E. (2001) *Distribution and sighting frequency of reef fishes in the Florida Keys National Marine Sanctuary*. Marine Sanctuaries Conservation Series MSD-01-1, US Department of Commerce, National Oceanic and Atmospheric Administration, Marine Sanctuaries Division, Silver Spring, MD, 51 pp.

Joiner, J.T. (ed.) (2001) *NOAA Diving Manual: Diving for Science and Technology*, 4th edn. Best Publishing Company, Flagstaff, AZ.

Jones, R.S. & Thompson, M.J. (1978) Comparison of Florida reef fish assesmblages using a rapid visual technique. *Bulletin of Marine Science*, **28**, 159–172.

Kenchington, R.A. & Morton, B. (1976) *Two Surveys of the Crown of Thorns Starfish over a Section of the Great Barrier Reef*. Australian Government Public Service, Canberra.

Kimmel, J.J. (1985) A new species-time method for visual assessment of fishes and its comparison with established methods. *Environmental Biology of Fishes*, **12**, 23–32.

Kimmel, J.J. (1993) Suggested modifications to diver-oriented point counts for fishes. *Proceedings of the 73rd Annual Meeting*, American Society of Ichthyologists and Herpetologists, Austin, TX.

Liddell, W.D. & Ohlhorst, S.L. (1987) Patterns of reef community structure, North Jamaica. *Bulletin of Marine Science*, **40**, 311–329.

Lincoln, R.J. & Sheals, J.G. (1979) *Invertebrate Animals – Collection and Preservation*. British Museum (Natural History) and Cambridge University Press.

Lobel, P.S. (2001) Fish bioacoustics and behavior: passive acoustic detection and the application of a closed-circuit rebreather for field study. *Marine Technology Society Journal*, **35**, 19–28.

Lundälv, T. (1971) Quantitative studies on rocky bottom biocoenoses by underwater photogrammetry. A methodical study. *Thalassia Jugoslavica*, **7** (1), 201–208.

Lundälv, T. (1985) Detection of long-term trends in rocky sublittoral communities: representativeness of fixed sites. In: *The Ecology of Rocky Coasts* (ed. P.G. Moore), Hodder and Stoughton, London, pp 329–345.

https://telegram.me/Geologybooks

Main, J. & Sangster, G.I. (1978) A new method for observing fishing gear using a towed wet submersible. *Progress in Underwater Science*, **3**, 259–267.

Marsh, L.M., Bradbury, R.H. & Reichelt, R.E. (1984) Determination of the physical parameters of coral distributions using line transect intersect data. *Coral Reefs*, **2**, 175–180.

Meier, O.W. & Porter, J.W. (1992) Methods comparison for monitoring Floridian reef coral populations. *American Zoologist*, **32**, 625–640.

Miller, S.L. & Cooper, C. (2000) The *Aquarius* underwater laboratory: America's "inner space" station. *Marine Technology Society Journal*, **34** (4), 69–74.

Moore, J., James, B. & Gilliland, P. (1999) Development of a monitoring programme and methods in Plymouth Sound cSAC: application of diver and ROV techniques. Unpublished Report by Cordah Limited to English Nature, Peterborough.

Moore, J. (2000) Development of a monitoring programme and methods in Plymouth Sound cSAC: application of diver techniques – 1999. Unpublished Report by Cordah Limited to English Nature, Peterborough.

Munro, C.D. (1992) An investigation into the effects of scallop dredging in Lyme Bay. Unpublished Report to the Devon Wildlife Trust, Exeter.

Munro, C.D. (1996) Lyme Bay potting impacts study. In: *A Study on the Effects of fish (Crustacea/ Mollusc) Traps on Benthic Habitats and Species* (eds N.C. Eno & D. MacDonald). Final Report to the European Commission Directorate General XIV Fisheries. Study Contract No. 94/076.

Munro, C.D. (1998) Lundy Marine Nature Reserve subtidal monitoring 3–6 May 1998. Unpublished Report by Marine Biological Surveys Consultancy to English Nature. ENRR No. 294. Peterborough.

Munro, C.D. (1999) Monitoring of the rocky sub-littoral of Milford Haven. May-July 1998. Unpublished Report by Marine Biological Surveys Consultancy to Milford Haven Waterway Environmental Monitoring Steering Group, Milford Haven.

Munro, C.D. (2000) Lundy Marine Nature Reserve subtidal monitoring 25–27 September 1999. Unpublished Report by Marine Biological Surveys Consultancy to English Nature, Peterborough.

Munro, C.D. & Munro, A. (2002) East Tennants Reef Seafan Study: Interim Report, September 2002. Reef Research Report 9/2002 ETR 06. Reef Research, Crediton, UK. http://www.marine-bio-images.com/RR_Eunicella_PDFS/RR_ReportETR 06Sep2002pdf.pdf (accessed 3 December 2012).

Munro, C.D. & Baldock, B.M. (2012) Lyme Bay closed area: measuring recovery of benthic species in cobble reef habitats – analysis of data collected by SCUBA divers September 2008, August 2009 and July 2010. A Marine Bio-images Report to DEFRA and Natural England. Marine Bio-images, Exeter.

Munro, L. (2001) East Tennants Reef Seafan Study – Report to Project AWARE (UK), August 2001. Reef Research Report 8/2001 ETR 03. Reef Research, Crediton, UK. http://www.marine-bio-images.com/RR_Eunicella_PDFS/RR_ReportETR 03Aug2001pdf.pdf (accessed 3 December 2012).

Nickell, L.A. & Atkinson, R.J.A. (1995) Functional morphology of burrows and trophic modes of three thalassinidean shrimp species, and a new approach to the classification of thalassinidean burrow morphology. *Marine Ecology Progress Series*, **128**, 181–197.

Nickell, L.A., Atkinson, R.J.A., Hughes, D.J., Ansell, A.D. & Smith, C.J. (1995a) Burrow morphology of the echiuran worm *Maxmuelleria lankesteri* (Echiura: Bonelliidae), and

https://telegram.me/Geologybooks

a brief review of burrow structure and related ecology of the Echiura. *Journal of Natural History*, **29**, 871–885.

Osbourne, K. & Oxley, W.G. (1997) Sampling benthic communities using video transects. In: *Survey Manual for Tropical Marine Resources* (eds S. English, C. Wilkinson & V. Baker), 2nd edn. Australian Institute of Marine Science. Townsville.

Parker, R.O. Jr., Chester, A.J. & Nelson, R.S. (1994) A video transect method for estimating reef fish abundance, composition and habitat utilisation at Gray's Reef National Marine Sanctuary, Georgia. *Fishery Bulletin*, **92**, 787–799.

Parrish, F.A. & Pyle, R.L. (2002) Field comparison of open-circuit Scuba to closed-circuit rebreathers mixed-gas diving operations. *Marine Technology Society Journal,* **36** (2). 82–91.

Pattengill-Semmens, C.V. & Semmens, B.X. (2003) Status of coral reefs of Little Cayman and Grand Cayman, British West Indies, in 1999 (part 2: fishes). In: *Status of the Coral Reefs of the Western Atlantic: Results of the Initial Survey, Atlantic and Gulf Rapid Reef Assessment (AGRRA) Program*, Atoll Research Bulletin, Vol. 496 (ed. J.C. Lang), Smithsonian Institution Press, Washington, DC, pp 204–226.

Pence, D. & Pyle, R.L. (2002) University of Hawaii dive team completes Fiji deep reef fish surveys using mixed-gas rebreathers. *The Slate*, 1–3 April.

Pinca, S., Beger, M., Peterson, E., Richards, Z., Reeves, E. (2002) Coral reef biodiversity community-based assessment and conservation planning in the Marshall Islands: baseline surveys, capacity building and natural protection and management of coral reefs of the atoll of Rongelap. RALgov public report. http://www.nras-conservation.org/nraslibrary/ReportRong2002full.pdf (accessed 3 December 2012).

Poulin, M. (2001) Marine biodiversity monitoring – protocol for monitoring sea-ice algae. A Report by the Marine Biodiversity Monitoring Committee (Atlantic Maritime Ecological Science Cooperative, Huntsman Marine Science Centre) to the Ecological Monitoring and Assessment Network of Environment Canada. http://ec.gc.ca/Publications/26B2176E-E90F-4A50-93B2-A5DDC7FF622D%5CMonitoringProtocolSeaIceAlgae.pdf (accessed 3 December 2012).

Pyle, R.L. (1992) The peppermint angelfish *Centropyge boylei*, n.sp. Pyle and Randall. *Freshwater and Maine Aquarium*, **15** (7), 16–18.

Pyle, R.L. (1996) How much coral reef biodiversity are we missing? *Global Biodiversity*, **6** (1), 3–7.

Pyle, R.L. (1998) Use of advanced mixed-gas diving technology to explore the coral reef "Twilight Zone". In: *Ocean Pulse: A Critical Diagnosis* (eds J.T. Tanacredi, & Loret, J.), Plenum Press, New York, pp 71–88.

Pyle, R.L. (1999) Mixed-gas, closed-circuit rebreather use for identification of new reef fish species from 200-500 fsw. In: *Assessment and Feasibility of Technical Diving Operations for Scientific Exploration* (eds R.W. Hamilton, D.F. Pence & D.E. Kesling), American Academy of Underwater Sciences, Nahant, MA, pp 53–65.

Pyle, R.L. (2000) Assessing undiscovered fish biodiversity on deep coral reefs using advanced self-contained diving technology. *Marine Technology Society Journal,* **34** (4), 82–91.

Pyle, R.L. & Randall, J.E. (1992) A new species of *Centropyge* (Perciformes: Pomacanthidae) from the Cook Islands, with a redescription of *C. boylei*. *Revue Francais Aquariologie*, **19** (4), 115–124.

https://telegram.me/Geologybooks

Radford, C.A., Jeffs, A.G., Tindle, C.T., Cole, R.G. & Montgomery, J.C. (2005) Bubbled waters: the noise generated by underwater breathing apparatus. *Marine and Freshwater Behaviour and Physiology*, **38** (5), 259–267.

Randall, J.E. & Pyle, R.L. (2001a). Three new species of labrid fishes of the genus *Cirrhilabrus* from islands of the tropical Pacific. *Aqua*, **4** (3), 89–98.

Randall, J.E. & Pyle, R.L. (2001b). Four new serranid fishes of the anthiine genus *Pseudoanthias* from the South Pacific. *Raffles Bulletin of Zoology*, **49** (1), 19–34.

Riley, J.D. & Holford, B.H. (1965) A sublittoral survey of Port Erin bay, particularly as an environment for young plaice. *Report of the Marine Biological Station of Port Erin*, **77**, 49–53.

Robinson, M. & Tully, O. (2000) Seasonal variation in community structure and recruitment of benthic decapods in a sub-tidal cobble habitat. *Marine Ecology Progress Series*, **206**, 181–191.

Rogers, C.S. & Miller, J. (2001) Coral bleaching, hurricane damage, and benthic cover on coral reefs in St. John, US Virgin Island: a comparison of surveys with the chain transect method and videography. *Bulletin of Marine Science*, **69**, 459–470.

Rogers, C.S., Garrison, R. & Grober-Dunsmore, R. (1997) A fishy story about hurricanes and herbivory: seven years of research on a reef in St. John, US Virgin Island. *Proceedings of the 8th International Coral Reef Symposium*, **1**, 555–560.

Rogers, C.S., Miller, J. & Waara, R.J. (2000) Tracking changes on a reef in the US Virgin Islands with videography and SONAR: a new approach. In: *Proceedings of the Ninth International Coral Reef Symposium*, Bali. 23–27 October 2000, Vol. 2 (eds M.K. Moosa, S. Soemodihardjo, A. Soegiarto, K. Romimohtarto, A. Nontji, Soekarno & Suharsono), pp. 1065–1070. http://www.reefbase.org/resource_center/publication/pub_14902.aspx (accessed 22 November 2012).

Rogers, C.S., Garrison, G., Grober, R., Hillis, Z.-M. & Franke, M.A. (1994) *Coral Reef Monitoring Manual for the Caribbean and Western Atlantic.* National Park Service, Virgin Islands National Park. St. Thomas, Virgin Islands. http://www.reefbase.org/resource_center/publication/pub_14902.aspx (accessed 12 December 2012).

Rostron, D.M. (2001) Procedural guideline no. 3-10: sampling marine benthos using suction samplers. In: *Marine Monitoring Handbook* (eds J. Davies (senior ed.), J. Baxter, M. Bradley, D. Connor, J. Khan, E. Murray, W. Sanderson, C. Turnbull & M. Vincent). Joint Nature Conservation Committee, Peterborough.

Russ, G.R. (1984a) Distribution and abundance of herbivorous grazing fishes in the Central Great Barrier Reef. I: levels of variability across the entire continental shelf. *Marine Ecology Progress Series*, **20**, 23–34.

Russ, G.R. (1984b) Distribution and abundance of herbivorous grazing fishes in the Central Great Barrier Reef. II: Patterns of zonation of mid-shelf and outer shelf reefs. *Marine Ecology Progress Series*, **20**, 35–44.

Sayer, M.D.J., Thurston, S.R., Nickell, L.A., Duncan, G.D. & Gale, A.V. (1996) Use of rebreathers in fish research. In: *Underwater Research and Discovery (Proceedings of the 3rd Underwater Science Symposium)*, Society for Underwater Technology, Aberdeen, pp 35–38.

Schinn, E.A. (1968) Burrowing in recent lime sediments of Florida and the Bahamas. *Journal of Palaeontology*, **42**, 879–894.

https://telegram.me/Geologybooks

Schmitt, E.F., Sluka, R.D. & Sullivan-Sealey, K.M. (2002) Evaluating the use of roving diver and transect surveys to asses the coral reef fish assemblage off southeastern Hispanola. *Coral Reefs*, **21**, 216–223.

Simenstad, C.A., Tanner, C.D., Thom, R.M. & Conquest, L.L. (1991) Estuarine Habitat Assessment Protocol. Puget Sound Estuary Program. Environmental Protection Agency Report 910/9-91-037, 201 pp.

Sinclair Knight Merz (1999) Video transect analysis of subtidal habitats in the Dampier Archipelago. Unpublished Report to the Western Australian Museum of Natural Science.

Squires, H.J., Ennis, G.P. & Dawe, G. (1999) On biology of the shrimp *Eualu pusiolus* (Kroyer, 1841) (Crustacea, Decapoda) at St. Chad's, Newfoundland. *NAFO Scientific Council Studies*, **33**, 1–10.

Stanton, G. (1991) The future of special gas mixtures in scientific diving at Florida State University. *Progress in Underwater Science*, **16**, 123–137.

Steneck, R.S., Ginsburg, R.N., Kramer, P., Lang, J. & Sale, P. (2000) Atlantic and Gulf Rapid Reef Assessment (AGRRA): a species and spatially explicit reef assessment protocol. *9th International Coral Reef Symposium*, Bali.

Thrush, S.F., Schultz, D., Hewitt, J.E. & Talley, D. (2002) Habitat structure in soft-sediment environments and abundance of juvenile snapper *Parus auratus*. *Marine Ecology Progress Series*, **245**, 273–280.

Whittington, M.W., Holt, R., Irving, R.A., Northen, K.O. & Stanwell-Smith, D. (2006) *Across-Wales Diving Monitoring Project Volume 2: Standard Operating Procedures.* Research Report 25b. Countryside Council for Wales.

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Chapter 5
Macrofauna Techniques

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Abstract

Recent concerns about the declining state of the sea, the depletion of marine resources, the impact of climatic changes and pollution have highlighted the demand for long-term and effective monitoring of the ecosystem in which marine benthos is a key component. For the study of the benthic macrofauna a range of commonly used sampling equipment such as grabs, corers, trawls, box corers and dredges are available and their technical features are reported in detail. The performance and efficiency of these samplers are discussed, enabling the user to select the sampler most suitable for the purpose of the survey. Descriptions of treatment and further processing of samples are included in this operational guide of macrofauna sampling. Information on storage of the data obtained for analysis and further use is also provided. The very extensive bibliography can guide the user to seek further information on the subject.

Keywords macrofauna, sampling equipment, methods and techniques, performance, efficiency, comparison of samplers, processing of samples, data storage

5.1 Littoral observation and collection

The study of the intertidal fauna and flora is in some ways easier than that of subtidal areas, but since the habitat is subjected to both aerial and aquatic climates, environmental factors influencing distribution and abundance are more complex.

When visiting the shore it should be remembered that low tide is a quiescent period for many animals that are active only when covered by the sea. Also, certain predators invade the shore at different periods of the tidal cycle: fish and crustaceans at high tide, and humans, birds, insects and, occasionally rats or cattle, at low tide.

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On sandy or muddy shores estimates of the standing crop of macrofauna and flora are made by driving a square sheet metal frame of appropriate area (e.g. 0.1 m^2 or 0.25 m^2) into the substratum (Bakus, 1990), the sediment within the frame being excavated to the desired depth. Plastic or metal tubes, which remove an undisturbed core of sediment (Desprez & Ducrotoy, 1987; Sylvand, 1995), are also widely used: a large version (0.1 m^2) that is slid into the sediment to remove a sample to a depth of 10 cm has been successfully employed on some beaches (Grange & Anderson, 1976).

Preliminary excavations should be made to find a suitable sampling depth. On some shores the majority of species and individuals occurs in the top 15 cm, in others it may be necessary to excavate to >30 cm, and even deeper for certain crustaceans and bivalves.

A plentiful supply of water is required for sieving; sometimes, a nearby stream may be utilised, but it should be noted that fresh water may damage the more delicate organisms. If there is no stream in the vicinity and the low-water mark is quite far away, it may be possible to arrange sampling at a time when the water's edge is fairly close to the sampling position. Alternatively, a hole excavated adjacent to the sampling position down to the level of the water table could supply the water required for sieving the samples. Subsequent sorting is made easier if all traces of fine material can be washed out of the sample when sieving.

Types of sieve mesh are discussed on pp. 222–223. On muddy or fine sandy shores it may be possible to use an aperture as small as 0.5 mm, but on coarse sand or gravel shores a coarser mesh of 1.0 or 1.4 mm may be necessary. The selected mesh size (0.5 mm) is indicative for the majority of benthic organisms. However, when sampling for benthic larvae or juveniles, it might be necessary to use a 0.25 mm or even a 0.125 mm mesh. Since most small individuals are found in the top 5–10 cm, it may be possible to use a fine sieve for the surface layers, passing the deeper layers through a coarser mesh (Pamatmat, 1968). However, in mixed sediments consisting of a range of different grades, it might be useful to use more than one mesh size in order to assess the loss of the smaller organisms. Such a procedure could be carried out mainly in the laboratory on unsieved samples. In this way much unnecessary labour may be avoided. Organisms that pass through the smallest mesh employed can be collected by taking smaller volumes of deposit with a Perspex or glass coring tube, and adopting the methods described for meiofauna in Chapter 6 where finer mesh sizes are used. The sieved material is preserved and further processed in the laboratory as described in Section 5.6.

At high tide, fish and crustaceans invade the intertidal zone. These may be sampled qualitatively by shrimping net, beam trawl (Edwards & Steele, 1968) or by a small sledge such as that described by Pullen *et al*. (1968). A small push-net operated by a single person (Riley & Corlett, 1965) may be used in shallow water, either just below low-tide mark, or on the flooded beach. It consists of a light metal frame construction seating on two skids with a net of 5.0–10.00 mm knot-to-knot mesh leading to a cod-end of finer mesh. The footrope is weighted down

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by lead weights and in front of the mouth a number of tickler chains are attached to the frame skids to help to stir up the organisms in the sediment. If operated at a standard speed and for a fixed time it can produce comparative data on small active animals such as shrimps or juvenile flatfish. Some small sand-burrowing crustaceans swim freely in the overlying water at high tide, returning to their zones in the sand as the tide retreats (Watkin, 1941). These animals can be sampled by nets such as those described by Colman and Segrove (1955), Macer (1967) and Eleftheriou (1979).

A relatively novel technique has been used for sampling the fauna of the surf zone by means of a motorised amphibious large tripod moving along the sandy beach and supporting a sampling platform 11 m above the water line. This samples the fauna and the epifauna down to 7 m depth by means of grab and beam trawl (Janssen *et al.*, 2008).

It is usual to survey an intertidal flat by means of traverses running from high- to low-water mark, with sampling stations sited at regular intervals and representing different tidal levels. Where possible, two or more samples should be taken at each station, as a measure of the variability of the populations. For intensive and repetitive investigations the shore can be divided by intersecting transects to provide samples at regularly spaced intervals. The belt transect method involves a contiguous area along a line transect. This may be considered as an expansion of the line transect or as a continuous line of quadrats. Because the surface of an intertidal flat is often more or less uniform in appearance, the question of possible bias in selecting the position of the traverses and of individual stations does not usually arise, although the presence of irregularities on the beach should be carefully observed, since these are often associated with turbulent conditions at particular tidal levels harbouring a different fauna. On smaller areas, such as pocket beaches, the profile may be much more obviously irregular, and the various features – ridges and runnels, streams, pools of fresh or salt water – should be taken into account when sampling. Examples of surveys are given by McIntyre and Eleftheriou (1968), Eleftheriou and McIntyre (1976) and Eleftheriou and Robertson (1988). On vegetated (dominated by eel grass) and unvegetated tidal flats, drop traps have been used for the study of mobile epifauna (Polte *et al*. (2005), from Pihl-Baden & Pihl (1984)).

In atidal beaches or on shores with a very small tidal range such as the Baltic or the Mediterranean, the littoral zone under investigation for the fauna and flora is greatly compressed to a very narrow zone along the edge of the water. Sampling techniques and overall methodology remain the same as described above, with stations taken above, at and below the swash zone extending a certain distance in the infralittoral but not exceeding 1–2 m depth. The very shallow water (1–2 m) in some shores can be investigated by hand-held devices such as core tubes (Maitland, 1969; Baker *et al*., 1977; Kanneworff & Nicolaisen, 1983). The top zone of a sandy beach, the supralittoral, situated above the drift line, occupied by a variety of supralittoral forms such as some crustaceans and insects, can be collected by pit trapping.

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On rocky shores, sampling is carried out by means of a square frame of heavy gauge wire laid on the substratum, the animals and plants within the frame being counted directly, or estimated in terms of percentage cover/abundance scale of the surface (for size of sampling unit, see Chapter 1), while photography provides an objective assessment of the community (see also Chapters 4 and 9). Non-destructive sampling may be carried out *in situ*, otherwise growths must be scraped off for subsequent examination in the laboratory. For larger organisms, a frame of 1 or 0.25 m^2 (often subdivided into smaller units) is suitable, but for smaller organisms or where the rock surface is irregular and habitat complexity and surface area in assessing biodiversity is required (Kostylev *et al*., 2005), frames of 0.1 m^2 (316 mm × 316 mm) should be used (see Chapter 9). A flexible frame might be appropriate on some rock surfaces. For the sampling of filamentous algae and their associated fauna in shallow water, a square-quadrat box sampler with a sampling area of 1109 cm^2 is described by the Finnish IBP-PM Group (1969), and other methods used by divers, as described in Chapter 4, may sometimes be appropriate (a comprehensive account of sampling methods for rocky intertidal and shallow subtidal areas is given in Chapter 9).

For counting small organisms such as barnacles, squares of 0.01 m^2 (100 mm × 100 mm) are suitable. For such counts a piece of thick (6 mm) Perspex exactly 100 mm square and etched with a grid of 10 mm squares is convenient, allowing smaller areas to be counted and also minimising the possibility of missing individuals or counting them twice. Besides organisms living on the rock surface, estimates should be made of crevice-living and boring species, and of those sheltering among weeds. The importance of photography and underwater video (Chapters 4 and 9) for non-destructive recording of seashore species and habitats must be emphasised.

However, the size of the sample (quadrat) should be determined by the population under investigation and in particular its spatial distribution. There is considerable debate concerning the choice of the optimal size of the sample, which could range from an arbitrary choice to a statistically valid replication. In Ecoscope (2000) it is pointed out that 'there is no simple rule for calculating the optimal size (quadrat)'. Andrew and Mapstone (1987) present a useful discussion on the subject, while Pringle (1984), having reviewed several papers, concluded that a 0.25 m^2 area should be appropriate for sampling marine organisms.

Stations should be spaced out at regular distances (or tidal height intervals) along traverses from high- to low-water mark. The lack of uniformity of most rocky shore habitats will often make it necessary to make a number of estimates in different types of habitat (e.g. rock pool, rocks exposed/sheltered from waves/sun, crevices and under stones) at each station. Since such habitats cannot normally be selected by predetermined measurements, the question of bias will arise. Bias cannot be entirely eliminated when making such estimates, and indeed on a rocky shore, which can have an almost infinite variety of microhabitats, it is questionable whether it is possible to take a sample that is in any degree representative of

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a wider area. Space does not permit detailed consideration of the literature on intertidal methods, but a useful discussion of methods for both sediment and rocky shores is given by Gonor and Kemp (1978), and in the separate contributions by Jones (1980) and Jones *et al*. (1980) (in Price *et al*., 1980). Methods for coral reefs are described in Stoddart and Johannes (1978), Marsh *et al.* (1984) and Bradbury *et al*. (1986) (see also Chapters 4 and 9).

A semi-quantitative method for surveying both intertidal and subtidal biota and organisms has been developed by the Joint Nature Conservation and published in a series of reviews (Marine Nature Conservation Reviews – MNCR). Details of this methodology can be found in Chapter 4 as well as in Hiscock (1996). On the other hand, a large-scale study of the narrow inshore zone of the world's oceans at depths of less than 20 m has been initiated by the Census of Marine Life (www.coml.org; accessed 8 November 2012) through the Natural Geography Inshore Areas project (NAGISA). Building on-site selection criteria and sampling protocols, now available online, this project's aims are to achieve coverage with standardised techniques that will provide a biodiversity baseline for future comparisons.

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Position fixing and levelling on the shore

The position of stations or transects on the shore may be fixed by standard surveying techniques as outlined, for example, by Southward (1965), and by Jones (1980). A cheap and simple level that may be used by one person is described by Kain (1958), and other levelling methods are described by Emery (1961), Stephen (1977) and Nelson-Smith (1979). Fuller treatment of levelling techniques is given by the Admiralty (1948), Ingham (1975) and Pugh (1975). Other relevant references are Kissam (1956), Morgans (1965) and Zinn (1969).

For repeat sampling, positions on a rocky shore may be marked with paint, marks chiselled into the rock, expanding bolts inserted into crevices, holes drilled in the rock or by small concrete blocks cast *in situ.* Boalch *et al*. (1974) used an underwater-setting resin compound for fixing metal bolts into holes drilled into rocks on the shore.

On sediment shores positions may be marked by posts driven deeply into the sediment. However, such posts may cause some alteration to the environment and may also attract the attention of beach users resulting in parts being removed or damaged as well as the sediment around the posts receiving more trampling than elsewhere. In such instances it is advisable to position reference marks on rocks or other permanent structures, from which locations are determined by tape measure, and/or transect lines. In the absence of any other solution, the position of a transect can be established by driving a metal pole into the sediment and this can be detected by a metal detector.

Since the second edition of this handbook (Holme & McIntyre, 1984), positioning technology has developed to the point where small, hand-held satellite-based positioning systems are readily available. The Global Positioning System (GPS),

and its more accurate sibling Differential GPS (DGPS) that uses a fixed terrestrial reference position, can give accuracies up to less than 5 m over the surface of the planet. The removal of 'Selective Availability' from the satellite system and the introduction of the European Geostationary Navigation Overlay System (EGNOS), a European version of the American Wide Area Augmentation System (WAAS), means that even small hand-held units can now achieve the same accuracy.

Systems such as those produced by Garmin International Inc. (www.garmin.com; accessed 31 October 2012) can log positions on demand and, therefore, can be used to plot the geography of beaches and bays accurately, by the simple expedient of the researcher walking along the boundaries she/he wishes to map. Position files can then be downloaded to a suitable computer for plotting (see p. 232), creating accurate and reproducible maps of the survey location upon which details of faunal or floral abundance can be superimposed. In addition, many of the units now carry altitude sensors and electronic compasses, which, once correctly calibrated, may be of use in various survey activities.

As an extension to these GPS instruments, the use of 'apps' to log relevant positions through smart phones can aid large-scale surveys covering huge geographic areas where data are transferred automatically to centralised databases when collected in real time. One example of this was during the Gulf of Mexico oil spill when data were collected on the distribution of marine reptiles and mammals; this could easily be adapted to deal with benthic data such as seaweed distributions.

A novel method of presentation has been adopted by the Scottish government to provide data and information on the research it has undertaken in support of marine renewables. Marine Scotland Interactive (MSI) makes use of the computer-based application Google Earth. (www.scotland.gov.uk/Topics/marine/science/MSInteractive; accessed 31 October 2012). Geographically correct locations identified by pointers are linked to video footage (stored on YouTube) and still photographs (stored on Picasa) in order to provide an overview of the local habitats and seabed conditions to interested parties. The system is also able to provide metadata information on bathymetry for areas of interest (www.scotland.gov.uk/Topics/marine/science/Publications/TopicSheets/MSInteractive; accessed 15 November 2012).

For intertidal organisms, the duration of exposure at each low tide is important. This may be roughly determined from the zonation of plants and animals on the shore, and by observations of the length of time for which the selected sites are exposed over a number of low tides (Lewis, 1964; McLachlan & Brown, 2006). Where tidal data are available the positions may be levelled to a benchmark or other fixed mark of known height, it will then be possible to calculate the mid-tide level of the beach as well as the exposure from data given in the tide tables. On sedimentary beaches the ensuing beach profiles can provide important information on the erosional and depositional cycles and, therefore, can be used to determine both the short- and long-term stability of the shore. Consideration should be given to the use of high-precision theodolites and laser-based levelling surveyors (such as

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those manufactured by Leica) employed by civil engineers and cartographers. These instruments are very expensive, but it may be possible to hire them for survey use.

In estuaries or in parts of the world where accurate tidal data are not available, a series of observations on a graduated tide pole should be carried out; positions on the shore can then be levelled in relation to this or to a fixed point above the shore. Allowance should be made for spring and neap tides, the effects of wind and barometric pressure, and for river outflow in estuaries. It should not be assumed that the tide follows a symmetrical harmonic curve, nor that tidal heights are necessarily the same on the two tides of one day (see official Admiralty Manual of Tides or various online tidal predictors, such as those provided by Admiralty EasyTide (http://easytide.ukho.gov.uk; accessed 31 October 2012)). On wave-exposed coasts, levels are elevated through spray and swash, so plants and animals tend to occur much higher than on sheltered shores (Lewis, 1964; Barnes & Hughes, 1982).

5.2 Remote collection

There are a number of reviews in which the equipment and techniques used for sampling the benthos are described (e.g. Gunter, 1957; Thorson, 1957; Holme, 1964; Hopkins, 1964; Longhurst, 1964; Reys, 1964; Bouma, 1969; Kajak, 1971; Lamotte & Bourliere, 1971; Clarke, M.R., 1972b; Eagle *et al*., 1978; Gray *et al*., 1991; Rumohr, 1999, 2009) and a comprehensive bibliography of benthic samplers given by Elliott *et al*. (1993). In a couple of reviews (Baker, 1987; Rumohr, 2009) a comprehensive account of a step-by-step procedure for sampling the macrofauna of soft sediments is provided, and information on the sampling equipment as well as the processing and treatment of the samples for the determination of the biotic indices has been dealt with in detail.

While choice of equipment depends largely on local conditions (size of ship, power and capabilities of lifting gear, whether sampling in exposed or sheltered conditions, depth of water, bottom deposit and type of sample required), the multiplicity of samplers that have been described is evidence not only of such factors, but also of a widespread dissatisfaction with existing methods of collection. Because the many samplers that are available have been described and discussed in reviews such as those listed above, this chapter will not attempt to cover the whole field nor to give a historical review, but rather to guide readers towards the most suitable instruments for their own particular purposes: to this end a summary of the attributes of selected samplers is given in Table 5.1. There are some grounds (notably those of rocks and boulders) that cannot be adequately sampled by any of the instruments listed here. At best, they can be sampled only by dredge, which may prove inadequate even as a qualitative sampler. Such habitats are often better investigated by diver observation from a submersible (Chapter 7), or by photography and television (Chapter 4).

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Table 5.1 Guide to literature in which efficiency of different sampling gear and methods is compared. Cross-referencing is obtained by reading vertically and horizontally from the name of each type of gear. The categories are broad, and where different sizes or variants of the same sampler have been tested against each other the references are shown in brackets after the name. However, comparative hauls between different types of otter trawl are not included. Descriptions and references to the different types of sampling gear are in the text.

Otter Trawl	Agassiz trawl	Beam Trawl	Dredge	Anchor Dredge	Epi-, suprabenthic sledge	Petersen grab	Campbell grab	Okean grab	van Veen grab [1,3,7]	Ponar grab	Hunter grab	Smith-McIntyre grab	Day grab	Orange-peel grab	Baird grab	Hamon grab*(67)
		18														
				16,53												
		18	18													
					31											
						24										
	27			16	16	7,13,17, 26,42,44, 55		2								
						13,34, 39			13,51, 62							
				11	65	17,34, 41,50,55			6,17,41 47,51	34,51	23					
												43				
									35,51	22,51		8,29, 51				
												19				
												54				

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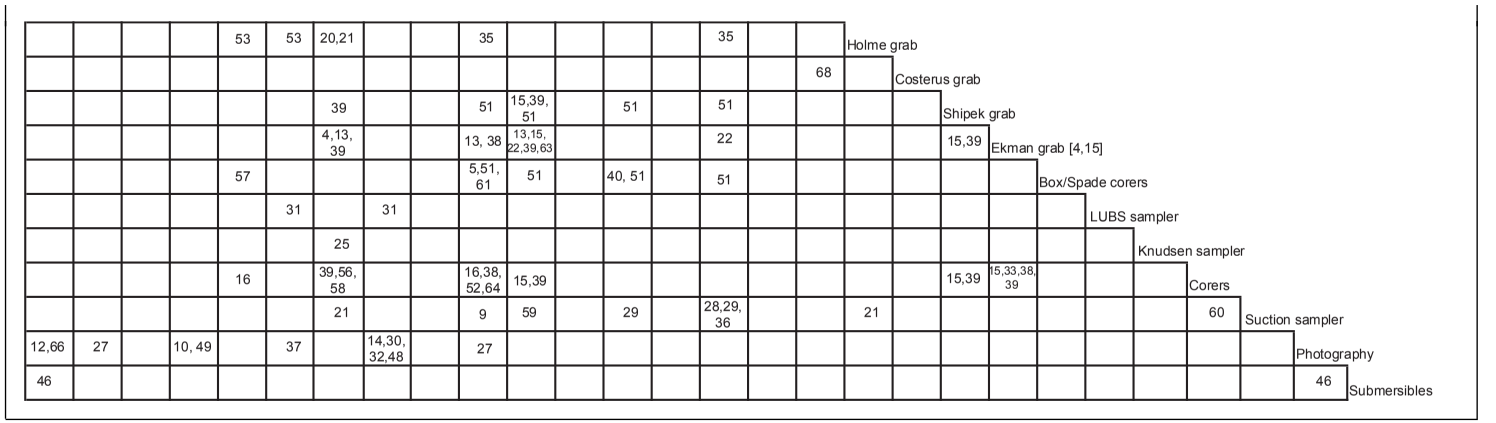

The references are numbered as follows: 1. Andersin and Sandler (1981); 2. Ankar *et al.* (1978); 3. Ankar *et al.* (1979); 4. Bakanov (1977); 5. Beukema (1974); 6. Bhaud and Duchene (1977); 7. Birkett (1958); 8. Bourcier *et al.* (1975); 9. Christie and Allen (1972); 10. Dickie (1955); 11. Dickinson and Carey (1975); 12. Dyer *et al.* (1982); 13. Elliott and Drake (1981); 14. Emery *et al.* (1965); 15. Flannagan (1970); 16. Gage (1975); 17. Gallardo (1965); 18. Gilat (1968); 19. Higgins (1972); 20. Holme (1949); 21. Holme (1953); 22. Hudson (1970); 23. Hunter and Simpson (1976); 24. Ivanov (1965); 25. Johansen (1927); 26. Kutty and Desai (1968); 27. McIntyre (1956); 28. Masse (1967); 29. Masse *et al.* (1977); 30. Menzies *et al.* (1963); 31. Menzies and Rowe (1968); 32. Owen *et al.* (1967); 33. Paterson and Fernando (1971); 34. Powers and Robertson (1967); 35. Reys (1964); 36. Reys and Salvat (1971); 37. Rice *et al.* (1982); 38. Rowe and Clifford (1973); 39. Sly (1969); 40. Smith and Howard (1972); 41. Smith and McIntyre (1954); 42. Thamdrup (1938); 43. Tyler and Shackley (1978); 44. Ursin (1954); 45. Ursin (1956); 46. Uzmann *et al.* (1977); 47. Wigley (1967); 48. Wigley and Emery (1967); 49. Wigley and Theroux (1970); 50. Wildish (1978); 51. Word (1976); 52. Jensen (1981); 53. Huberdeau and Brunel (1982); 54. Dauvin (1979); 55. Riddle (1989); 56. Bagge *et al.* (1994); 57. Probert (1984); 58. Baker *et al.* (1977); 59. Long and Wang (1994); 60. Stoner *et al.* (1983); 61. Heip *et al.* (1985); 62. Ellis and Jones (1980); 63. Howmiller (1971); 64. Thayer *et al.* (1975); 65. Koulouri *et al.* (2003); 66. Cranmer *et al.* (1984); *67 Boyd *et al.* (2006); 68 Barrio Frojan and Mason (2010).

Therefore, with the continuing improvements of technology, a combination of different techniques (high-resolution sidescan sonar, underwater television, sediment profile imaging, GPS) and the application of different samplers is essential for obtaining a meaningful synthesis of the different biota and animal populations. In the search for more information, sampling is quite frequently not confined to the limits of the continental shelf (200 m) but well beyond it in the deeper areas of the continental slope (>200 m). Consequently, some of the sampling gear used has a universal application, as it is used as much in 'shallower' water as in deeper areas (Agassiz and otter trawls, some sledges and sleds, multicorers and box corers). The methods and samplers used mainly or only in the deep sea can be found in Chapter 7.

Trawls

Beam, Agassiz, otter and shrimp trawls may be used for qualitative sampling of the epifauna. These nets are designed to skim over the surface of the bottom, and because of the large area covered, are useful for collecting scarcer members of the epifauna (<10 mm), and species of fish, echinoderms, gastropods, cephalopods and crustaceans associated with the bottom. The efficiency of such gear, in terms of numbers of animals captured in relation to those in the area swept by the net, is generally low, and is selective for particular species. Attempts at quantifying results from trawl catches are considered on pp. 192–193. Towards this, beam trawls are frequently equipped with cameras and real-time television cameras (in order to improve the qualitative assessment) and depth-finding sonar (in order to determine the exact time and position of contact with the seabed) (Neumann *et al*., 2008).

The *Agassiz* or *Blake* trawl (Fig. 5.1a) is virtually a double-sided beam trawl with a double-weighted chain designed to collect samples in places where it is not possible to control which way up the trawl lands on the bottom (Agassiz, 1888). Compared to the beam trawl it suffers from the disadvantage that the ground and head ropes, being interchangeable in function, are necessarily of the same length. Consequently, few fish are caught by the Agassiz trawl, which is not, therefore, used commercially.

The *beam trawl* (Fig.5.1b) is still used commercially for fishing for shrimps, prawns and flatfish. The mouth of the net is held open by a wooden or metal beam of 2–10 m length, with metal runners at either end. The net is a fairly long bag, of mesh about 12.5 mm knot-to-knot, the lower leading edge of which is attached to a weighted chain, forming a ground rope that curves back behind the top of the net attached to the beam so that fish and invertebrates disturbed by the ground rope cannot escape upwards. In commercial beam trawls, which are also used in research studies, several rows of tickler chain are fixed in front of the ground rope in order to increase sediment disturbance and the emergence of fish and invertebrates (Creutzberg *et al*., 1987; Kaiser *et al*., 1994). A beam trawl developed by Gunderson and Ellis (1986) for the stock assessment of demersal

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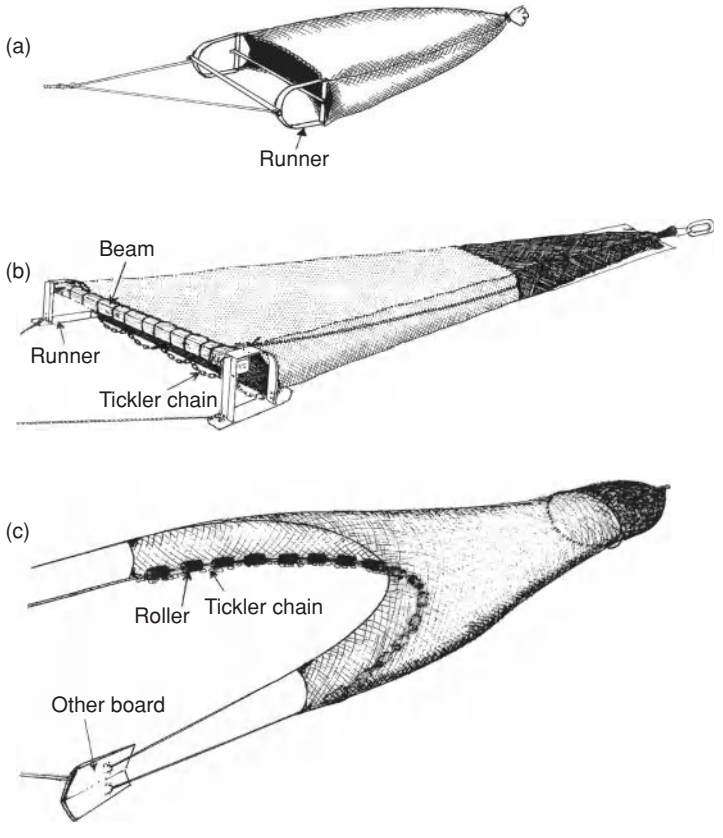

Fig. 5.1 (a) Two-metre wide Agassiz double-sided beam trawl. (b) Beam trawl showing timber beam, steel runners and tickler chain. (c) Otter trawl showing otter boards, tickler chain rigged to the footrope and the rollers to help avoid the trawl biting into the sediment.

fish and invertebrates has been modified by Abookire and Rose (2005). In the new design, the tickler chains are replaced by disc ground gear in order to be used for sampling over rough grounds. However, this design aims at sampling demersal fish in a variety of habitats and although it also accidentally catches larger epifaunal organisms such as crabs, it cannot be considered as suitable for benthos sampling.

Both beam and Agassiz trawls can be towed on a pair of bridles attached to a single tow rope or wire. Unfortunately, the cross-bars or beams are liable to be damaged if they meet an obstruction, and a weak link should be used, especially when working unexplored grounds. Carey and Heyamoto (1972) describe a variation of the standard beam trawl (ORE trawl), but heavier and without any tickler chains, designed mainly for deeper-water sampling. There is a flexible connection between beam and runners, which allows momentary collapse of the net when an obstruction is encountered and subsequent easy retrieval from a single bridle.

Otter trawls (Fig. 5.1c) used for commercial fishing also capture members of the invertebrate epifauna, but because of the rather large mesh size only the larger

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animals are retained. Besides those animals retained in the cod-end of the trawl, many small organisms may be found enmeshed in the body of the trawl. These must be removed. The range of otter trawls and their rigging, shooting and working is a specialised subject, which is comprehensively discussed in Chapter 7; instructions for making up a small trawl are given by Steven (1952) while scaled-down otter trawls with finer meshes have effectively been used for the collection of small fish and epifaunal invertebrates (Eleftheriou, 1979). Details of various types of fishing gear designs have been published in an FAO (1972) catalogue while further references are given by von Brandt (1978). Galbraith *et al.* (2004) give a beautifully illustrated introduction to commercial fishing gears and methods used in Scotland. One important development in estimating the capture efficiency of commercial trawls was the application of underwater photography or television. Dyer *et al*. (1982) and Cranmer *et al*. (1984) successfully applied underwater photography to their studies on the benthic invertebrate of the North Sea. On the other hand, in order to improve catchability and retention of benthic fauna, McConnaughey *et al*. (2000) modified an otter trawl equipped with mechanical and electronic devices as well as tickler chain and liner on the whole length of the trawl.

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Bottom sledges

Many types of sledge have been designed for sampling the epifauna and the hyperbenthos as well as larger members of the plankton immediately above the bottom. Some are little more than plankton nets on runners (e.g. Myers, 1942), but Ockelmann's detritus-sledge has a tickler chain to stir up newly settled benthic invertebrates (Ockelmann, 1964). Others have opening and closing mechanisms, and sometimes a flow meter to measure the quantity of water filtered (Bossanyi, 1951; Wickstead, 1953; Beyer, 1958 (illustrated in Holme, 1964); Frolander & Pratt, 1962; Macer, 1967; Bieri & Tokioka, 1968; Yocum & Tessar, 1979; Sorbe, 1983). A sledge net for hand towing in the intertidal zone is described by Colman and Segrove (1955).

An improved version of Macer's sled-mounted 'supra-benthic' sampler (Macer-GIROQ) has been developed by Brunel *et al*. (1978), who used it successfully to depths of 200 m (Fig. 5.2). It was used to provide quantitative estimates of megabenthos in the Georges Bank with a sheet steel collection box sampling at a depth of 25 cm (Thouzeau & Vine, 1991). Further changes were made by Dauvin *et al*. (1995), while Rowell *et al*. (1997) made further modifications by the addition of stabilising wings, wider runs, a video system and a double odometer.

A modified version of the Rothlisberg and Pearcy (1977) epibenthic sledge (Fig. 5.3) was constructed by Fossa *et al*. (1988) for sampling the epifauna at moderate depths (40–120 m). The sledge, which is of standard design, is equipped with a pneumatic opening and closing device that ensures sampling occurs only when the sledge is in contact with the seafloor. Other modifications of the Rothlisberg and Pearcy (1977) epibenthic sledge were provided by Buhl-Jensen

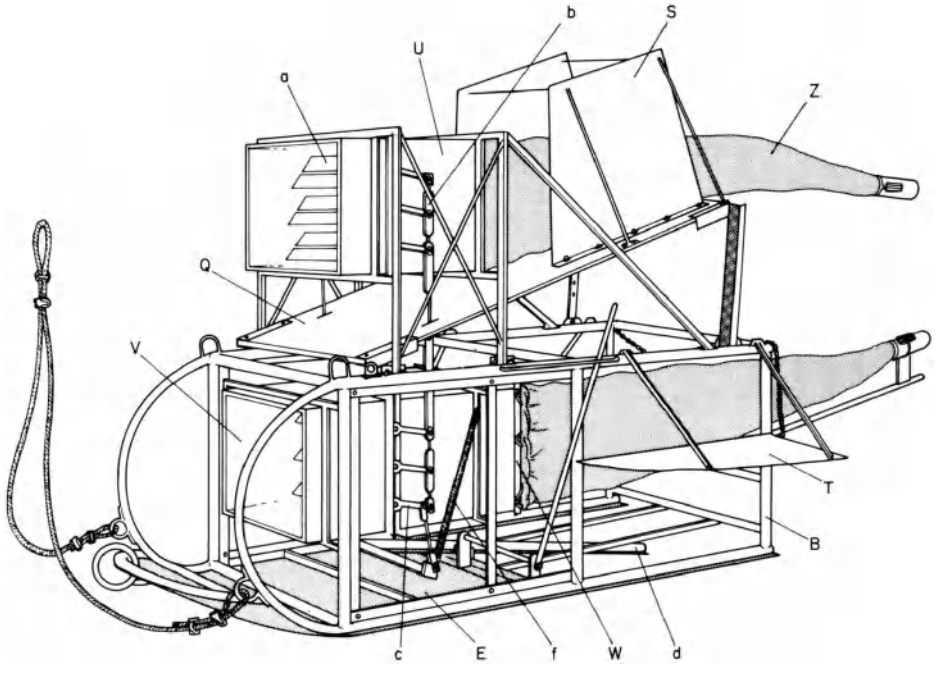

Fig. 5.2 The Macer-GIROQ epibenthic sledge as developed by Brunel *et al*. (1978). B, tubular chassis; E, sheet metal gliding plate, turned upward at front; Q, adjustable wooden depressor; S, vertical fin; T, horizontal fin; U, wooden box at front of upper net; V, wooden box at front of lower net; W, metal strip for attachment of net; Z, zooplankton net; a, shutter closing mechanism; b, adjustable control link; c, crank lever; d, lever operating closing mechanism; f, closing spring.

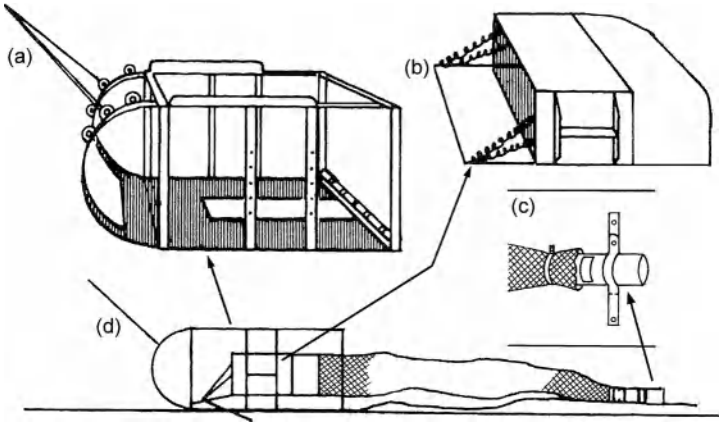

Fig. 5.3 Modified Rothlisberg–Pearcy (RP) epibenthic sled sampler. (a) Sled frame, with rubber chafing apron attached to the rear lower frame; (b) sampling box with door open; (c) cod-end with collector pot strapped to the rubber chafing apron; (d) side view of assembled sled on the bottom, with the door held open by friction with the bottom. (After Buhl-Jensen (1986).)

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(1986) and Brattegard and Fossa (1991) for sampling the peracarid epifauna in greater depths (130–380 m). The use of weights, an opening and closing mechanism and a protective mat ensured a more efficient sampling and easier operational handling. Another variation of the Rothlisberg and Pearcy (1977) sledge, but with superimposed net boxes (epibenthic and suprabenthic nets), has been designed by Brenke (2005). However, this is a combination of a bottom plankton net and a dredge, equipped with flaps for opening and closing the nets, and is able to operate both in shallow or deep water, in sandy or rocky grounds.

A number of other workers used samplers with superimposed nets (Hesthagen, 1978; Sorbe, 1983; Dauvin & Lorgere, 1989; Elizalde *et al*., 1991; Dauvin *et al*., 1995) or with additional nets mounted on either side of the original nets (Oug, 1977), in order to study the distribution of hyperbenthos in the first metre above the seafloor. Koulouri *et al*. (2003), using the basic frame of the Shand and Priestley (1999) photosled (Towed Trawl Simulator Sledge, TTSS2), developed a benthic sledge with superimposed nets, opening and closing mechanism, odometer and underwater television system for the study of the small invertebrate communities living above the sediment/water interface, which is severely disturbed by fishing gear activities (Fig. 5.4). In a similar way, Sirenko *et al.* (1996) studied the

Fig. 5.4 The Towed Trawl Simulator Sledge (TTSS2; Koulouri *et al*., 2003). 1, Ground rope; 2, opening–closing mechanism; 3, electronic controller; 4, television camera; 5, collectors; 6, odometer; 7, superimposed nets.

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suprabenthos by a benthopelagic sampler net frame attached to the upper part of a 3 m Agassiz trawl. It should be pointed out that all the epibenthic sledges sample the bottom layers at various distances from the ocean floor. Inevitably, sampling too near the bottom sediment results in contamination of the samples with both sediment and organisms living near the sediment surface. Many authors overcome this problem by combined or additional sampling (by grab or corer), which allows them to apportion the organisms to the appropriate biota.

Epibenthic sledges have a heavy frame enclosing the net, and are particularly useful for deep-sea sampling. There are also several instruments designed for deep-sea sampling that are intermediate between the Agassiz trawl and dredge in that they consist of a net attached to a rectangular steel frame fitted with runners (Menzies, 1962; Rice *et al*., 1982).

Dredges

Dredges have a heavy metal frame and are designed for breaking off pieces of rock, scraping organisms off hard surfaces or for limited penetration and collection of sediments and fauna. A small dredge with runners used at Plymouth (Fig. 5.5) has been extensively employed for sampling the epifauna on the continental shelf.

The *naturalists'* or *rectangular dredge* (Fig. 5.6) is a useful instrument for exploratory purposes as it can obtain samples on a variety of grounds. One of the dredge arms is attached directly to the tow rope, the other being joined to it by a few turns of twine, which act as a weak link to release the dredge should it become fast on the seabed. It is important not to use too many turns of twine, since, particularly with synthetic twine, it may then be too strong to part should the need arise. More sophisticated links, involving a metal shear pin, are available commercially. An alternative arrangement is to attach a safety line consisting of a piece of chain or wire from the towing point to some point towards the back of the dredge, for retrieval when the weak link parts. A swivel should always be inserted between towing warp and the dredge (or any other sampling gear), and this must be of a ball- or roller-bearing type, turning freely under load, when the towing warp is of wire.

The dredge net is usually about half as deep as it is wide, the mesh varying according to circumstances. Machine-made netting of mesh 10–12 mm knot-to-knot is generally suitable, but material used for shrimp trawls is usually too light in construction. For collection of sediment the bag can be lined with an inner bag of sacking, stramin or burlap. Impervious material such as canvas should not be used, as water must drain away when the dredge comes on board. Where a net bag is required (e.g. to avoid washing out of the sample when hauling), the net should be an open-ended sleeve with a cod-end tied at the bottom with a rope that is untied to release the sample. Another simple but effective dredge widely used in the Mediterranean for the collection of organisms and bottom material is the Charcot dredge (Picard, 1965).

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Fig. 5.5 Dredge with runners as used by the Marine Laboratory at Plymouth. The width is 1 m and the net is protected by canvas strips on either side of the net.

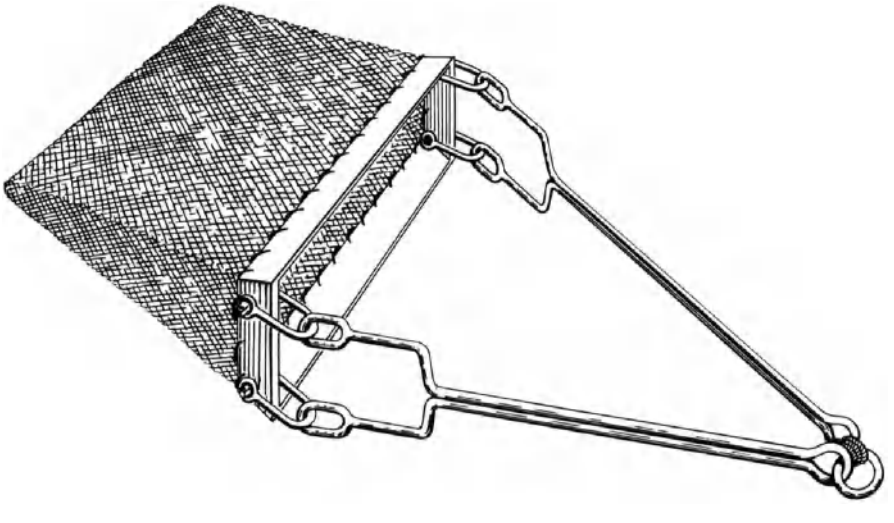

Fig. 5.6 Naturalist's or rectangular dredge. Note weak link of twine joining one arm to ring.

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On some types of sea bottom a net may raise an unnecessarily large sample and burst when a heavy load is brought up. Chafing of the net while being towed along the bottom can be minimised by fitting sheets of rubber, hide or canvas (Fig. 5.5) on either side of the net bag.

When operating from a small boat where the dredge is hand-hauled, a rectangular dredge frame of length 300–380 mm is large enough. For ease of hand-hauling, this should be towed on a rope of at least 10 mm, and preferably 12 mm, diameter. For larger boats with a power hoist a dredge frame of 450–600 mm is suitable, and 750–1300 mm is used for trawler-sized vessels. On ships equipped with winches, wire ropes will be used for working the dredge, the diameter of the wire being related to the size of the ship and the depth of water, bearing in mind that, in spite of weak links, very severe strains can be exerted on the towing warp from time to time. On the continental shelf it is usual to tow with warp equal to 2.5–3 times the depth of water, but in the deep sea a factor <1.5 times may be possible by using heavy weights or diving plates on the towing line in front of the gear and where an acoustic pinger (Gaunt & Wilson, 1975) is used to show when the dredge is on the bottom (see Chapter 7). When dredging is being carried out, the ship should drift or steam slowly (1–2 knots), a check being made on the performance of the dredge by the angle of the warp and any jerks or vibrations, strain being recorded if possible on a strain gauge. The dredge should normally be towed on the bottom for 5–10 minutes, but on some grounds it may fill up at once and can be hauled up almost immediately. Special techniques for deep-sea dredging are given in Chapter 7.

Many types of dredge have been produced for different purposes. For geological sampling there are sturdy rock dredges, often with teeth (Nalwalk *et al.*, 1962; see Fig. 5.7). Boillot (1964) and Clarke, A.H., (1972a) have described a heavy dredge for sampling in mixed boulder and mud substrata. Rock dredges typically have

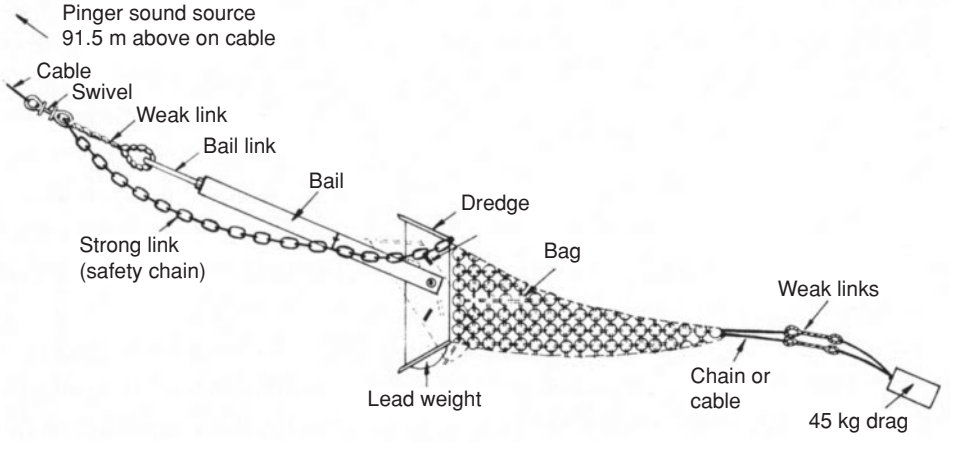

Fig. 5.7 Rock dredge with bag of interlaced metal rings. Note the arrangement of weak link, safety chain and swivel. (Redrawn from Nalwalk *et al*., 1962.)

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bags of metal rings or wire grommets, similar to those used on oyster and scallop dredges. These may be lined with a finer mesh of synthetic netting if desired. This is also a feature of Brenke's (2005) epibenthic sledge, the lower net box of which operates as a dredge (see the section entitled 'Bottom sledges') for sampling on all types of grounds but especially on very uneven rocky bottoms. Where digging into the sediment is required, dredges that are bowed, oval or circular in shape are more effective than those with a straight edge, but the penetrating powers of most are limited, except in soft mud, so that they typically sample only the shallower-burrowing members of the infauna. Dredges with teeth are more effective for certain purposes (Baird, 1955, 1959), and the use of inclined steel diving plates to make the dredge sink more quickly and to help maintain contact with the bottom is worth considering.

A specialised dredge mounted on a sledge adapted for the study of juvenile molluscs at the superficial sediment, designed by Thouzeau and Hily (1986) and subsequently improved by Thouzeau and Lehay (1988) was used for the study of young scallops (Thouzeau & Lehay, 1988; Thouzeau & Vine, 1991). The dredge, which is equipped with video camera, odometer and interchangeable nets, had a tested efficiency of >80% according to the size of the target organisms.

Although seldom providing satisfactory quantitative samples, trawls and dredges are indispensable sampling instruments and should always be carried on board ship. Dredges, in particular, are invaluable for preliminary surveys to discover the nature of the bottom and its fauna and may be the only instruments that can be used under adverse sea conditions; being mechanically unsophisticated they are a standby when more complex equipment has broken down, and on some grounds they may be the only means of obtaining a sample and would then be the prime means of investigation.

Notwithstanding the misgivings felt about the semi-quantitative aspect of dredges, quantitative collection of the larger-sized epifauna and infauna of low abundance on soft sediments has been satisfactorily achieved by Bergmann and van Santbrink (1994). By using the Deep-Digging Dredge (Triple D) rigged with an interchangeable blade to collect a slice of the benthic sediments, accurate and reliable results on the benthic assemblies of the soft sediments were obtained. The sediments are sieved and retained in a net attached to the frame of the metal box connected to the dredge frame provided with a pair of runners and a measuring wheel.

Semi-quantitative estimates with trawl and dredge

When trawling it may be possible to standardise conditions and duration of tow, so as to obtain estimates of population density, which are of value for comparative purposes, and such hauls are commonly used for estimating demersal fish populations. However, it is not easy to estimate the exact time during which gear is on the bottom; both trawls and dredges will continue to drag along the bottom for some time after hauling has commenced. Carey and Heyamoto (1972) used a time-depth

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recorder to monitor the behaviour of a trawl on the bottom, and methods involving acoustic signals are described by Laubier *et al.* (1971) (also see Fig. 7.2) and also by Rice *et al.* (1982). In order to determine the actual bottom contact of otter trawls, a digital timing device (BENSEM – Benthic Sampler Effectiveness Measurer) has been developed and mounted on the otter trawl door, improving trawl reliability and consistency of sampling (Diener & Rimer, 1993). It is well known that trawls and dredges sample only a fraction of the fauna lying on the surface of the seabed (e.g. Mason *et al*., 1979) and very few of the burrowing animals, so that results merely represent minimum densities on the ground. Several trawls and dredges (Rothlisberg & Pearcy, 1977; Sorbe, 1983; Rice *et al.*, 1982; Fossa *et al*., 1988; Brenke, 2005) have a mechanically activated device for opening and closing the sampler.

Several workers have fitted measuring wheels on trawl or dredge frames in order to measure distance covered (e.g. Belyaev & Sokolova, 1960; Riedl, 1961; Wolff, 1961; Gilat, 1964; Richards & Riley, 1967; Bieri & Tokioka, 1968; Carey & Heyamoto, 1972; Pearcy, 1972; Carney & Carey, 1980; Rice *et al*., 1982). The performance and success of such wheels seem to be variable, and is complicated by the fact that some sampling instruments tend to progress by leaps and bounds (Baird, 1955; Menzies, 1972). In addition, the wheel may jam or malfunction for all or part of the tow, giving a false reading. The fitting of an odometer wheel on each side of the frame might seem to overcome this problem, but in spite of this, Carney and Carey (1980) found considerable variation between readings from wheels fixed either side of a beam trawl. They considered that slippage might result in under-reading of distance by as much as 40%. If a continuous record of rotation can be obtained, so that it is known that the wheel is rotating throughout the tow, a reasonably satisfactory measure of distance should be obtained (Holme & Barrett, 1977; Thouzeau & Hily, 1986; Thouzeau & Lehay, 1988), although Rice *et al.* (1982) have some doubts over the accuracy of a continuously recording wheel attached to their epibenthic sledge. In some cases, the sampling gear equipped with an underwater camera with real-time video or photography system (Holme & Barrett, 1977; Dyer *et al*., 1982; Cranmer *et al*., 1984; Thouzeau & Hily, 1986; Thouzeau & Lehay, 1988; Thouzeau & Vine, 1991) provides direct assessment of the ocean floor morphology and of the abundance and distribution of the larger epibenthic organisms. These can be compared against the samples taken by the sampling gear.

During sampling, the distance trawled or dredged can be measured by an on-board DGPS. This is feasible for such calculations in shallow waters, but it becomes less accurate with depth (Brenke, 2005).

Where the dredge bag is lined with closely woven material to retain the sediment, semi-quantitative data in terms of numbers of animals per unit volume of sediment can be obtained. The results are naturally dependent on depth of penetration, but under difficult conditions where grab samples cannot be taken this may be the best that can be achieved.

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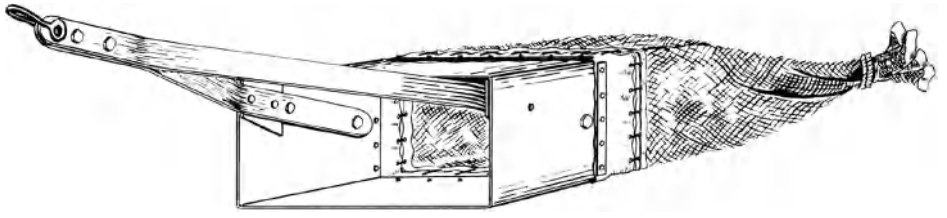

Fig. 5.8 Double-sided anchor dredge with wishbone towing arms free to swivel or can be locked to one side if required.

Anchor dredges

Forster's anchor dredge (Forster, 1953) is an invaluable instrument for semi-quantitative sampling of sands and other firmly packed deposits. This dredge has an inclined plate intended to dig in deeply at one place, and it is not towed, as are other dredges. It is shot by allowing the ship to drift while warp equal to 5 times the depth is gradually paid out; the warp is then made fast, and the strain exerted as the ship is brought to a standstill drives the dredge into the sand to a depth of up to 25 cm, taking several litres at a time. This dredge is best used from a small launch fitted with a power hoist, because if used from a larger ship there is a tendency for it to be jerked out of the sediment. This also occurs if insufficient length of warp is paid out. The sample theoretically taken by Forster's anchor dredge is wedge-shaped in section and approximately the same area as the digging plate, so that it may be used for semi-quantitative studies. A double-sided anchor dredge (Holme, 1961) capable of working either way up is shown in Fig. 5.8.

Thomas (1960) describes a modified anchor dredge with adjustable digging plate and with a self-sifting mesh net. Because this dredge is intended to be towed through the sediment the results obtained are non-quantitative, but it appears to be a useful collecting instrument for deeper-burrowing animals.

Sanders *et al*. (1965) describe a rather different type of anchor dredge (Fig. 5.9) for use on the shelf and the deep sea. This is double-sided with two angled digging plates between which is a wide horizontal plate that limits penetration to 11 cm. Since the dredge is very heavy it is assumed to sink consistently to this depth in the sediment, and, therefore, to sample to a constant depth. Once the bag is

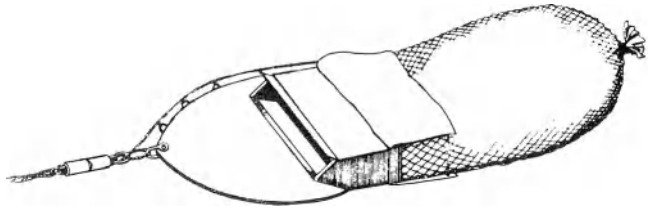

Fig. 5.9 Semi-quantitative anchor dredge used by Sanders *et al.* (1965).

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full, further material is rejected out of the mouth of the dredge. Because of the observed consistency of operation, at least on silt–clay grounds, this dredge has been employed for quantitative studies. A lighter model is described in Sanders (1956).

A modified version of the Sanders' anchor dredge (the *anchor-box dredge*) was developed by Carey and Hancock (1965). This samples an area of 1.3 m^2, to a depth of 10 cm, and has been successfully used to depths of 2800 m, where it was worked with a warp to depth ratio of 1.39:1. A smaller version of this dredge with a total capacity of 40 litres was used by Probert (1984).

Grabs

Quantitative samples of animals inhabiting sediments are usually taken by grab. The grab, which is lowered vertically from a stationary ship, captures slow-moving and sedentary members of the epifauna and infauna to the depth excavated.

There has been much discussion on the depths to which animals burrow into the seafloor (MacGinitie, 1935, 1939; Thorson, 1957; Holme, 1964). The majority inhabit the top 5–10 cm, but some burrow more deeply (Barnett & Hardy, 1967; Kaplan *et al*., 1974; Thayer *et al.*, 1975). Exceptionally, some crustaceans have been found to burrow to depths >3 m (Pemberton *et al.*, 1976; Myers, 1979). Few grabs are designed to dig deeper than 15 cm, and in practice many dig to less than 10 cm in firmly packed deposits (Table 5.2). Thus, a grab may be an adequate sampling instrument for some grounds (Ankar, 1977b), while on others it may leave significant elements of the fauna unsampled below the depth of bite.

If a grab is used as the prime means of investigation it would be advisable, at the pilot survey stage, to make comparative hauls with a deeper-digging instrument such as the Forster anchor dredge (p. 194), a box corer (pp. 204–207) or suction sampler (pp. 207–210) in order to check whether there are deeper-burrowing individuals out of the reach of the grab. If this should prove to be the case, one of these methods should be used from time to time to supplement the information obtained by grab. The sampling efficiency of grabs is further discussed on pp. 217–219.

For sampling the macrofauna, grabs covering a surface area of 0.1 or 0.2 m^2 (the conventional sample unit) are commonly employed, several samples being taken to aggregate to 0.5 or 1.0 m^2 per station. Samples of this total size are usually considered adequate for quantitative determinations of the commoner species, measurements of population abundance and biomass, but do not adequately sample scarcer animals, which are often members of the epifauna. Moreover, some fast-moving species escape the grab altogether. It is, therefore, advisable to supplement grab estimates of the epifauna by hauls with an epifaunal bottom sledge, an Agassiz or beam trawl or by underwater photography, television or diving.

The *Petersen grab* used by C.G.J. Petersen for investigations in the Danish fjords at the beginning of the century is the prototype from which many modifications and improvements have been made (Petersen & Boysen Jensen, 1911). It consists

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Table 5.2 Suitability of different sampling gear for various applications.

Gear	Weight	Sample: Width (m)	Sample: Area (m^2)	Quantitative?	Depth of sample (firm sand)	Deposit: Rock/ stones	Deposit: Firm sand	Deposit: Mud	Sea depth: Shallow	Sea depth: Shelf	Sea depth: Deep sea	Difficult sea conditions	Ship size
Beam trawl	L–M	2–10		SQ[†]	0	O	+	+	+	+		+	SML
Agassiz trawl	L–M	2–4			0	O	+	+	+	+	+	+	SML
Otter trawl	H	10			0	O	+	+	+	+		+	SML
Macer-GIROQ sampler	H	0.5		Q	0	O	+	+	+	+			SML
Epibenthic sled (Hessler & Sanders)	H	0.8			0	O	+	+			+		ML
Epibenthic sledge (TTSS2)	M	0·58		Q	0	O	+	+	+	+	+	O	SML
Triple D	H	1.5		Q	1	O	+	+	+	+	+	O	ML
Rectangular dredge	L	0.3–1.3			0	+	+	+	+	+	+	+	SML
Small biology trawl (Menzies)	L	1.0			0	O	+	+	+	+	+		ML
Anchor dredge (Forster)	L	0.5		SQ	3	O	+	+	+	+	O		SM
Anchor dredge (Thomas)	L	0.6			2	O	+	+	+	+			SM
Small anchor dredge (Sanders)	L	0.29		SQ	1	O	+	+	+	+	O		SM
Anchor dredge (Sanders *et al.*)	H	0.57		SQ	2	O	+	+		+	+		ML
Anchor-box dredge	H	0.5	1.33	SQ	1	O	+	+		+	+		ML
Petersen grab	L		0.1[*]	Q	1	O	O	+	+	+		O	SM
Campbell grab	H		0.55	Q	2	O	+	+		+	+		ML
Okean grab	L		0.08[*]	Q	1	O	+	+	+	+	+		SML
van Veen grab	L		0.1[*]	Q	1	O	+	+	+	+		O	SM
Ponar grab	L		0.055	Q	1	O	+	+	+	+	O		SM
Hunter grab	L		0.1	Q	1	O	+	+	+	+	O		SM
Smith–McIntyre grab	L		0.1	Q	1	O	+	+	+	+			SM
Day grab	L		0.1	Q	1	O	+	+	+	+			SM

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Orange-peel grab	M		Various	Q	1	O	+	+	+	+	+		ML
Baird grab	L		0.5	Q	2	O	+	+	+		O	O	SM
Hamon grab	H		0.29	SQ	2	O	+	+	+	+			ML
Holme grab	M		2 × 0.05	Q	1	O	+	+	+	+			M
Shipek grab	L		0.04	Q	1	O	+	+	+	+	O		SM
Birge-Ekman grab	L		0.04	Q	M1	O		+	+	+			SM
Reineck box sampler	H		0.06*	Q	3	O	+	+	+	+	+		ML
LUBS sampler	M		0.06–0.25	Q	M2	O		+		+	+		ML
Haps corer	L		0.015	Q	M3	O		+	+	+			SM
Knudsen sampler	M		0.1	Q	3	O	+		+	+	O	O	SM
Suction sampler (True *et al.*)	L		0.1	Q	3	O	+		+	+	+‡	O	SM
Suction sampler (Kaplan *et al.*)	L		0.1	Q	3	O	+	+	+	O	O	O	S
Suction sampler (Thayer *et al.*)	L		0.07	Q	3	O	+	+	+	O	O	O	S
Flushing sampler (van Arkel)	L		0.02	Q	3	O	+	+	+	O	O	O	S
Photography	L		<2	Q	0	+	+	+	+	+	+	O	ML
Television	L	1–2		Q	0	+	+	+	+	+		O	ML
Submersible observation	H	2–10		SQ†	0	+	+	+	+	+	+		
Traps	L	×	×		0	+	+	+	+	+	+		SML

General applications: +, suitable; blank, possible application; O, unsuitable.
Weight (total with any additional weights included): L, <100 kg; m, 100–200 kg; H, >200 kg.
Sample width and area: *Other sizes available. ×, traps sample an indefinite area.
Quantitative?: Q, quantitative; SQ, semi-quantitative; SQ†, semi-quantitative if odometer wheel fitted.
Depth of sample (penetration of sampler into firm sand): 0, surface sample only; 1, 1–10 cm penetration; 2, 10–20 cm penetration; 3, >20 cm penetration; m, above penetration depths but in soft mud only.
Sea depth: shallow, diving depth (i.e. <30 m); shelf, 30–200 m; deep sea, >200 m (i.e. slope and abyss); ‡from submersible.
Difficult sea conditions (most sampling gear cannot be used under severe conditions of swell, waves or currents): +, instruments likely to obtain a sample under such conditions; O, these instruments can only be used under calm conditions and/or the absence of strong currents.
Ship size: S, launch with power hoist; M, trawler; L, large research vessel.

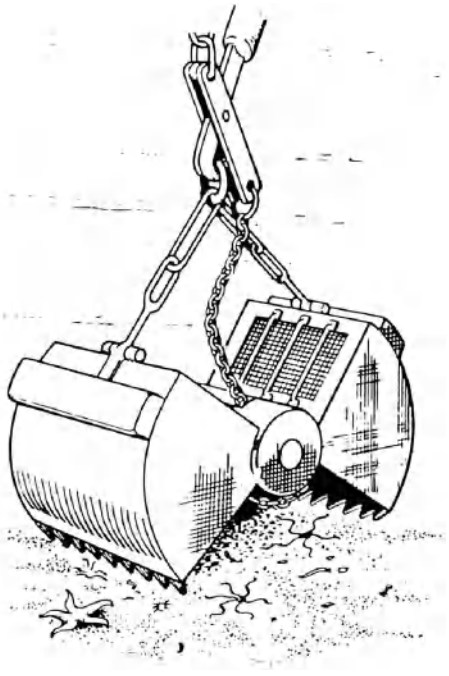

Fig. 5.10 Petersen grab in sampling position on the seafloor. After the release hook has actuated, an upward pull exerted on the central chain closes the two buckets of the grab. (After Hardy, 1959.)

of two buckets (Fig. 5.10) hinged together, which are held in an open position during lowering. The top of each bucket has a gauze-covered window to allow water to escape while the grab is closing. However, this offers some resistance to the rapid lowering of the gear, which is desirable when sampling in deeper water. When on the bottom, the lowering rope slackens, allowing a release hook to operate so that on hauling the two buckets close together before the grab leaves the bottom. The disadvantages of the Petersen grab for sampling in other than soft muds and sheltered waters have often been discussed (e.g. Davis, 1925; Thorson, 1957; Holme, 1964). These relate to premature operation of the release during descent due to momentary slackening of the rope as the ship rolls, failure to penetrate sufficiently deeply into the sediment, losses due to the jaws not closing completely and inadequate sampling due to an oblique upward pull when closing due to drift of the ship on station. For these reasons the Petersen grab has not been much in use in recent years, many workers choosing alternative sampling gear. Nevertheless, it has been used, mostly in comparative studies (Pearson *et al.*, 1985; Bagge *et al.*, 1994) with satisfactory results.

The *Campbell grab* (Hartman, 1955) is similar to the Petersen grab, but its greater efficiency is due to its larger size (0.55 m^2 sampling area, contrasted with 0.1 or 0.2 m^2 for the Petersen grab), and greatly increased weight (410 kg).

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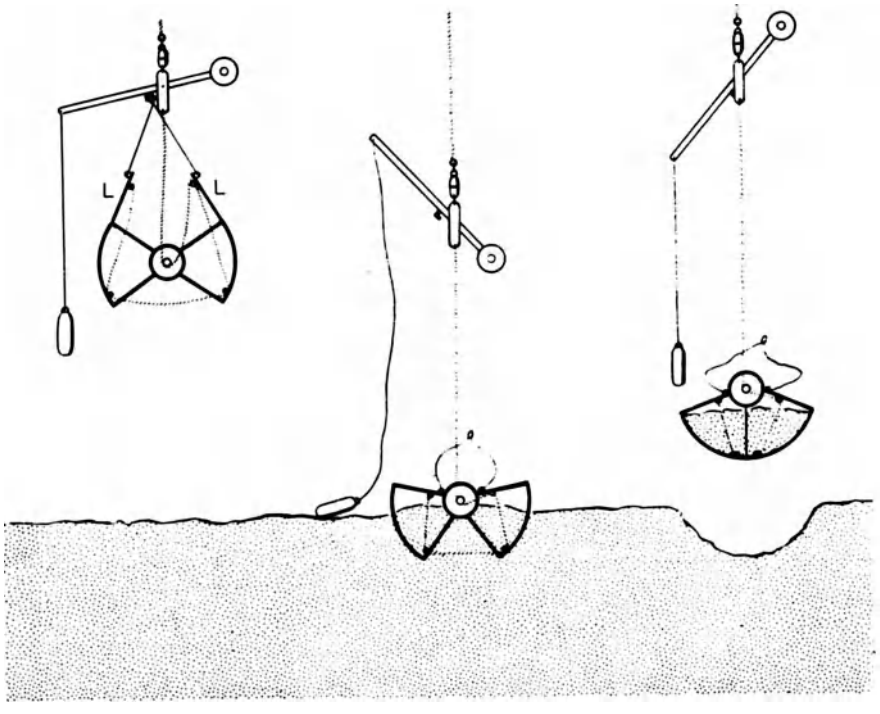

Fig. 5.11 Okean grab. Sequence in sampling operation. Note the counterweight release and the lids (L) of the two buckets, which are open during descent. (Redrawn from Rumohr, 1999.)

The *Okean grab* (Lisitsin & Udintsev, 1955) is basically a Petersen grab but with the tops of the buckets having hinged doors that are held open during the descent, and that close when the grab reaches bottom (Fig. 5.11). Very rapid rates of lowering are possible with the Okean grab, which has, in addition, a counterweight mechanism to prevent tripping in mid-water. Nevertheless, it does suffer from the same problems as the Petersen grab.

The *van Veen grab* (van Veen, 1933) improves on the Petersen grab in having long arms attached to each bucket, so giving better leverage for closing (Fig. 5.12). The arms also tend to prevent the grab from being jerked off the bottom should the ship roll as the grab is closing. On the other hand, the arms may pull the grab to one side if, through drift of the ship, the upward pull for closing is oblique. The sampling efficiency of this grab in different sediments has been tested by Christie (1975), Ankar (1977a), Ankar *et al*. (1979) and Kingston (1988). The van Veen grab was adopted as the standard sampler for benthic investigations in the Baltic Sea (Dybern *et al.*, 1976), but some improvements to the mechanism have been proposed by Sjolund and Purasjoki (1979). Under open-sea conditions, the counterweight release mechanism described by Lassig (1965) can be employed.

The *Ponar grab* (Powers & Robertson, 1967), basically a sediment sampler used in limnological work, is closed by a scissor action of the arms attached to

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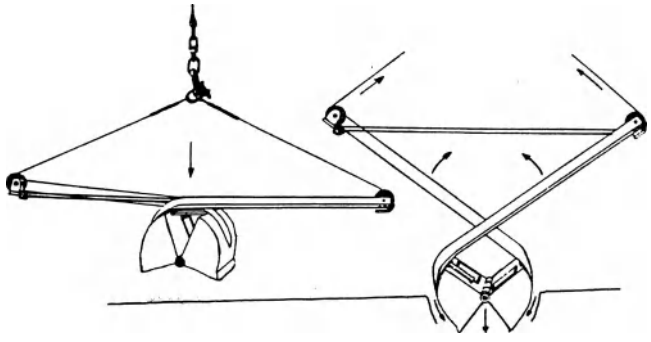

Fig. 5.12 Long arm, warp-rigged van Veen grab in sampling operation. (Redrawn from Lisitsin & Udintsev, 1955.)

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the buckets. It appears to be an easy-to-handle efficient sampler under sheltered conditions, but the release mechanism is not suited for open-sea use. It has been increasingly but erroneously used for macrofaunal sampling.

The *Hunter grab* (Hunter & Simpson, 1976) is a robust and compact grab with jaws that are extended to form levers, with the upper surfaces providing lids that allow a free-flow of water through the sampler during the descent. There is no evidence that it is used by the main body of researchers.

The *Smith–McIntyre grab* (Smith & McIntyre, 1954) was designed for sampling under the difficult conditions often encountered when working from a small boat in the open sea. This grab has hinged buckets mounted within a stabilising framework (Fig. 5.13) and powerful springs to assist penetration of the sediment. Trigger plates on either side of the frame ensure that the grab is resting flat on the bottom before the springs are released. Closing of the grab is completed as hauling commences by cables linked to arms attached to each bucket. The Smith–McIntyre grab covers an area of 0.1 m^2, and on firm sands penetrates to about the same depth as the 0.1 m^2 van Veen grab, but the greater reliability of its release makes it preferable for open-sea use. Several workers have adopted the Smith–McIntyre grab as their standard sampling instrument, but some consider it complicated and, because of the spring-loaded mechanism, occasionally dangerous to use.

The *Day grab* (Day, 1978; Fig. 5.14) is a popular alternative to the Smith–McIntyre grab as it represents an attempt to simplify the design of this type of instrument. It incorporates a frame to keep the grab level on the seabed, and two trigger plates for actuating the release, but there are no springs to force the hinged buckets into the bottom. Penetration is assisted by the greater weight of the sampler. The Day grab seems to sample as efficiently as the Smith–McIntyre grab (Tyler & Shackley, 1978), and is preferred by some workers because of its greater simplicity.

The *Video grab* is a large hydraulically-powered grab, with a sampling area of 0.5 m^2, mounted on a frame and equipped with a video imaging system consisting of two video cameras and a high-resolution acoustic imaging system

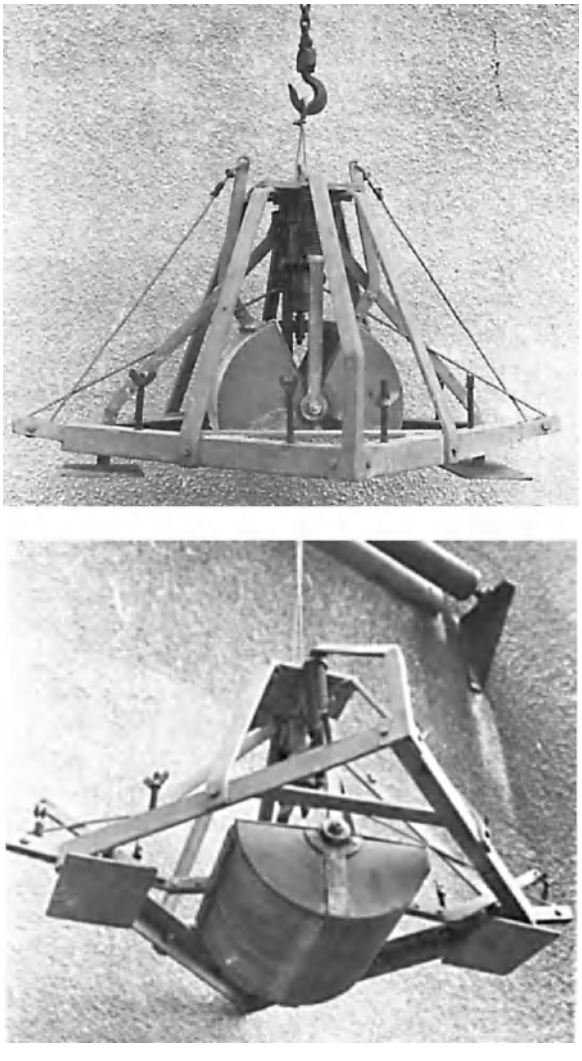

Fig. 5.13 Smith–McIntyre grab. (a) Above in open position ready for lowering. (b) Below in closed position. Note the trigger plates on either side, both of which must be in touch with the bottom before the release is actuated. The threaded studs with butterfly nuts are for attachment of lead weights. (Photograph by A.D. McIntyre.)

(DRUMS – Dynamically Responding Underwater Matrix Scanner). It is designed to take very large volumes (average 38 litres) of undisturbed samples (Kenchington *et al*., 2006) of the upper 10–25 cm of the ocean floor and has the distinct advantage for the operator to have visual contact with the ocean floor and, therefore, control the operation and take reliable samples (Rowell *et al*., 1997; Gordon *et al*., 2000).

The *orange peel grab* described by Reish (1959b) and modified by Briba and Reys (1966) is a large sampler that Thorson (1957) did not consider as a satisfactory

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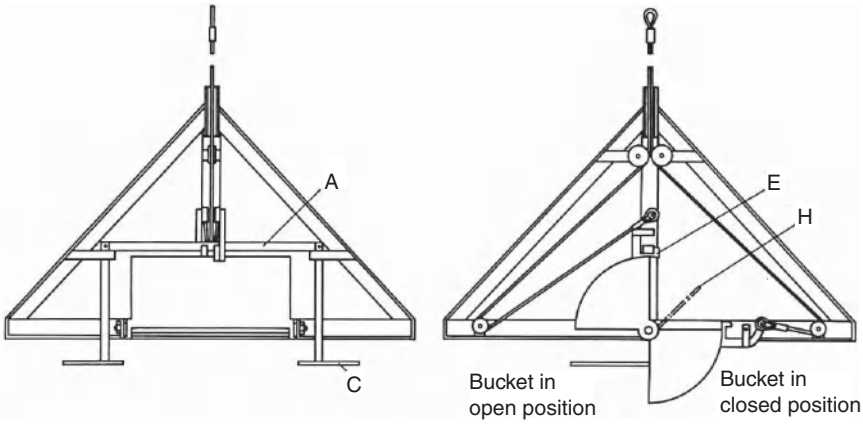

Fig. 5.14 The Day grab. Left, end view: open for lowering; right, side view: one bucket open, the other closed. On reaching the seabed the two pressure plates (C) are pushed upward, releasing the transverse beam (A) so that the hooks (E) holding the buckets open are released. The buckets are closed by tension on the two cables, the hinged flap (H) allowing water to escape during the descent but acting as a cover during hauling. Reproduced from Day (1978).

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quantitative sampler. As there are no reports of its use in the scientific literature, it may be considered as obsolete.

The *Baird grab* (Baird, 1958) was designed for sampling the epifauna of oyster beds, but has also been used in the open sea. It has two inclined digging plates that are pulled together by springs and levers (illustrated in Holme, 1964). It covers an area of 0.5 m^2 and has been found to dig into sediments quite well, having applications for sampling the infauna where a sample of large area is required. Since the surface of the sample is not covered, there may be some washing out during hauling. It had a very limited use and there are no recent references to the use of this sampler.

The *Hamon grab* (Fig. 5.15), originally designed by Oele (1978) for geological work, has been particularly effective in sampling coarse loose sediments. It is a robust device that samples an area of 0.29 m^2 by a rectangular scoop rotating through 90°. At the end of the movement the scoop locates onto an inclined rubber-covered steel plate, sealing it completely, thus reducing any loss of material from the scoop, a feature of many conventional grabs (Boyd *et al*., 2006). A smaller version of the grab, sampling an area of 0.1 m^2, introduced by CEFAS (Centre for Environment, Fisheries and Aquaculture Science; Boyd, 2002), has been widely used in many benthic surveys of the continental shelf. Dauvin (1979) reports that in spite of its considerable weight (350 kg) and size (height 2 m) it is easy to handle on board ship, and is capable of sampling the deeper infauna of sediments below 10 cm.

The *Holme grab* (Holme, 1949) samples by means of a single semi-circular scoop rotating through 180°. This design minimises loss of material while hauling, and a

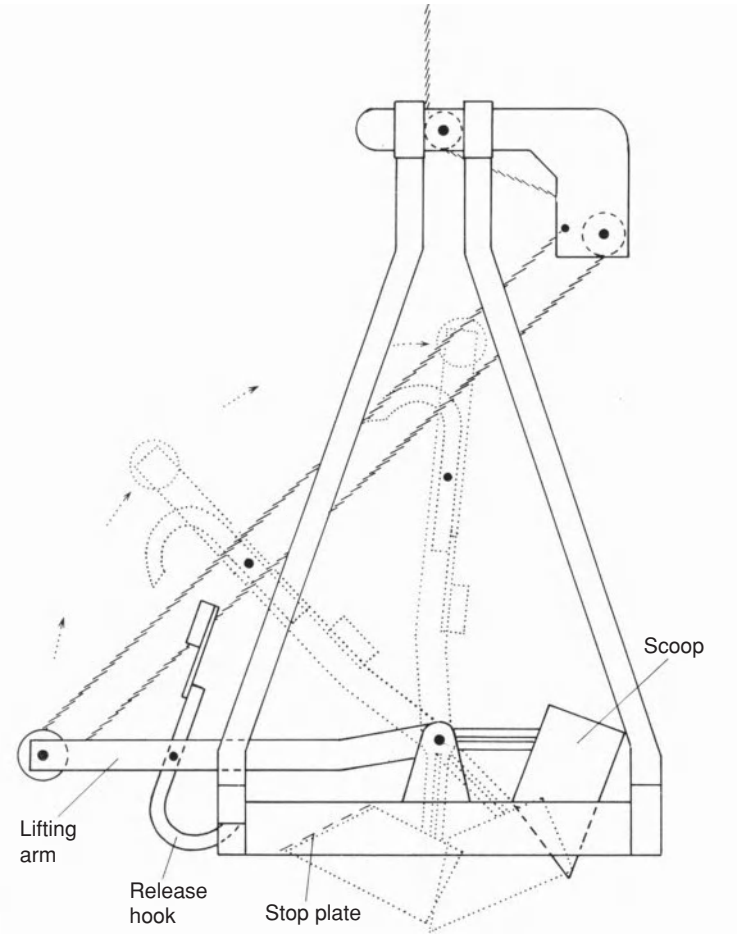

Fig. 5.15 Hamon grab, showing mode of action. The lifting arm rotates through 90° to drive the scoop through the sediment, closing against the stop plate. (After Dauvin, 1979.)

later model (Holme, 1953) with two independently-operating scoops reduces any tendency for sideways movement during digging. However, there is no evidence that this sampler has been extensively used.

The *Grizzle–Stegner grab* (Grizzle & Stegner, 1985) is another quantitative grab for shallow water sampling that combines features of several grabs such as the design of a van Veen grab mounted on a Smith–McIntyre frame. The grab has many attractive characteristics but despite the authors' assurances about its overall performance and reliability, it could be said that it has remained untried by the rest of the scientific community. Similarly, the Kingston Hydrostatic grab, designed by the Heriot Watt Institute of Offshore Engineering (Remote Technology Ltd, The Design Council) utilising hydrostatic pressure to open and close specially improved buckets, has also remained untried.

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The *Costerus grab*, a twin grab recently developed under the Marine Aggregate Levy Sustainability Fund (Barrio Frojan & Mason, 2010), is designed to improve sampling of coarse sediments while maintaining compatibility with existing data sets. The sampler takes two independent 0.1 m^2 samples at each deployment allowing separate samples for both biotic and abiotic parameters to be collected. The grab action is independent of the cable-pulling action common to many grabs and is reliant on a compressed air cylinder mounted within the grab body to complete the scooping action of the sampling buckets. When the grab lands on the seabed, a pressure trigger forces compressed air to push against independently-operated pistons forcing the twin sampling buckets through the sediment until they press against the fixed closing plates, effectively scooping up the sediment sample. In comparison tests against the mini Hamon grab, the Costerus consistently sampled a greater volume in each bucket and as a result biotic metrics depending on abundance and numbers of species were altered significantly, although particle size distribution patterns were not significantly different (Barrio Frojan & Mason, 2010).

The *Shipek sediment sampler* (Hydro Products Spec.Bull.3 pp) has a single semicircular scoop activated by powerful springs. Covering an area of only 0.04 m^2, this instrument is rather small for macrofauna investigations, and its sampling action tends to destroy any layering present in the sediment. Its main use is by geologists to obtain a small sample of bottom sediment. A similar spring-activated grab was described by Franklin and Anderson (1961), which, despite the large sample volume (2.5 litres) and the powerful closing mechanism, nevertheless, results in substantial loss of the superficial sediments.

Many small grabs such as the *Ekman* and *Birge-Ekman* grab (Blomqvist, 1990; see bibliography in Elliott *et al*., 1993) are suitable for use from a small boat on soft sediment, but since they cover an area of only about 0.04 m^2, they are not very suitable for macrofauna sampling. However, a modified Birge-Ekman grab has been designed by Rowe and Clifford (1973) for use by SCUBA divers or from deep submergence vessels, while Håkanson (1986) improved the Ekman grab by incorporating an automatic closing mechanism.

Box samplers and corers

Box corers and samplers, because of their reliability, have been extensively used in the deep sea (see Chapter 7). However, their use in shallower water studies is equally relevant; hence, a short outline of these samplers is given here.

The *Reineck box sampler* (Reineck, 1958, 1963) consists of a rectangular box corer supported in a pipe frame with a hinged cutting arm that is pulled down to close the bottom of the tube (Fig. 5.16). Designed and used mainly for geological studies, this instrument has also been used for the study of the benthic macroinfauna of the continental shelf and beyond. It samples an area 20 cm × 30 cm to a depth of 45 cm and weighs 750 kg, weighted for use. A similar instrument is described by Bouma and Marshall (1964). There have been a number of other modified versions

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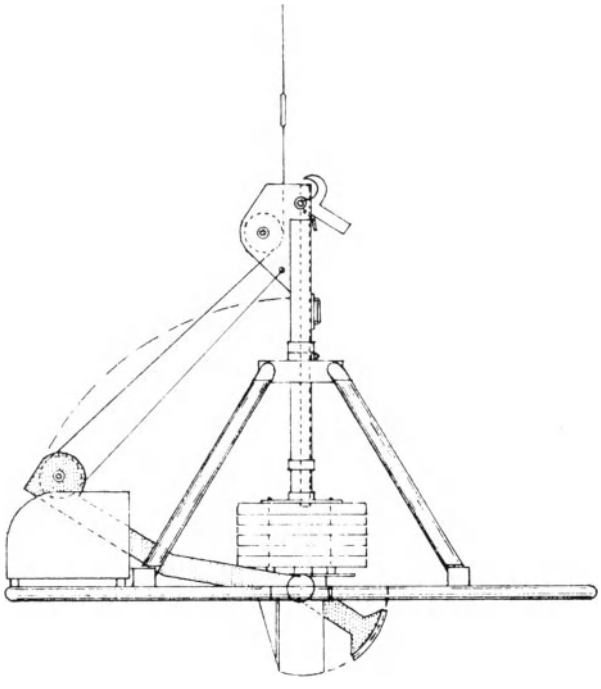

Fig. 5.16 Reineck box sampler. The rectangular coring tube is closed by a spade actuated by pulling up on the lever on the left. (Redrawn from Reineck, 1963.)

of the original box samplers: Hessler and Jumars (1974) describe a 'spade corer' (referred to as the USNEL Mark II box corer; Fig. 7.7), covering an area of 0.25 m^2, and further modified by Jumars (1975) in having the sample subdivided into 25 contiguous subcores. The main feature of this box sampler is the removable spade from the lever arm, which reduces handling time on board (Rumohr, 2009). Farris and Crezee (1976) improved the sealing of the corer so that it gave better retention of coarse sand cores, together with the overlying water. Another type of box corer, sampling 30 cm × 30 cm, is described by Jonasson and Olausson (1966). Another version (*GOMEX*) of this box corer was designed by Boland and Rowe (1991) with considerable improvements in its tripping and closing mechanisms. Box corer samples compared very favourably with other quantitative samplers. The *IOS box corer* described by Peters *et al*. (1980) has direct lowering control from the ship for penetration of the sediment, allowing elimination of the main framework and of a piston assembly previously needed to control penetration.

Box corers provide a means of obtaining deep and relatively undisturbed samples suitable for evaluation of macrofauna from a variety of sediments and are also suitable for deep-sea use. The surface of the bottom, and supernatant water, appear to be taken without undue disturbance, as evidenced by the persistence of animal tracks and burrows and sessile forms in their living positions in the samples (Hessler & Jumars, 1974).

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Owing to their large size and great weight, box corers are difficult to work, requiring a large vessel and calm conditions for safe deployment. The above authors state that launch and recovery are always challenging and potentially dangerous. Finally, box corers are very expensive, although they are rarely lost and seldom damaged.

Another much lighter box corer has been designed by the Institute of Oceanographic Sciences Deacon Laboratory (IOSDL) of the United Kingdom. The closing mechanism is activated upon contact with the bottom by two closely fitting shovels mounted on pivot arms. The extent of usage of the apparatus is not known.

Nevertheless, despite the opinions of several authors that they consider that deployment of box corers is difficult and potentially dangerous, because of the large sampling area, the undisturbed sample and their deep penetration, box corers have become standard oceanographic samplers widely used in many benthic surveys.

In order to overcome the problem of effective and meaningful sample replication, essential for providing better basis for describing benthic assemblages, a new generation of multiple corers initially inspired by the Craib corer prototype (Craib, 1965) has been designed. Barnett *et al.* (1984) pioneered the development of a multiple corer by producing a sampler in a range of sizes and weight and capable of taking 4–12 core tubes. The sampler is lowered into the sediment by a hydraulic damper mounted on a supporting frame enabling sampling to be carried out with minimum disturbance. The very positive characteristics of the Craib corer such as reliability in operation, penetration depth and minimum disturbance are maintained. However, having recognised that the Craib corer takes rather too small samples, mainly for meiofaunal and/or geo-chemical work, it was subsequently modified by Barnett (1998–1999) in order to take cores of different diameters from 65 to 114 mm. Despite some of the drawbacks encountered, the corer has been successfully used, and there is the prospect of still further improvements. A version of the multiple corer designed by Barnett *et al.* (1984) was refined and developed by Bowers and Connelly (Ocean Scientific International), who produced different sizes of the multicorer design, the largest of which, the megacorer, is equipped with 12×80 cm^2 cores (Glover *et al.*, 2008) and used for deep-sea work (see Fig. 7.8).

A much smaller and lighter sampler, the minicorer, nearly identical to the Barnett multiple corer (Barnett *et al.*, 1984), has been designed and used by the Alfred-Wegener Institute of Bremerhaven. However, the triggering mechanism is different and it can take simultaneously up to four deep and undisturbed samples. It has been successfully used in conjunction with a CTD system and an acoustic pinger.

A nine-core multibox corer, attached to a circular frame, sampling an area of 0.22 m^2 over 2–3 m^2 of seafloor is described by Gerdes (1990). The sampling boxes, activated by wire cables, penetrate into the sediment supported by the weight of the frame and they are sealed by a shear device upon lifting. It has been tried successfully in Arctic waters, but it does not appear to have received wide acceptance.

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There are a number of other corers that can be used in certain situations. These include the *Haps corer*, a frame-supported bottom corer with a round box and a flat spade (Kanneworff & Nicolaisen, 1973) and a number of narrower diameter corers more appropriate for meiofauna or geological studies – these are referred to in Chapter 6. However, a Haps corer with the same characteristics was successfully used for the study of macrofauna by Bagge *et al*. (1994).

Large diameter (>10 cm) gravity, piston or vibrocorers designed for geological work (McManus, 1965; Reineck, 1967; Burke, 1968; Pearson, 1978; see also Blomqvist, 1991; Elliott *et al*., 1993) are, on the whole, untried for biological work. However, their deep penetration, relatively large sampling area and effective core catchers (Kermabon *et al*., 1966; Burke *et al*., 1983) are features that could make them suitable for macrofauna sampling.

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Suction samplers

A number of samplers employ suction, either to force a coring tube down into the substratum or to draw the sediment and its fauna up into a tube leading to some form of self-sieving collector.

The *Knudsen sampler* (Knudsen, 1927) was the first sampler to use suction to take a core of a size suitable for sampling the macrofauna. A pump attached to the top of a wide coring tube (36 cm diameter × 30 cm long) is actuated by unwinding cable from a drum when the sampler is on the seabed. On lifting, the coring tube is inverted as it comes out of the bottom, so retaining the sample. The Knudsen sampler meets many of the specifications for the perfect sampler but as it falls over on the ocean floor it can be used only under calm conditions. Despite the improvement made by Barnett (1969), there is no evidence that this sampler has been used in recent years.

The same principle of suction sampling was adopted by Kaplan *et al*. (1974) who describe a manual and an automatic sampler for use to water depths of 3.5 m, and by Thayer *et al.* (1975), who describe a more complex device with a similar depth capability. Brown *et al*. (1987) also designed a vacuum sampler for the sampling of diverse types of substrata in shallow streams. A significant advantage of this sampler over other suction samplers lies in its successful recovery of undamaged organisms during pumping.

A number of samplers use pumped water to suck up samples of sediment and fauna. For example, the *Benthic Suction Sampler* of True *et al.* (1968) employs a jet of water acting through a venturi to suck a coring tube of 0.1 m^2 area into the bottom, the sediment and fauna being drawn up into a wire mesh collecting basket. The instrument is powered by a submerged electric motor or by pressure hose from the ship, and has even been successfully operated in deep water from a submersible. Other remotely controlled suction samplers are described by Emig and Lienhart (1971) and Emig (1977) (Fig. 5.17), while diver-controlled suction

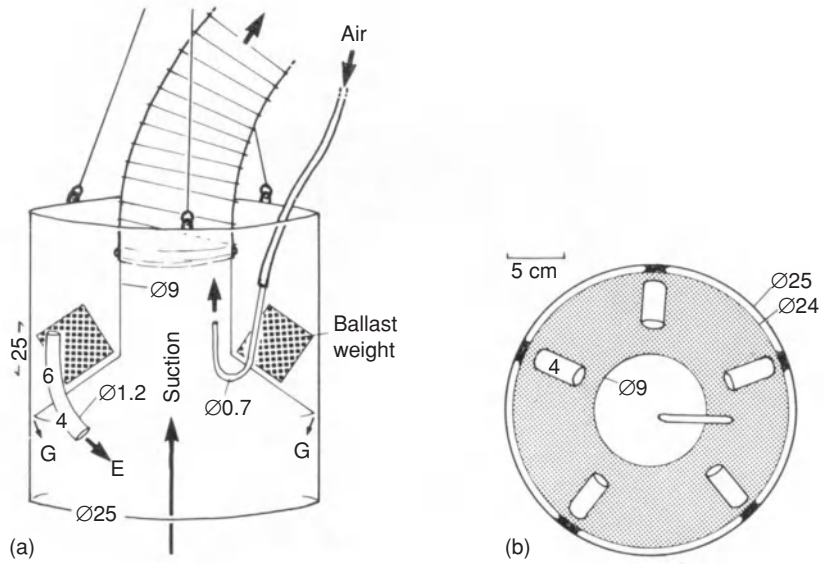

Fig. 5.17 Suction sampler of Emig (1977). (a) In profile; (b) from below. Air introduced through the small tube produces suction in the central tube, through which sediment and fauna is drawn up. The five compensating tubes (E) and the gab between cylinder and cone (G) are provided not only to enable the tube to dig into the substratum but also to help bring the sediment into suspension so that it is more easily collected. Diameters of the various tubes, in centimetres, are shown. Reproduced from Emig (1977), with permission from Springer and Business Media.

samplers are described in Chapter 4. Van Arkel and Mulder (1975) describe a hand-held corer, working on a counterflush system, which can be used from a small boat in shallow water (Fig. 5.18). A modification of this system, which enables the corer to suck itself into the sediment, is described by Mulder and van Arkel (1980). An instrument for deep sampling to 65–70 cm in intertidal sands is described by Grussendorf (1981), and another shallow water sampling system is described by Larsen (1974). Remote sampling by suction devices on difficult substrata has also been designed and successfully applied. The Drake and Elliott (1982, 1983) sampler has been designed to sample stony bottoms in fresh water biota, while a portable lightweight dredge for quantitative sampling was successfully used in a range of substratum material from very fine to very coarse gravel and coralline rubble (Brook, 1979). It should be noted that a large number of suction samplers are designed for sampling in the shallow waters of inshore areas in estuaries, lagoons and in fresh water habitats.

Some suction samplers are towed slowly along the bottom, sediment and fauna being sucked into a collecting bag. These include the vacuum sled of Allen and Hudson (1970), used for making quantitative estimates of young pink shrimps buried in the sand. In addition, there are hydraulic dredgers used for commercial harvesting of shellfish (e.g. Pickett, 1973; Morello *et al*., 2005). There are a number of diver-operated suction samplers (see Chapter 4).

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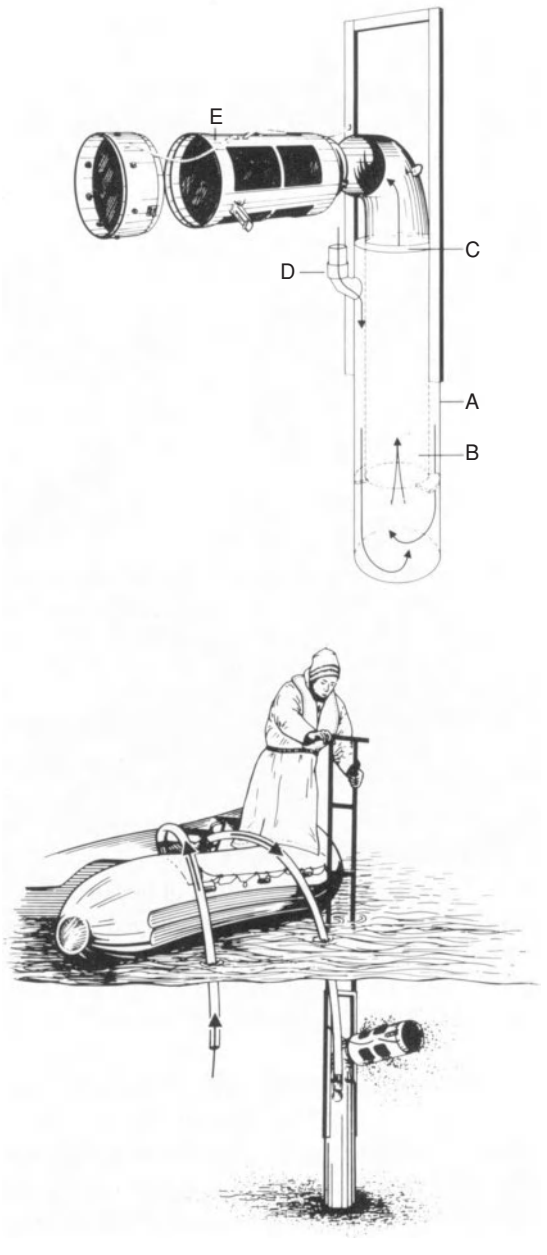

Fig. 5.18 Suction sampler of van Arkel and Mulder (1975). The sampler consists of two concentric pipes (A and B) united at the top (C). Water is injected through D. During operation the sampler is pushed into the sediment, a mixture of water, sediment and organisms passing up pipe B to the cylindrical sieve E.

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Other methods of sampling

Some species are not readily taken by conventional sampling gear such as dredges, trawls or grabs, either because they occur too sparsely to be represented in samples covering a limited area or because they live in habitats inadequately sampled by the instrument employed. Alternative methods, which do not necessarily sample other members of the fauna, are available for such species. Techniques for underwater photography and television, of special value in estimating scarce members of the epifauna, are described in Chapter 4.

After storms, burrowing animals are often washed in from shallow water on to the beach, and this may be the best means of obtaining some deep-burrowing species not readily taken by ship-borne samplers. Empty mollusc shells and other recent remains cast up on the beach are usually some guide to the nature of the shallow-water offshore fauna. Occurrence of burrowing species on the sediment surface following dinoflagellate blooms has been noted by Dyer *et al*. (1983) and others.

Fish stomachs often contain deep-burrowing or active members of the benthos seldom taken by sampling instruments, but as the fish are likely to have been feeding selectively, little idea of the abundance of the prey species can be obtained by this means. Similarly, the stomachs of birds feeding in estuaries and salt marshes may show the presence of otherwise unreported species.

Species of fish and crustaceans that hide away in rock crevices may be taken by using one of the chemical fish collectors that drive them out of their hiding places. Some such as Rotenone are toxic, but the Quinaldine compounds (Gibson, 1967) have an anaesthetic action and usually cause no permanent damage. The range of chemical techniques available, together with other methods such as use of explosives, are reviewed by Lagler (1978) and by Russell *et al*. (1978) for coral reefs. An electrofishing technique, claimed to be less selective for sampling rock lobsters in that 'soft' individuals that have moulted are also taken, is described by Phillips and Scolaro (1980).

Free-living invertebrates may be captured in baited traps or with light lures. Baited traps have been used to capture mainly scavenging crustaceans in shallow water (Groenewold & Fonds, 2000; Morton & Lee, 2012). Baited traps have also been used to study the effects of benthic scavengers on the damaged benthos resulting from beam trawl fishery (Lindeboom & de Groot, 1998; Groenewold, 1999; Groenewold & Fonds, 2000). Different types of trap are described in the commercial fisheries literature, including Davis (1958), von Brandt (1972), FAO (1972), Kawamura and Bagarinao (1980) and Motoh (1980). A net trap for sampling epibenthic crustaceans of coral reef systems operated by a diver was designed by Carleton and Hamner (1989). The trapping effectiveness of this device was found to be several orders of magnitude greater when compared with towed sleds and plankton nets. Traps for invertebrates and small fish, some with light, are described by Zismann (1969), Beamish (1972), Espinosa and Clark (1972), Ervin and Haines

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(1972), Thomas and Jelley (1972) and Haahtela (1969, 1974). Traps also have been used in the deep sea (see Fig. 7.4).

There are many active animals, both fish and invertebrates, that are poorly sampled because they actively avoid trawls and other towed gear. There is still a need for the development of methods for their assessment, although underwater photography and television also give good results for some species (e.g. Kanneworff, 1979). An attempt to sample such populations by a cage lowered on to the bottom is described by van Cleve *et al.* (1966). On a smaller scale, the use of throw traps for sampling small fish in shallow marshes is described by Kushlan (1981) while Polte *et al*. (2005) used metal drop traps in inundated tidal flats for sampling the mobile epibenthos and fish. References to small drop and pull-up traps and drop nets are given by Aneer and Nellbring (1977). Pots and creels of different design are baited traps set down on the seabed to catch primarily commercially important species such as crabs, lobsters, etc. However, they also inadvertently catch many other invertebrates such as gastropods, starfish and other predators. Collectors of benthic animals settling on hard substrata have been used experimentally, the results of which can be found in a large bibliography. Substrate settlement units allow the assessment of recruitment responses of epifaunal and infaunal invertebrates (Martel & Chia, 1991; Whitlatch & Osman, 1998).

5.3 Working sampling gear at sea

Continental shelf

In the earlier part of this chapter (p. 189), comments were made on the working of dredges and other towed gear. Grabs and other instruments operated vertically require a different technique. When using a grab of moderate weight (up to, say, 100–150 kg) it is important not to use too thick a wire for lowering: many grabs are not hydrodynamically shaped and sink rather slowly, so that if too heavy a wire is used it may form a loop below the grab, causing kinking or entanglement. For the same reason the grab should be lowered at steady speed with gentle braking (or reverse torque) on the winch. This also helps to prevent the wire slackening as the ship rolls, which can trip the release prematurely. A light grab can be worked on a 6 mm wire, but some workers prefer to use a neutrally buoyant rope, which overcomes some of the above difficulties.

As soon as bottom is reached, paying out should be stopped, and hauling should commence. Any delay will increase wire angle if the ship is drifting, causing the instrument to be pulled out obliquely, so that it samples less effectively. However, it is important to haul in slowly and steadily until the sampler has left bottom because, with most grabs, closing is completed as hauling commences, so that if the warp is suddenly pulled up the grab will tend to be jerked out of the bottom while it is still closing. In addition, great strains are produced in releasing a sampler that has dug deeply into the sediment, and these may cause the gear to buckle or the warp to part.

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On the other hand, many grabs are not stable on landing and direct observation by photography or video have shown sideways tipping and somersaulting of the grab, which can put serious doubts on the quality of sampling (Kingston, 1988). However, it should be noted that grab sampling from a drifting ship introduces an important discrepancy when replicates are also taken from that station. Nevertheless, the ship can be maintained in position during grab deployment using the Dynamic Positioning System and the sampling location can be fixed by the Differential Global Positioning System (DGPS).

The main problems with towed gear relate to the rate of paying out of the wire, the total length paid out, and the ship's course and speed throughout the operation. Failure to take a sample may be due either to paying out too much warp, or at too high a speed, so that the wire becomes entangled with itself or with the gear, or too little may be paid out so that bottom is never reached. For dredges and Agassiz trawls the gear can either be lowered from a stationary ship, using a dynamic positioning system, or the ship can go ahead slowly while shooting is underway, which is essential for working otter trawls (Laubier *et al.*, 1971). A heavy weight attached to the wire some distance ahead of the dredge aids descent, and gives a more horizontal pull on the gear, thus reducing the length of wire to be paid out. Little and Mullins (1964) have shown that diving plates increase the speed of descent of a beam trawl, and reduce the length of wire required.

Even when the trawl or dredge has reached bottom it may not function satisfactorily. Menzies (1972) (also, Menzies *et al.*, 1973) describes results from mounting a forward-facing time-lapse camera in the mouth of a 1.52 m trawl. It was found that in a 90-minute tow the net was fishing normally for only about four minutes, and that most of the time was spent 'twisting, flipping and flopping' over the seabed, spilling its contents back on the seafloor, or being buried mouth first in the mud. The use of pingers and direct video observation would seem to overcome many of the uncertainties attached to the achievement of satisfactory results with such gear.

Exploration of canyons on the continental slope requires special care. Where the bottom is steeply sloping it may not be possible to get a reliable depth sounding, because of echoes from the side walls of the canyon, and it may be difficult to place the gear in the required location on the seabed. Unless the canyon is particularly well charted, and accurate position fixes can be obtained, working any type of gear may be hazardous. Because of the risk of loss it is inadvisable to use expensive instruments such as pingers, and indeed pinger signals may give a false impression by failing to give a return echo from vertical cliff-faces towards which the gear may be drifting.

For general purpose collecting on the slope and in canyons, a sturdy rock-collecting dredge (Fig. 5.7) with safety link can be used. This should be lowered vertically from the ship, which then drifts or steams slowly towards the canyon wall. Manoeuvring of the ship at slow speeds is more easily accomplished if the ship is steaming against the surface current. Exploration of canyons can only be carried out in good weather, and even then frequent losses of gear are to be expected.

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Diving submersibles and Remotely Operated Vehicle (ROVs) have been used for exploration of the seafloor and have been instrumental in important discoveries of sea life and conditions on the seafloor (see Chapter 7 concerning their importance in the exploration and investigation of hydrothermal vents).

Recovery of lost gear

With the increasing sophistication of underwater gear and the development of 'instrument packages' of electronic equipment, often purpose-made, the action to be taken should such valuable gear be lost or become fouled on the seabed ought to be anticipated. The advent of widely available and easily used multi-beam sonar systems means that it is now possible (and advisable) to survey with great accuracy sampling locations prior to starting a sampling programme. Such surveying can identify areas possibly unsuited to the deployment of such instrument packages and, possibly more importantly, with their sub-metre resolution should be able to locate 'lost' equipment if necessary.

Loss of gear can be minimised by ensuring that all wires, shackles, swivels, etc., are in good condition and of appropriate size, and that weak links will fail when required to do so. Very often loss occurs through parting of the wire or associated components close to the underwater towing point either: (i) at the sea surface, through increasing strains as the gear and sample are brought out of the water, or the gear is accidentally hoisted right up into the towing block through failure to stop the winch; (ii) on the seabed through entanglement with underwater obstructions, the side walls of canyons, etc. or (iii) when recovering gear from on or in the seafloor, being hoisted by an oblique upward pull thus increasing the strain, which may result in the wire parting.

Apart from precautions to minimise loss, the following guidelines are suggested to aid recovery:

(1) Name, address, fax and telephone numbers and e-mail should have been conspicuously marked on the gear.
(2) On towed instruments, a light line (length equal to 2–3 times depth) with marker float is attached to the gear. This will greatly aid underwater location by a diver, particularly near the limit of diving depth.
(3) A similar pop-up marker, automatically released after a time-delay, with light and radio beacon, may be used.
(4) The possibility of designing the equipment so that an instrument package can be released independently of the main framework should be considered. This might be brought up on a light line, or on a pop-up buoy system with preset time-delay.
(5) On vertically lowered gear it may be difficult to attach a marker line because of the likelihood of its twisting around the lowering wire. Under such circumstances, and whenever valuable equipment is involved, an acoustic transponder

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(pinger) that can be interrogated by sonar from the ship will allow location after an interval of weeks or even months. Below diving depths the possibility of recovery will depend on the value of the lost gear, and whether a submersible can be deployed for salvage.

It is suggested that by common practice, if not in law, gear on the seabed that has been clearly marked as indicated above is not 'lost', and could not be the subject of a salvage claim. It is assumed that at the time of loss the ship's master would log the position, taking account of any transit lines to the shore. In the event of none of the suggested procedures being carried out an anchored marker float (which should have been made ready in advance) should be dropped over the side without delay. Grapnels and trawls have, on occasion, been used with success for recovery of lost gear. For more expensive instruments and samplers, insurance cover, if available, should be considered although premiums may prove to be uneconomic for many projects.

5.4 Efficiency of benthos sampling gear

The efficiency with which a sampler operates is related both to its design and to its mode of operation. Consequently, there are many variations and modifications of the standard sampling equipment, made by many workers in an attempt to improve a sampler's efficiency. Sampling efficiency is a useful concept only when referring to quantitative or semi-quantitative gear.

Dredges and trawls

Most dredges, defined as collecting instruments that are towed along the bottom, are at best semi-quantitative. When used to collect fauna living on or just above the bottom, the efficiency of a dredge, judged by its ability to capture all the animals within its sweep, is usually low. The performance will vary with the configuration and nature of the bottom and since several types of ground may be encountered on any given tow, it is difficult to allow for such variations. Other complicating factors are the behaviour of the ship, the length of warp and the speed of towing – increased speed above a low level usually reduces catches. Further, the type of warp used can affect performance. Wire, with a weight in water greater than the drag, can increase the effective weight of the dredge on the bottom, while rope, with the drag greater than the weight and a consequent backward and upward catenary, can produce a lift. The fitting of depressors or diving plates, tickler chains and the proper use of teeth on the leading edge can increase efficiency (Baird, 1959). Attempts have been made to employ odometers to measure the exact distance covered by the dredge on the bottom (see p. 192), which should help to quantify results.

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The behaviour of the animals themselves is also of significance and if sampling is related to only one species, with consequent reduction in the range of habitat and behaviour encountered, it should be possible to select the appropriate sampler or to make appropriate gear modifications to increase efficiency. Yet Dickie (1955) has shown that dredges specifically designed to capture scallops had an efficiency of only 5% on uneven inshore grounds and just over 12% on smoother offshore areas. However, somewhat higher efficiencies were reported by Chapman *et al*. (1977). Again, juvenile stages of a flatfish have been the subject of population studies in Britain, and gear has been developed for their capture. Since they occur on relatively flat sandy grounds in shallow water where their behaviour can be observed by divers, it may be expected that high gear efficiencies should be possible. Riley and Corlett (1965) used a 4 m beam trawl and found it worked best when towed at a speed of 35 m min^{-1} with three tickler chains attached. Creutzberg *et al*. (1987) studied the effect of different numbers of tickler chains on beam trawl catches. Efficiency ranged from 33% to 57%, and even for fish in their first year of life it varied considerably at different times of the year, depending on the size and age of the fish. In other experiments, Edwards and Steele (1968) found a catching efficiency of a 2 m beam trawl for plaice to range between 23% and 37%. However, efficiencies of this order seem to be exceptional for dredges, and when total fauna is considered, a value nearer to 10% is probably more realistic (Richards & Riley, 1967). One important development in estimating the capture efficiency of commercial trawls lay in the application of diving techniques (Hemmings, 1973; High *et al*., 1973), originally by divers clinging on the headline of the trawl, and subsequently by the use of a two-man submersible. This permits easy and safe observation as well as the use of underwater television and photography (Chapter 4). Caddy (1971) and Chapman *et al*. (1979) made field observations concerning the swimming behaviour of pectinid bivalves in response to dredging while Eleftheriou and Robertson (1992) assessed experimentally the efficiency of the scallop dredges.

Grabs

The concept of efficiency is more meaningful for grabs, which are lowered on (ideally) a vertical warp from a stationary ship with dynamic positioning, to take a deposit sample of a given surface area. In this context, the term ‘efficiency’ tends to be used loosely to cover various purely functional aspects of an instrument’s general performance and digging characteristics, as well as its ability to produce an acceptable picture of animal density and distribution. Although all these uses of the term are related, they should not be confused.

Performance

Considering first the functional aspect, this refers primarily to the ability of a grab to perform consistently and correctly, according to its design, in all conditions of

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deposit, depth and weather. These features are perhaps better covered by the word 'reliability' rather than 'efficiency' and are best judged by the volume of deposit collected, so that an instrument that is filled to capacity on every haul would be regarded as completely reliable within the limits of its design. The first requirement for reliability is that the grab must land on the bottom in a condition in which it will operate properly. Any grab that is activated by the slackening of the warp when the gear strikes bottom will tend to be set off in mid-water by the roll of the ship, and so may be difficult to use in bad weather or deep water. An instrument that is not stable when in an upright position on the bottom or that must remain at rest for a time to collect the sample may be upset by strong currents or by an oblique upward pull on the warp. Once correctly on the seabed, a grab-type instrument covers a known surface area, and, assuming that it can be raised by a reasonably vertical warp and not pulled laterally off the bottom (the skill and experience of the operator is often important here, and comments on the use of grabs at sea are given on pp. 214–215), then the extent to which it attains its maximum depth and, therefore, greatest volume of sample, depends largely on the weight and the design of the instrument and the nature of the substratum. However, it should be noted that a grab, such as the Costerus twin grab (Barrio Frojan & Mason, 2010) operated by compressed air, is entirely independent of the pulling action of the winch cable and likely to take consistently deeper and larger samples. On soft mud, most grabs will fill completely, but on the most difficult grounds of hard-packed sand conventional grabs will merely scrape the surface unless they are adequately weighted, and even those instruments that achieve some initial penetration by spring loading (Smith & McIntyre, 1954) will be raised off the bottom if they are not heavy enough. However, Riddle (1989) showed that the ratio of initial penetration to weight indicated that weight is not the only factor that influences the grab performance. If all of these factors are satisfactory the volume of deposit will serve as an index of the depth of penetration, but will not give an absolute measure unless the exact profile of the bite is known.

Given that a particular grab is fully reliable, as discussed above, one would further wish to know, still dealing with the functional aspect, how well its design allows it to collect the deposit below the surface area that it covers. This is the sense in which Birkett (1958) used the term 'efficiency', which he defined as the ratio between the volume of sediment collected and the theoretical volume, which is calculated by multiplying the area covered by the deepest penetration depth. This could perhaps be called the index of digging performance.

In recent years, a generation of suction samplers has been introduced, working on the principle that the deposit can be raised by jets of air or water (Drake & Elliott, 1982). Such samplers tend to have a high digging performance (especially if operated by divers – see Chapter 4) since they can lift all of the deposit to a given penetration depth from within their area of operation. In contrast, it was considered until recently that the biting profile of grabs with horizontally placed spindles was more or less semi-circular and that the digging performance of such grabs was,

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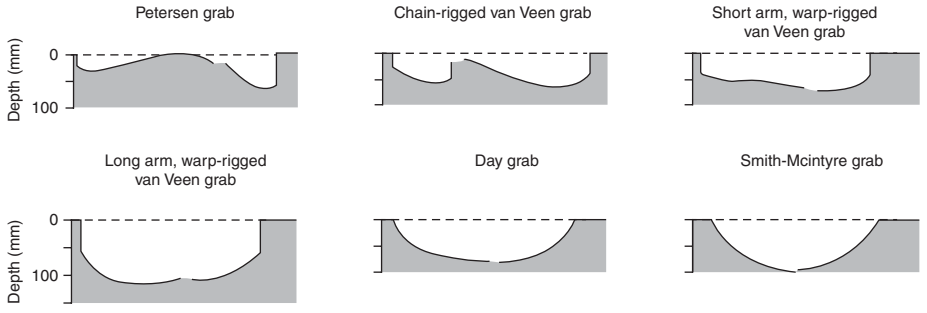

Fig. 5.19 The bite profiles of six grab samplers tested on medium to fine sands (only one bite profile is shown out of three for each type of grab). (Redrawn from Riddle, 1989.)

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therefore, low, since the deepest part of the bite sampled only a fraction of the surface area spanned by the open jaws. However, observations by Gallardo (1965), Lie and Pamatmat (1965) and Ankar (1977a) indicate that the profile is more nearly rectangular, and that divergences from a true rectangle can be explained in terms of the closing mechanisms of the various grabs. Thus, the Petersen and the chain-rigged van Veen, which have an upward leverage as the jaws close, tend to leave a hump of deposit in the middle of the sampling area, while the Smith–McIntyre, with a downward pull on the arms as the jaws close, digs deeper in the middle of the sampling area and thus takes a rather larger volume than the chain-rigged van Veen, for the same degree of penetration. These grabs thus appear to have higher digging performance than had been supposed, and on hard-packed sands this can be increased by the addition of extra weights. In contrast, Riddle (1989) (Fig. 5.19) studying the bite profiles of six grabs (Petersen, chain-rigged van Veen, short arm warp-rigged van Veen, long arm warp-rigged van Veen, Day, Smith–McIntyre) concluded that only the two designs of warp-rigged van Veen grabs took deep and parallel-side bites. The long arm warp-rigged van Veen performed best by taking deep samples over the whole of the sampling area. Riddle (1989) also concluded that bucket design is of primary importance in the performance of grabs. Redesigning the bucket shape in order to optimise penetration and upward movement on closure is of primary importance.

Evidence from published accounts shows that heavily weighted single bucket grabs (such as the Hamon or the Costerus) perform well with deep penetration even in mixed or coarse deposits (Dauvin, 1979; Barrio Frojan & Mason, 2010).

Efficiency of capture

The second aspect of efficiency is more complex and relates to the ability of the gear to collect the fauna so as to give a reasonably accurate picture of its density and distribution. This may be called the efficiency of capture. Few studies have been made of how efficient a particular grab is in capturing all the fauna below a

given surface area. This was attempted by Lie and Pamatmat (1965), who compared collections taken with a 0.1 m^2 van Veen grab at high tide with hand-dug samples of the same unit area at low tide. They showed that for the most abundant species there were significant differences between the two sets of samples in only 8 out of 37 cases.

Efficiency of capture may be defined as the ratio of the number of animals in the volume of deposit collected by the grab to the number present in the same volume *in situ.* By using as the denominator of this ratio the same volume as was collected rather than the theoretical volume based on a rectangular bite, the ratio excludes the functional efficiency of the grab and deals only with its ability to capture the fauna available to it. Long and Wang (1994), comparing the capture efficiency of benthic sampling devices, recommended that differences in abundance (minimum detectable difference) is based on the mean to the standard deviation ratio rather than on absolute differences.

One possible cause of low efficiency of capture is the downrush of water caused by the grab's descent, which may disturb the surface of the deposit and result in the loss of superficial fauna. Smith and McIntyre (1954) considered that the use of gauze windows on the upper surface of their grab reduced this, producing higher catches of small crustaceans on sandy grounds, and this is supported by experimental work by Wigley and Emery (1967) who studied the behaviour of grabs by motion pictures. On muddy grounds disturbance of the surface layers may present a serious problem, as has been shown by Andersin and Sandler (1981): comparison of the efficiency of two types of van Veen grab showed that large windows at the upper surface of the buckets minimised the shock wave, resulting in increased efficiency by an average of 50% for small amphipods and by 80% for small polychaetes, as compared to a grab with small windows. It should be noted that many grabs and corers in use have been provided with gauze windows on the upper surface of the instrument. It could be pointed out that single bucket grabs such as the Hamon grab have a distinct advantage concerning the shock wave produced by other grabs, while a rubber-sealed metal plate prevents the loss of material experienced with other conventional samplers (Kenny & Rees, 1994; Robinson *et al*., 2005). It is, nevertheless, still probable that most sampling instruments, even when landed carefully on the bottom, will not sample the fine surface layer adequately. Apart from this aspect, even a sampler that fulfils the criteria of reliability as described above, i.e. that consistently takes its maximum volume, may not be the most suitable for every task. For example, one with a low or medium maximum penetration will not sample adequately the deep-burrowing animals, and an instrument that covers only a small surface area, no matter how efficient as a machine, may be quite unsuitable for sampling widely dispersed species. Boyd *et al*. (2006), when comparing the efficiency of two sizes of Hamon grabs (0.1 and 0.25 m^2) in sampling the infauna of coarse deposits, came to the same conclusion. However, comparison of 0.1 m^2 Hamon grab with the same size Costerus grab (Barrio Frojan & Mason, 2010) showed that the latter collected

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larger volumes of sediment, resulting in a greater biomass, abundance and number of species.

In conclusion, selection of the most 'efficient' grab involves consideration of reliability, digging performance and capture efficiency, which are, in turn, influenced by such factors as the size of the sample required, type of deposit, depth of water, prevailing weather conditions, handling facilities available and the experience of the operator. The influence of such factors described above were exemplified in Heip's work (Heip *et al*., 1985) where no statistical difference between the macrofauna of the silty sediments of the North Sea, sampled by van Veen and box corer, was found.

Corers

From the purely functional aspect, coring devices have a higher digging performance and most tend to be relatively reliable in that a particular instrument of a given weight will usually provide consistently similar lengths of core, depending on the type of sediment sampled. The main difficulty may be loss of the sample during ascent, and on sandy grounds a core retainer is usually required.

As a means of providing quantitative data on the fauna, the main criterion of a corer's efficiency must be the accuracy with which the collected core represents the sediment column as it was *in situ*. Only the larger versions of the different types of corer used in meiofaunal and geo-chemical studies are suitable for the efficient sampling of macrofauna. With wide cores (>10 cm diameter), core shortening due to wall friction, observed with the narrow diameter corers, is not noticeable and undisturbed cores can be obtained. Design of the more recent corers minimises the downwash effect, which reduces the risk of losing the superficial layer of sediment.

In conclusion, the most efficient corer will be one that takes relatively undisturbed samples, penetrates deeply, and shows the smallest loss of surface layers. Box corers conforming to most of these characteristics have a distinct advantage over many other core samplers (see also Blomqvist, 1991).

Comparative efficiency

Many workers have tested sampling devices and provided information as to their comparative efficiency. The criteria used in these comparisons are:

(1) The digging characteristics of the sampler (depth of penetration, volume of sediment, degree of disturbance).
(2) The efficiency of capture in order to give a representative picture of the density and distribution of the fauna.
(3) Technical characteristics of the samplers (ease of manipulation, weight, ease of access to the sample, safety aspects, mechanical reliability, etc.).

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A selection of works dealing with the comparative efficiency of different samplers and sampling methods is given in Table 5.1 and a more complete bibliography of works on the comparative efficiency of samplers can be found in Elliott *et al*. (1993).

5.5 Choice of a sampler

The choice of an appropriate sampler must necessarily be a compromise, based on the overall aims of the survey (a comprehensive account of the practical considerations relating to the choice of sampler is given by Lampadariou *et al*. (2005) and Boyd *et al.* (2006)), working conditions and the availability of suitable gear (see Blomqvist, 1991). Table 5.2 represents an attempt to show the main characteristics of the samplers described in this chapter. Choice may often be restricted by weight, and the heavier equipment commonly used for deeper work (large grabs, epibenthic sledges, box corers) can only be worked from large research vessels that have the necessary lifting equipment (see Chapter 7). Many of the lighter samplers, on the other hand, have serious limitations imposed by their being less efficient, having more limited penetration into firm sediments or sampling too small an area.

Sampling of firmly packed sediments presents particular problems, since many instruments fail to penetrate sufficiently deeply to sample all the fauna. If the objective is to sample to $\geq$ 20 cm, choice is limited to a few samplers, all of which have restrictions on their suitability. The Forster anchor dredge is a useful instrument for this purpose, but it samples most effectively when used in shallow water and from a small launch, and its samples must be considered as only semi-quantitative. Box corers and multicorers are only suitable for use from large ships, and some grabs and corers take samples that are of too small an area (0.015 m^2) for general macrofauna studies. Others take samples of adequate depth and surface area, but they are unsuitable for open-sea conditions and for deeper water. Some suction samplers, whether remote- or diver-operated, are mostly restricted to shallow water, and generally sample less well in cohesive muds.

Where quantitative samples are required, using an instrument of only moderate weight, it must be accepted that penetration of firm sediments is unlikely to be adequate to capture the deeper burrowers. Choice is limited to a few grabs, notably the warp-rigged van Veen, Smith–McIntyre and Day grab, some corers and the smaller version of box corers. Most other grabs sample too small an area, do not dig sufficiently deeply or have other drawbacks not necessarily indicated in Table 5.2. The Baltic Marine Biologists have adopted the 0.1 m^2 van Veen grab as the standard sampling instrument for macrofauna (Dybern *et al*., 1976), but this is not the best choice under more open-sea conditions, for which purposes some workers have adopted the Smith–McIntyre grab, being substituted by its modified version of the Day grab. The latter is of simpler construction than the former, and is without springs, making it safer in use. It appears to dig about as deeply as

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the Smith–McIntyre grab. For the sampling of the macrofauna of offshore coarse sediments, the Hamon grab has been adopted by many workers as it can take large samples and sample the deeper infauna (Robinson *et al*., 2005; Boyd *et al*., 2006; Bolam *et al*., 2008). The recently designed Costerus grab (Barrio Frojan & Mason, 2010) has some positive features and sea trials showed that it performed well in sampling mixed sediments.

5.6 Treatment and sorting of samples

A benthos sample usually consists of a volume of sediment from which the animals must be extracted. Macrofauna samples may vary in size from a few to many litres, and the extraction process is often divided into two stages, the first being carried out in the field with a view to reducing the bulk of material to be taken back to the laboratory, where the second stage of separation takes place.

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Initial treatment

At sea, the standard procedure is to receive the bottom sampler on deck on a wooden or metal-lined sieving table or hopper, which should be designed in such a way that the sampler can be emptied and the contents washed through a sieve without loss of material. A measure of the total volume of deposit collected is often required, since this helps in assessment of the performance of the gear. The volume may be measured either by using a dip stick before emptying the sampler, or by arranging that the contents pass into a graduated container before being sieved. Methods of dealing with the sample have been reviewed by Holme (1964), and vary from the simple arrangement of McNeely and Pereyra (1961), which consists of washing the sample through a nest of sieves with a hose, to the elaborate set-up described by Durham (in Hartman, 1955) in which a mechanical shaker agitates a graded series of screens under a set of sprinkler heads. Similarly, Rumohr (1999) provided a useful overview of sieves and sieving procedures for the sediments. A small hopper (Fig. 5.20) for general use has been described by Holme (1959) but there is probably no single set-up that would be satisfactory for the full range of sampling instruments and working conditions, and it may be desirable to modify an existing pattern appropriately to suit particular needs, or to construct a new unit such as the combined grab cradle and wash trough (Fig. 5.21) designed by Carey and Paul (1968) for the Smith–McIntyre grab. A more recent design of a sieving table with hand-controlled water sprinkler is given by Rumohr (1999) on a design provided by G. Fallesen.

Other mechanical means for washing large numbers of samples are given by Pedrick (1974), who describes a non-metallic device suitable for pollution studies. For the processing of large samples (over 20–30 litres), an elaborate system has been used by M.H. Thurston and R.G. Aldred (Institute of Oceanographic Sciences) (Fig. 5.22). The sample is placed in a large tub and, after preliminary hand picking

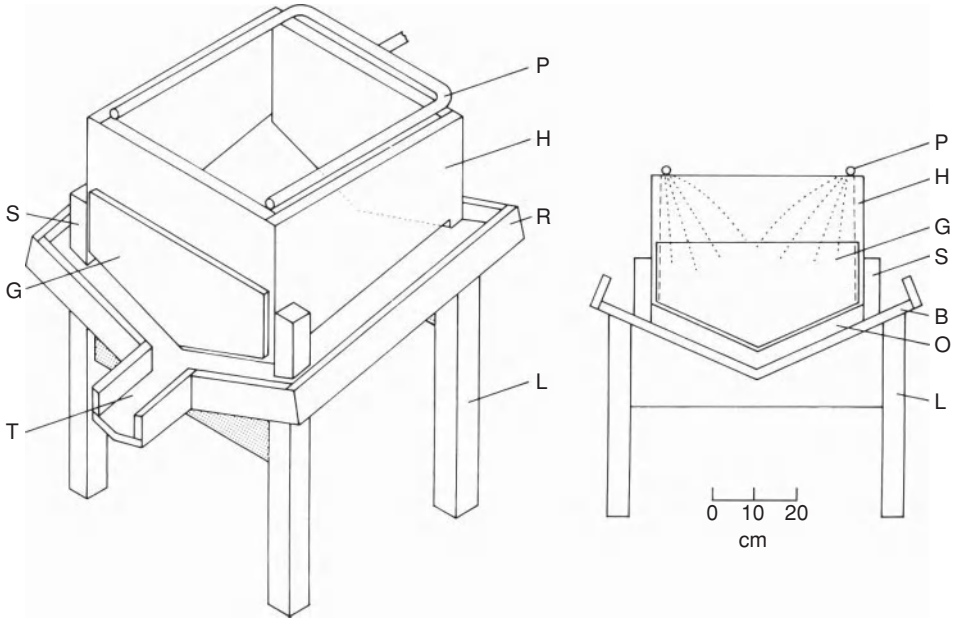

Fig. 5.20 Small hopper for general treatment of sediment samples (Holme, 1959). Left, general view; right, cross-section. P, pipes supplying jets along the top of hopper; H, side wall of hopper; R, retaining wall at side of base (B); T, spout; G, rising gate; S, short legs supporting hopper off base; L, legs; O, gap between hopper and base.

of the larger animals, it is passed through a series of sieve baskets (16, 2 and 0.5 mm mesh) where it is separated by washing and agitation into three separate size classes. Each size class is kept separately and is transferred to containers for fixation. The system, which is operated by one or two persons, enables large samples (200–300 litres or more) to be processed in a reasonably short time.

The Wilson Autosiever (Fig. 5.23), described in Proudfoot *et al.* (2000), employs a water sprinkling system that gently washes the underside of the sieve. This is

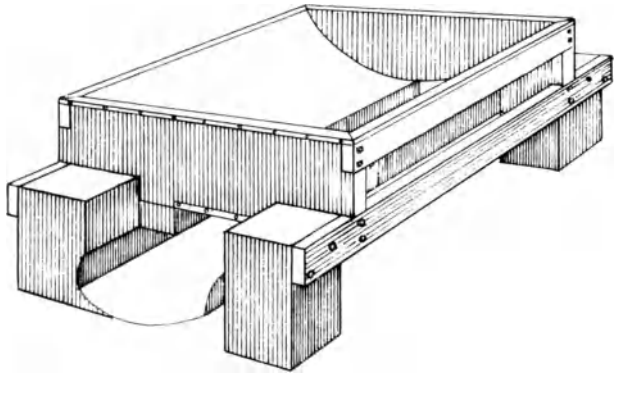

Fig. 5.21 Combined grab cradle and wash trough. (Redrawn from Carey & Paul, 1968.)

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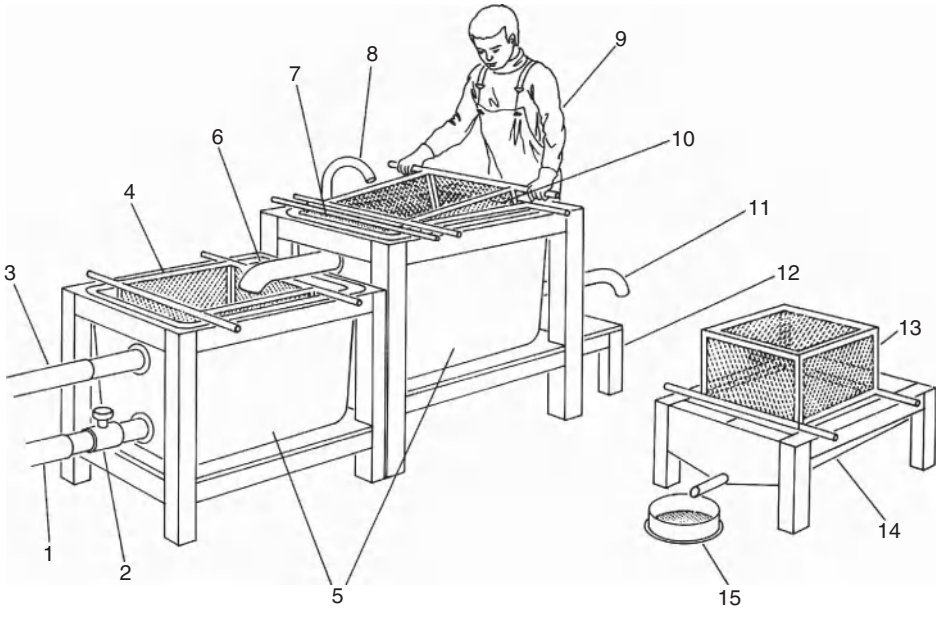

Fig. 5.22 Equipment for washing and sieving sediment samples (Institute of Oceanographic Sciences). 1, Hose for draining lower tank; 2, valve; 3, outflow hose for lower tank; 4, 500 μm mesh basket; 5, fiberglass tanks; 6, outflow for upper tank; 7, 2 mm mesh basket; 8, water supply; 9, operator agitating sieve basket; 10, 16 mm mesh basket; 11, outlet and valve for draining upper tank; 12, retractable step; 13, inverted sieve basket; 14, basket washing trough with V-section, sloping bottom; 15, sieve.

claimed to lead to more efficient operation in terms of time and the condition of the retained specimens and in maintaining the apertures clear can also minimise retention of meiofaunal organisms. The autosiever introduces, for the first time, a level of reproducible automation, which can also reduce the potential safety risk of back problems for operators. Proudfoot *et al*. (2000) did not find significantly increased faunal statistics, but they describe a case study from IRTU in Northern Ireland that found increased numbers of species being returned from samples processed through the autosiever.

The surface area of the sieve may depend to some extent on the design of the sieving table or hopper, but, unless the sample is to be washed through slowly in small sections, the sieve must be of a certain minimum size to allow an adequate sieving area and to prevent clogging by the sediment. For samples of more than a few litres a sieve of at least 30 cm × 30 cm is desirable. Washing can be done by single or multiple jets from a hose, but unless this is very gentle and, therefore, time-consuming, it can damage the animals. A gentler method, described by Sanders *et al.* (1965), utilises a large vessel with spout about one-third of the way up. A continuous stream of water passes through the vessel, carrying the animals over into a sieve. If the animals are required in especially good condition sieving should

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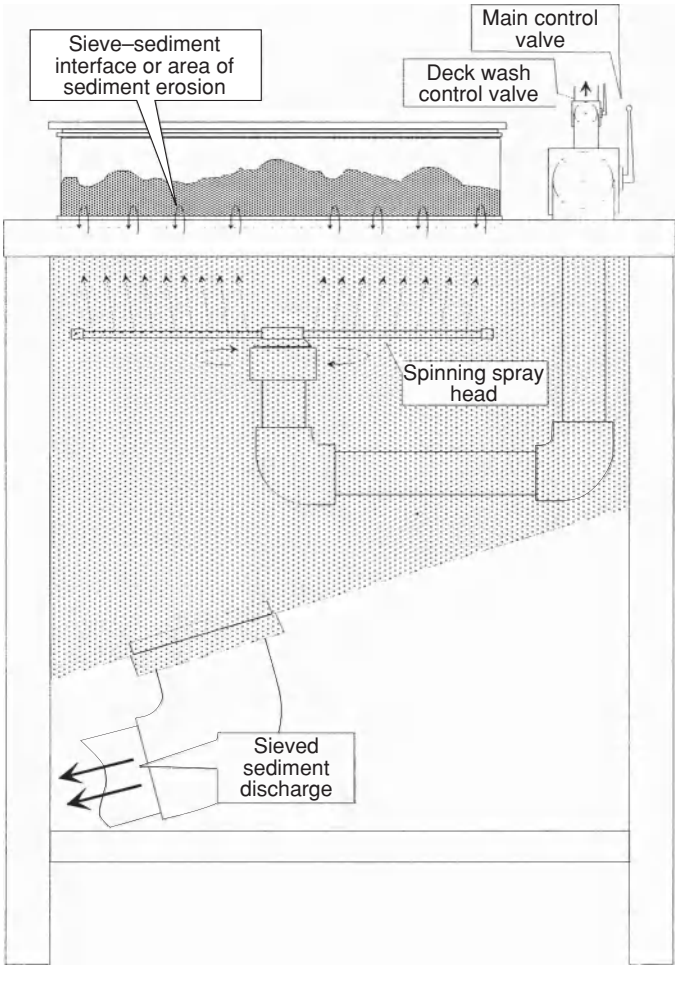

Fig. 5.23 Operational diagram of the Wilson Autosiever© (Gardline Surveys Ltd). Reproduced from Proudfoot *et al*., (2000); information © Environment Agency.

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be done by hand, the sieve being gently agitated in water so that flow takes place from below as well as from above. Rumohr (1999) describes a small sieve holder that allows the transfer of the sieve residue to the sample container while retaining the quality of the sampled specimens.

It could be assumed that the devices described above for the initial treatment of macrofauna sampling are still being used, although no reference has been made in the literature.

The mesh size of the sieve is of critical importance and should be determined at an early stage of planning. Sieves with either round or square holes may be used, but a square mesh is to be preferred since it has a higher percentage open area, and this type is in general use for soil analysis so that a wide range of sizes to

standard specifications is available. However, Rumohr (2009) remarks that some researchers have been changing to round mesh sieves owing partly to improved conditions of the animals retained thereby and partly to the theoretical improvement in mesh selectivity. The square mesh size is considered as introducing erroneous information as the diagonal width of the mesh is greater than the nominal mesh width. In practice, mesh sizes have varied from 2 to 0.5 mm, and even finer apertures have occasionally been used to capture juvenile stages or to make maximum use of deep-sea samples. The effects of different meshes on results have been referred to by McIntyre (1961) and Driscoll (1964). Jonasson (1955) showed that for one particular species a small decrease in mesh size, from 0.62 to 0.51 mm, resulted in a 47% increase in numbers, and stressed that the use of too large a mesh could produce an erroneous picture of seasonal peaks in animal numbers. Lewis and Stoner (1981), testing the retention of 0.5 and 1.0 mm meshes, found that only 55–77% of the total macrofauna was retained by the 1.0 mm mesh. Nalepa and Robertson (1981) found that use of a 0.595 mm mesh resulted in serious underestimates of the abundance of fresh water macrobenthos, although 97% of the biomass was retained. They concluded that the optimum mesh size chosen should be small enough (e.g. 0.10 mm) to retain all or most of the individuals of the taxa studied. In a more detailed study Reish (1959a) passed five grab samples from a shallow water muddy ground through a series of 11 sieves with apertures ranging from 4.7 to 0.15 mm. His data have been recalculated in Table 5.3 to show cumulative percentages for the main species and groups. If only molluscs were required, a screen of 0.85 mm, which separated about 95% of the individuals in his samples, would have been suitable (see also Schlacher & Wooldridge, 1996; Dukerschein *et al*., 1996), but a very much finer mesh was needed for nematodes and crustaceans. Analyses of polychaetes into species show the variation that can occur within a single taxonomic group: about 95% of the *Lumbrineris* were found on the 1.0 mm mesh, but to attain this level of separation for *Cossura candida* required a mesh of 0.27 mm. When the overall assemblage is considered it appears that a 0.27 mm mesh was needed to collect about 95% of all individuals. Pavithran *et al*. (2009), testing the effectiveness of sieve size, found that the use of a larger mesh size (0.5 mm) resulted in an important reduction in the number of species (39%), density and biomass (90% and 78%, respectively, for polychaetes and nematodes) of small deep-sea organisms than the finer mesh size of 0.3 mm. On the other hand, if only biomass is required, Reish (1959a) found that >90% was retained on the 1.4 mm sieve. While these results clearly apply only to the particular ground studied, they emphasise the importance of correct selection of sieve mesh, according to the purpose of the survey.

Degraer *et al.* (2007) investigating the qualification and quantification of the mesh size and sieving procedure using 1 mm mesh size on sieving a sample live or after fixation concluded that there are noticeable differences in the levels of ecological organisation, but that the results depended on the aspects under investigation.

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Table 5.3 Number of specimens retained on graded screens and cumulative percentages.

	Mesh sizes (mm)											
	4.7	2.8	1.4	1.0	0.85	0.7	0.59	0.5	0.35	0.27	0.15	Total
Nematoda						1	2	6	26	456	90	581
%						0.2	0.5	1.5	6.0	72.0	100.0	
Nemerta	2	7		6	3	5	2		1			
%	7.7	34.6	57.7	69.2	88.5	96.2	100.0					
Polychaeta												
Lumbrineris spp.	8	10	33	9	3							63
%	12.7	28.6	81.0	95.2	100.0							
Dorvillea articulata			22	52	30	5	4	2	2	2		119
%			18.5	62.2	87.4	91.6	95.0	96.6	98.3	100.0		
Prionospio cirrifera	1	6	23	100	115	30	18	10		1		304
%	0.3	2.3	10.0	42.8	80.6	90.1	96.4	99.8	99.8	100.0		
Capitita ambiseta	3	6	29	104	109	23	21	13	2			310
%	1.0	2.9	12.3	45.8	81.0	88.4	95.2	99.4	100.0			
Cossura candida			1	11	129	100	157	265	88	105	10	866
%			0.1	1.4	16.3	27.8	46.0	76.6	86.7	98.8	100.0	
Other Polychaeta	10	23	27	38	31	10	11	7	4	5	2	168
%	6.0	19.6	35.7	58.3	76.8	82.7	89.3	93.4	95.8	98.8	100.0	
Crustacea	3				2		1	3	5	3		17
%	17.6	17.6	17.6	17.6	29.4	29.4	35.3	52.9	82.4	100.0		
Mollusca	5	4	7	5	2			1				24
%	20.5	37.5	66.7	87.5	95.8	95.8	95.8	100.0				
Pisces	1											
%	100.0											
Total	33	56	148	322	426	171	214	308	127	572	102	2479
%	1.3	3.5	9.7	22.5	39.7	46.6	55.3	67.7	72.8	95.9	100.0	

Source: Calculated from Reish (1959a).

The Baltic Marine Biologists have standardised the mesh used in their studies at 1.00 mm (Dybern *et al*., 1976; Ankar *et al.*, 1979), with the recommendation that a 0.50 mm mesh should be used in addition whenever possible. Rumohr (1999) recommends the use of a 1.00 mm mesh for descriptive surveys and the use of a 0.5 mm mesh for special purposes such as the determination of production of bottom-living organisms.

In general, it is suggested that a 0.5 mm sieve should be used for macrofauna separation, but since this may retain too large a volume of material on coarse grounds, a compromise may have to be made, the final mesh selected being related to the grade of deposit and the size of the organisms to be separated. The use of a mesh corresponding to one of those in International Standard (ISO) is recommended, and a choice should be made from one of the following apertures: 2.00, 1.40, 1.00, 0.71 or 0.50 mm.

In areas where coarse deposits necessitate the use of wide-meshed sieves, it is recommended that additional small samples be sieved through a finer mesh to assess the losses occurring through the coarser sieve.

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Preservation

Having reduced the sample to a manageable size by initial sieving, the sorting of animals from the residue can proceed. If the final extraction is carried out soon after collection, and near the sampling site, sorting of living material may be possible, with the advantage that movement of the animals helps in their detection, especially if they are small. But it is frequently necessary, after initial sieving in the field, to preserve the collections for later sorting. In such circumstances, it is important to ensure adequate labelling. It may be convenient to number the tops or sides of jars with a waterproof marker, but even if this is done, a properly annotated paper, or synthetic paper label, strong enough to withstand water and preservatives should be placed inside the jar. Alternatively, sheet plastic with a matt surface that can be marked with pencil can be used, and this is particularly suitable for sediment samples.

Formalin is normally used for the initial preservation, and this can conveniently be diluted with seawater. While a 10% solution of commercial formalin (equivalent to 4% formaldehyde) is suitable for histological purposes, the strength may be reduced to between 2.5% and 5%, formalin for general storage, provided that the volume of preserving fluid is considerably greater than that of the specimens (Rumohr, 1999). In very large samples, perhaps containing much gravel, care should be taken to see that not only is there sufficient preservative, but that it is also adequately mixed through the sample. Since formalin tends to become acid with storage and so cause damage to the specimens, a buffer such as borax (sodium tetraborate) or hexamine (hexamethylene tetramine = urotropine) is often added to the formalin. Unbuffered formalin is known to erode molluscan shells and affect biomass as a result of dissolving lipids and fatty tissues. These substances have

been criticised for causing disintegration of labels, or for producing a precipitate. And for plankton samples sodium acetate is sometimes used. The addition of marble chips to the formalin is often used as minimum precaution to prevent the development of acidic conditions. It should be noted that formaldehyde is a toxic compound and should be handled with caution. Material exposed to formaldehyde should be thoroughly washed in tap water and ventilation should be provided in confined spaces in order to eliminate any formalin vapours.

Although bulk treatment is satisfactory for general samples, particular animals required in good condition should be extracted and dealt with individually. It is an advantage to narcotise highly contractile animals before fixation, allowing subsequent preservation in an extended condition. An account of anaesthetic agents is given by Steedman (1976) and by Lincoln and Sheals (1979). Alcohol is often used for later storage of samples, but it is less satisfactory for initial field preservation because of its volatility, because mixing with seawater causes a precipitate, and because it may cause the separation of lamellibranchs from their shells. A mixture of 70% ethanol and 5% glycerine is often used for permanent storage.

The use of 'Dowcil 100' in 10% solution or Kohrsolin releases formaldehyde only in the presence of proteins and has many advantages over formalin because it does not give off irritant fumes. It is also preferable to alcohol because it is neither flammable nor volatile. Its rather high cost has prevented its wider usage so far. Moreover, the use of an alternative preservative other than the traditional formaldehyde has been resisted by most researchers because of the possibility of its producing different effects, thus potentially compromising valid comparisons between sample series.

Detailed information on the use of fixatives, preservatives and buffering agents is provided by the SCOR working group (Steedman, 1976) and by Lincoln and Sheals (1979). Recovery of the organisms from the sediment sample is followed by further processing involving identification, enumeration, weighing, etc., for general or specific purposes. Some of the taxonomic work is greatly assisted by the application of different chemical agents. Berlese's fluid, lactophenol and glycerol have all been used by histologists to clear tissues of soft-bodied macrofauna. In common with most fixatives and preservatives there are safety concerns that must be addressed in the use of these materials. For all, the use of a ventilation system in close proximity to the workstation is recommended. Safety practices vary from country to country but the manufacturers' recommendations contained within the materials safety data sheets should be followed explicitly. After the final processing has been completed the organisms should be transferred to a preservation fluid, such as 70–80% alcohol, or a solution of propylene phenoxetol for storage.

Subsequent sorting

If the study is restricted to major species or to large individuals, hand sorting may be straightforward. This is best done in glass trays below which black or white

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material can be inserted to provide varying backgrounds suitable for distinguishing different types of animals. If every individual must be extracted this can be a time-consuming task, which may severely restrict the extent of sampling. It is often possible to divide a sample into fractions by agitating the light material into suspension and pouring it through a fine sieve. This separates small animals (such as crustaceans and polychaetes) together with fine debris, leaving large or heavy animals behind in the main sample.

Bulk staining of samples with vital stains (Rose Bengal, rhodamine B, eosin, etc.) to facilitate sorting has sometimes been used, and a counterstaining technique for samples containing large quantities of detritus is described by Williams and Williams (1974), where the primary stain, Rose Bengal or Lugol's iodine, is counterstained with chlorazol black E to provide a high colour contrast between the animals and the detritus in the samples. Hamilton (1969) used fluorescence for faster sorting of fresh water organisms from sediment and detritus: organisms stained with a dye (rhodamine B) fluoresce when examined under long-wave ultraviolet light. Of all the stains, Rose Bengal (4 g/litre of 36% formaldehyde) is the most widely used, although there is some opposition to its widespread application as it may obscure diagnostic features used in species identification. The use of methylene blue during the identification process allows analysts to determine fine difference in some integuments in soft-bodied macrofauna, particularly polychaetes, and has the advantage of being a temporary stain with only a short-lasting effect. See also methods for meiofauna in Chapter 6.

Two methods sometimes used to ease the work of sorting macrofauna are flotation and elutriation. Flotation is based on differences in specific weight between the organisms and the sediment – application of a medium of a suitably high density causes the animals to float free of heavier debris. Liquids such as carbon tetrachloride (Birkett, 1957; Dillon, 1964; Whitehouse & Lewis, 1966), sugar solutions (Anderson, 1959; Kajak *et al.*, 1968; Fast, 1970; Lackey & May, 1971), ZnCl (Sellmer, 1956; Mattheisen, 1960), and many others have been applied with varying degrees of success. Unfortunately, organic detritus also floats, making these methods unsuitable for muds and silts without adjustment of the specific gravity of the flotation medium. Other disadvantages are that most of these techniques are messy, and, in the case of carbon tetrachloride, dangerous if inhalation of vapour occurs (Dillon, 1964). Thus, the liquid must always be covered with water and the operation must be carried out with adequate ventilation. De Jonge and Bouwman (1977) used a colloidal silica polymer (Ludox^{TM}) to separate nematodes from sediment and detritus (see Chapter 6); this technique has also been used for the successful separation of macrofauna species.

Elutriation involves passing a continuous upward stream of water through the sample in a container with an overflow to a fine collecting screen. The flow rate is adjusted so that the water agitates the sample and carries up the small animals but not the sediment. Several apparatuses suitable for macrofauna (Lauff *et al.*, 1961; Pauly, 1973; Worswick & Barbour, 1974) utilise both water and air jets. Barnett's

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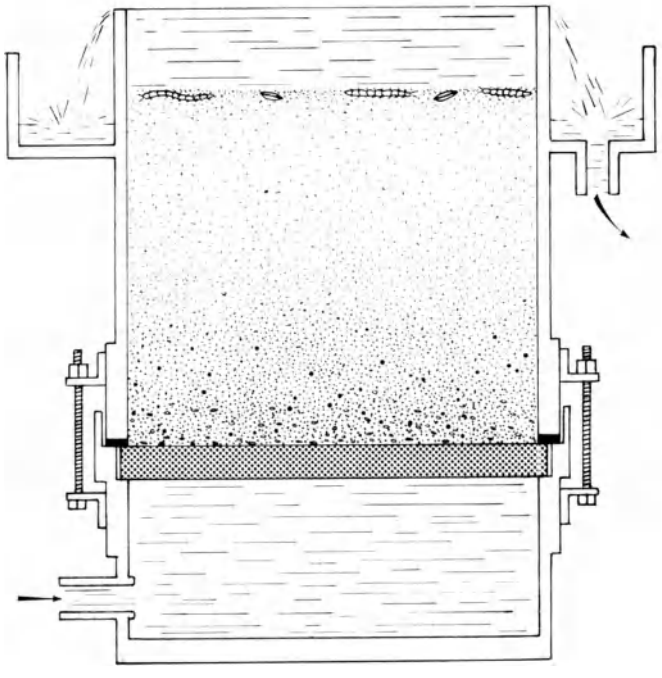

Fig. 5.24 General diagram of Barnett's fluidised bath. Water entering at the bottom passes upward via a ceramic sheet through the sand bed.

fluidised sand bath (Fig. 5.24) uses an upward current of water to separate animals from large quantities (up to 20 litres) of sediment. Sand uniformly fluidised by the passage of water provides a dense flotation medium into which the sample is tipped. Organisms float at the sand/water interface and are collected with a special sieve while the lightest ones are retained on the fine screen in the overflow. The average separation time is approximately 10 minutes and the claimed efficiency, 98–100%. However, this apparatus has been used by some benthos researchers, although there is no mention of it in the literature.

5.7 Data recording

Analysis of benthic infaunal surveys often results in large volumes of data in the form of species by sample (or station) matrices and the supporting environmental physical and chemical data. Rees *et al*. (1990) suggest that initial recording of such data should be in the form of the open book method, which will prevent more inexperienced workers from forcing identification data into previously defined sheets.

Where large amounts of information on species occurrence, with supporting environmental data, are collected, there is likely to be a need for use of a computerised

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system for information storage, sorting and retrieval. Modern desktop computers effectively offer infinite storage solutions for benthic surveys, with 40–80GB hard drives now being common, offering more storage space than could be filled in any lifetime of analysis (raw data derived from *all* extant oil related benthic surveys in the North Sea (from 1974 to 2000), and their associated chemical parameters have been produced on a single CD-ROM (640MB) with space to spare (UKOOA, 2001)).

The use of databases and spreadsheets has become a daily occurrence since the first edition of this book and this technology continues to develop faster than any other aspect of benthic methodology, perhaps, apart from developments in acoustic mapping.

Databases provide a robust and reliable method of storing data and, when properly used, can ensure such details as taxonomic nomenclature are entered both efficiently and correctly. This can be achieved in a number of ways including the use of code lists for species names. When entered into the database, these codes are translated into the full, correct taxonomic binomial. Many laboratories find the use of a multi-level system as the most efficient method, with an in-house system relating to those species actually encountered in their analyses being based on three or four letter couplets (in general, it is easier for the human brain to recall a letter-based code than a purely number-based system). This in-house code is then related to a comprehensive taxonomic listing, usually based on a numerical code, for correct hierarchical taxonomic ordering for tabulation and presentation purposes.

Taxonomic listings such as that produced by the Marine Conservation Society for the United Kingdom can provide excellent reference material for localised regions but for a fully hierarchical list, the NODC Taxonomic list from NOAA is of most use. Although apparently tortuous, the majority of this manipulation is conducted in the background and the analyst is unaware of the complex operations being performed; however, some initial effort in setting up the system is required.

Standardisation of records requires agreement on the scientific names for genus and species. Reference can be made to names used in a standard text, or a list, with author references, must be specially prepared. Quality assurance systems such as the Nation Marine Biology Analytical Quality Control scheme, conducted under the auspices of the MPMMG has sought to standardise species identification within active laboratories within the United Kingdom, with some degree of success.

Earlier editions of this book recognised that there was a requirement for textual data to be available in relation to many of the records maintained in most surveys. These data are often required for the expert interpretation of survey data and also to provide a method for historical comparison of possible anomalies between years. Modern database applications will allow the storage of such information and the addition of fields after defining the original database is now much more simple. Search and selection facilities and relating benthic faunal data with environmental factors also held in the same database application is also easily done.

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Database applications such as UNICORN from Unicomarine are widely used for data input and storage and can also be used as an aid to identification by holding taxonomic details of specific characters, along with a photographic record of such characters. Databases are not designed to perform complex mathematical tasks but they can be easily programmed to export selected data for analysis either in a standard spreadsheet or in a specialised analytical application.

Spreadsheets are invaluable for the data manipulation required to produce basic faunal indices, but more complex analyses are best undertaken within dedicated applications such as those produced by Plymouth Marine Laboratory (PML) and Cornell University. The increase in storage power of the desktop computer has also been accompanied by an increase in their computing power. This means that powerful multivariate analytical tools are now available to most benthic ecologists. It is not the purpose of this chapter to describe in detail such applications but we would refer the reader to PRIMER (Plymouth Routines In Multivariate Ecological Research) from PML (which includes MultiDimensional Scaling (MDS) and Canonical Analysis).

Another important tool is the use of Geographic Information Systems (GIS) in the presentation of benthic faunal data. This allows spatial display of data at a variety of resolutions, from global to local. Applications such as ArcView/ArcInfo and MapInfo can access data directly from database and spreadsheet applications and display them in a variety of spatial resolutions and geographical projections. Some even allow data to be presented against the backdrop of Admiralty charts where these are available. Certain relationships within the data can also be displayed within these applications.

In recent years, the use of habitat classification systems has been adopted as a useful tool in managing the marine environment, mainly for conservation purposes. The leading classification system in the United Kingdom is the JNCC Marine Habitat Classification (www.jncc.defra.gov.uk/MarineHabitatClassification; accessed 29 October 2012) but the European Nature Information System (EUNIS), though mainly concerned with non-marine environments, is also of importance (http://eunis.eea.europa.eu/about.jsp; accessed 29 October 2012). These systems define marine habitats in a hierarchical manner using the environment, the broad habitat type, habitat and biotope complexes and sub-biotopes giving a shorthand definition such as SS.SCS.ICS.HchrEdw for a habitat that is Sublittoral/Infralittoral coarse sediment with *Halicampa chrysantellum* and *Edwardsia timida* being present.

These classification systems can be applied in situations where no macrofaunal samples have been taken and can often be derived from visual information derived from either television images or diver observations and a computer-based biotope decision tool, Bioscribe, is available on the Internet (www.jncc.gov.uk/page-5776; accessed 29 October 2012).

There are many applications that can undertake, in an objective manner, the previously subjective technique of contouring data over survey areas, allowing

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environmental factors to be directly compared with biological data and to elicit possible relationships that have previously been latent.

Many of the above techniques are very robust and will frequently continue an analysis even in the absence of some elements of the data. It is, therefore, imperative that an element of expert interpretation must continue to be involved in data analysis.

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References

Abookire, A.A. & Rose, C.S. (2005) Modifications to a plumb staff beam trawl for sampling uneven complex habitats. *Fisheries Research*, **71**, 247–254.

Admiralty (1948) *Admiralty Manual of Hydrographic Surveying*, 2nd edn, Hydrographic Department, Admiralty, London, 572 pp.

Agassiz, A. (1888) Three cruises of the United States Coast and Geodetic Survey Steamer Blake. *Bulletin of the Museum of' Comparative Zoology at Harvard College*, **14**, 314 pp.

Allen, D.M. & Hudson, J.H. (1970) A sled-mounted suction sampler for benthic organisms. *United States Fish and Wildlife Service Special Scientific Report-Fisheries*, No. 614, 5 pp.

Andersin, A.B. & Sandler, H. (1981) Comparison of the sampling efficiency of two van Veen grabs. *Finnish Marine Research*, **248**, 137–142.

Anderson, R.O. (1959) A modified flotation technique for sorting bottom fauna samples. *Limnology and Oceanography*, **4**, 223–225.

Andrew, N.L. & Mapstone, B.D. (1987) Sampling and the description of spatial pattern in marine ecology. *Oceanography and Marine Biology Annual Review*, **25**, 39–90.

Aneer, A. & Nellbring, S. (1977) A drop-trap investigation of the abundance of fish in very shallow water in the Asko area, northern Baltic proper. In: *Biology of Benthic Organisms. Eleventh European Symposium on Marine Biology. Galway, October 1976* (eds B.F. Keegan, P. O'Ceidigh & P.J.S. Boaden), pp. 21–30, Pergamon Press, Oxford, 630 pp.

Ankar, S. (1977a) Digging profile and penetration of the van Veen grab in different sediment types. *Contributions from the Asko Laboratory, University of Stockholm. Sweden*, **16**, 22 pp.

Ankar, S. (1977b) The soft bottom ecosystem of the Northern Baltic proper with special reference to the macrofauna. *Contributions from the Asko Laboratory, University of Stockholm, Sweden*, No. 19, 62 pp.

https://telegram.me/Geologybooks

Ankar, S., Andersin, A.-B., Lassig, J., Norling, L. & Sandler, H. (1979) Methods for studying benthic macrofauna. An intercalibration between two laboratories in the Baltic Sea. *Finnish Marine Research*, **246**, 147–160.

Ankar, S., Cederwall, H., Lagzdins, G. & Norling, L. (1978) Comparison between Soviet and Swedish methods of sampling and treating soft bottom macrofauna. Final report from the Soviet-Swedish expert meeting on intercalibration of biological methods and analyses. Asko 5–12, July 1975. *Contributions from the Asko Laboratory, University of Stockholm, Sweden*, No. 23, 38 pp.

Bagge, O., Nielsen, E. & Mellergard, S. (1994) Comparative bottom sampling using Haaps and Petersen samplers. *International Council for the Exploration of the Sea (ICES) Shellfish Committee, Stat. Meeting*, *CM* 1994/k:42, 20 pp.

Baird, R.H. (1955) A preliminary report on a new type of commercial escallop dredge. *Journal du Conseil Permanent international pour I'Exploration de la Mer*, **20**, 290–294.

Baird, R.H. (1958) A preliminary account of a new half square metre bottom sampler. *International Council for the Exploration of the Sea, Shellfish Committee, CM* 1958/70, 4 pp.

Baird, R.H. (1959) Factors affecting the efficiency of dredges. In: *Modern Fishing Gear of the World* (ed. H. Kristjonnson), pp. 222–224, Fishing News, London, 607 pp.

Bakanov, A.I. (1977) Comparative evaluation of the effectiveness of different dredges. *Gidrobiologicheskii Zhurnal, Kiev*, **13**(2), 97–103.

Baker, J.H., Kimball, T. & Bedinger, C.A. (1977) Comparison of benthic sampling procedures: Petersen grab vs. Mackin corer. *Water Research*, **II**, 597–601.

Baker, S. (1987) Recommended protocols for sampling and analyzing subtidal benthic macroinvertebrate assemblages in Puget Sound. *US Environmental Protection Agency, Washington DC*, Contract No. 68-01-6938.

Bakus, G.J. (1990) *Quantitative Ecology and Marine Biology*, 157 pp. Balkema, Rotterdam.

Barnes, R.S.K. & Hughes, R.N. (1982) *An Introduction to Marine Ecology*, Blackwell Scientific Publications, London, 339 pp.

Barnett, P.R.O. (1969) A stabilizing framework for the Knudsen bottom sampler. *Limnology and Oceanography*, **14**, 648–649.

Barnett, P.R.O. (1998–1999) *The core issue?* University Marine Biological Station, Millport 30th Annual Report, 1999–2000, 14 pp.

Barnett, P.R.O. & Hardy, B.L.S. (1967) A diver-operated quantitative bottom sampler for sand macrofaunas. *Helgolander wissenschaftliche Meeresuntersuchungen*, **15**, 396–398.

Barnett, P.R.O., Watson, J. & Connelly, D. (1984) A multiple corer for taking virtually undisturbed samples from shelf, bathyal and abyssal sediments. *Oceanologica Acta*, **7**(4), 399–408.

Barrio Frojan, C.R.S. & Mason, C. (2010) A quantitative comparison between the sampling efficacy of the mini Hamon grab and the Costerus twin grab. *Marine Aggregate Levy Sustainability Fund*, MEPF Ref. No. MEPF REC 08/P04, 9 pp.

Beamish, R.J. (1972) Design of a trapnet for sampling shallow-water habitats. *Technical Report, Fisheries Research Board of Canada*, No. 305, 14 pp.

Belyaev, G.M. & Sokolova, M.N. (1960) On methods of quantitative investigation of deep-water benthos. *Trudy Instituta Okeanologii, Akademiya nauk SSSR*, **39**, 96–100 (in Russian).

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Bergmann, M. & van Santbrink, J.W. (1994) A new benthos dredge (Triple D) for quantitative sampling infauna species of low abundance. *Netherlands Journal of Sea Research*, **33**(1), 129–133.

Beukema, J.J. (1974) The efficiency of the van Veen grab compared with the Reineck box sampler. *Journal du Conseil Permanent international pour I'Exploration de la Mer*, **35**, 319–327.

Beyer, F. (1958) A new bottom living Trachymedusa from the Oslo Fjord. Description of the species, and a general discussion of the life conditions and fauna of the fjord deeps. *Nytt Magasin for Zoologi*, **6**, 121–143.

Bhaud, M. & Duchene, J.-C. (1977) Observations sur l'efficacité comparée de deux bennes. *Vie et Milieu, Series A*, **27**, 35–54.

Bieri, R. & Tokioka, T. (1968) Dragonet II, an opening-closing quantitative trawl for the study of microvertical distribution of zooplankton and the meio-epibenthos. *Publications of the Seto Marine Biological Laboratory*, **15**, 373–390.

Birkett, L. (1957) Flotation technique for sorting grab samples. *Journal du Conseil Permanent International pour I'Exploration de la Mer*, **22**, 289–292.

Birkett, L. (1958) A basis for comparing grabs. *Journal du Conseil Permanent International pour I'Exploration de la Mer*, **23**, 202–207.

Blomqvist, S. (1990) Sampling performance of Ekman grabs – *in situ* observations and design improvements. *Hydrobiologia*, **206**, 245–254.

Blomqvist, S. (1991) Quantitative sampling of soft bottom sediments: problems and solutions. *Marine Ecology Progress Series*, **72**, 295–304.

Boalch, G.T., Holme, N.A., Jephson, N.A. & Sidwell, J.M.C. (1974) A resurvey of Colman's intertidal traverses at Wembury, South Devon. *Journal of the Marine Biological Association of the United Kingdom*, **54**, 551–553.

Boillot, G. (1964) Geologie de la Manche occidentale. Fonds rocheux, dépôts quaternaires, sédiments actuels. *Annales de l'lnstitut Océanographique*, **42**, 1–219.

Bolam, S.G., Eggleton, J., Smith, R., Mason, C., Van Staen, K. & Rees, H. (2008) Spatial distribution of macrofaunal assemblages along the English Channel. *Journal of Marine Biological Association of the United Kingdom*, **88**(4), 675–687.

Boland, G.S. & Rowe, G.T. (1991) Deep-sea benthic sampling with the GOMEX box corer. *Limnology and Oceanography*, **36**(5), 1015–1020.

Bossanyi, J. (1951) An apparatus for the collection of plankton in the immediate neighbourhood of the sea-bottom. *Journal of the Marine Biological Association of the United Kingdom*, **30**, 265–270.

Bouma, A.H. (1969) *Methods for the Study of Sedimentary Structures*, John Wiley & Sons, New York, 458 pp.

Bouma, A.H. & Marshall, N.F. (1964) A method for obtaining and analysing undisturbed oceanic bed samples. *Marine Geology*, **2**, 81–99.

Bourcier, M., Masse, H., Plante, R., Reys, J.P. & Tahvildari, B. (1975) Note préliminaire sur l'étude comparative des bennes Smith-McIntyre et Briba-Reys. *Rapports et procés-verbaux des Réunions. Commission Internationale pour I'Exploration scientifique de la Mer Mediterranée*, **23**(2), 155–156.

Boyd, S.E. (2002) *Guidelines for the Conduct of Benthic Studies at Aggregate Dredging Sites*, Department for Transport, Local Government and the Regions, CEFAS, Lowestoft, 117 pp.

https://telegram.me/Geologybooks

Boyd, S.E., Barry, J. & Nicholson, M. (2006) A comparative study of a 0.1 m^2 and 0.25 m^2 Hamon grab for offshore marine gravels. *Journal of the Marine Biological Association of the United Kingdom*, **86**, 1315–1328.

Bradbury, R.H., Loya, Y., Reichert, R.E. & Williams, W.T. (1986) Patterns in the structure topology of benthic communities on two coral reefs of the central Great Barrier Reef. *Journal of Wildlife Management*, **4**, 161–167.

Brattegard, T. & Fossa, J.H. (1991) Replicability of an epibenthic sampler. *Journal of the Marine Biological Association of the United Kingdom*, **71**, 153–166.

Brenke, N. (2005) An epibenthic sledge for operations on marine soft bottom and bedrock. *Marine Technology Society Journal*, **39**(2), 10–15.

Briba, C. & Reys, J.P. (1966) Modification d'une benne 'orange peel' pour les prélèvements quantitatifs du benthos de substrats meubles. *Recueil des Travaux de la Station Marine d'Endoume*, **41**(57), 117–121.

Brook, I.M. (1979) A portable suction dredge for quantitative sampling in difficult substrates, *Estuaries,* **2**(1), 54–58.

Brown, A.V., Schram, M.D. & Brussock, P.P. (1987) A vacuum benthos sampler suitable for diverse habitats. *Hydrobiologica*, **153**(3), 241–247.

Brunel, P., Besner, M., Messier, D., Poirier, L., Granger, D. & Weinstein, M. (1978) Le traineau suprabenthique Mater-GIROQ; appareil améliore pour l'échantillonage quantitatif étage de la petite faune nageuse au voisinage du fond. *Internationale Revue der Gesamten Hydrobiologie*, **63**, 815–829.

Buhl-Jensen, L. (1986) The benthic amphipod fauna of the west Norwegian continental shelf compared with the fauna of five adjacent fjords. *Sarsia*, **71**, 193–208.

Burke, J.C. (1968) A sediment coring device of 21-cm diameter with sphincter core retainer. *Limnology and Oceanography*, **13**, 714–718. Collected Reprints, Woods Hole Oceanographic Institution, 2149.

Burke, J.C., Casso, S.A. & Hamblin, R.E. (1983) Tripod modification of sphincter corer: construction, operation, core extrusion and sampling efficiency. Technical Report, Woods Hole Oceanographic Institute, 18 pp.

Caddy, J.F. (1971) Efficiency and selectivity of the Canadian offshore scallop dredge. *ICES Shellfish and Benthos Committee*, No. K 25, 8 pp.

Carey, A.G. & Hancock, D.R. (1965) An anchor-box dredge for deep-sea sampling. *Deep-Sea Research*, **12**, 983–984.

Carey, A.G. & Heyamoto, H. (1972) Techniques and equipment for sampling benthic organisms. In: *The Columbia River Estuary and Adjacent Ocean Waters: Bioenvironmental Studies* (eds A.T. Pruter & D.L. Alverson), pp. 378–408, University of Washington Press, Seattle, WA, 882 pp.

Carey, A.G. & Paul, R.R. (1968) A modification of the Smith-McIntyre grab for simultaneous collection of sediment and bottom water. *Limnology and Oceanography*, **13**, 545–549.

Carleton, J.H. & Hamner, W.M. (1989) Resident mysids: community structure, abundance and small-scale distributions in a coral reef lagoon. *Marine Biology*, **102**(4), 461–472.

Carney, R.S. & Carey, A.G., Jr (1980) Effectiveness of metering wheels for measurement of area sampled by beam trawls. *Fishery Bulletin, National Marine Fisheries Service, NOAA, Seattle, USA*, **18**, 791–796.

https://telegram.me/Geologybooks

Chapman, C.J., Main, J., Howell, T. & Sangster, G.I. (1979) The swimming speed and endurance of the queen scallop *Chlamys opercularis* in relation to trawling. *Progress in Underwater Science*, **4**, 57–72.

Chapman, C.J., Mason, J. & Kinnear, J.A.M. (1977) Diving observations on the efficiency of dredges used in the Scottish fishery for the scallop, *Pecten maximus* (L.). *Scottish Fisheries Research Report*, No. 10, 15 pp.

Christie, N.D. (1975) Relationship between sediment texture, species richness and volume of sediment sampled by a grab. *Marine Biology*, **30**, 89–96.

Christie, N.D. & Allen, J.C. (1972) A self-contained diver-operated quantitative sampler for investigating the macrofauna of soft substrates. *Transactions of' the Royal Society of South Africa*, **40**, 299–307.

Clarke, A.H. (1972a) The arctic dredge, a benthic biological sampler for mixed boulder and mud substrates. *Journal of the Fisheries Research Board of' Canada*, **29**, 1503–1505.

Clarke, M.R. (1972b) A brief review of sampling techniques and tools of marine biology. In: *A Voyage of Discovery* (ed. M. Angel), pp. 439–469, Pergamon Press, Oxford, 669 pp.

Colman, J.S. & Segrove, F. (1955) The tidal plankton over Stoupe Beck Sands, Robin Hood's Bay (Yorkshire, North Riding). *Journal of Animal Ecology*, **24**, 445–462.

Craib, J.S. (1965) A sampler for taking short undisturbed marine cores. *Journal du Conseil Permanent International pour l'Exploration de la Mer*, **30**, 34–39.

Cranmer, G.J., Dyer, M.F. & Fry, P.D. (1984) Further results from headline camera surveys in the North Sea. *Journal of the Marine Biological Association of the United Kingdom*, **64**(2), 335–342.

Creutzberg, F., Duineveld, G.C.A. & van Noort, G.J. (1987) The effect of different numbers of tickler chains on beam-trawl catches. *Journal du Conseil International pour l'Exploration de la Mer*, **43**, 159–168.

Dauvin, J.-C. (1979) *Recherches Quantitatives sur le Peuplement des Sables Fins de la Pierre Noire. Baie de Morlaix, et sur la Perturbation par les Hydrocarbures del'AMOC0-CADIZ'*, Thèse du Diplome de Docteur de 3'Cycle, Universite Pierre et Marie Curie, Paris, 251 pp.

Dauvin, J.-C. & Lorgere, J.-C. (1989) Modifications du traineau Macer-GIROQ pour l'amélioration de l'échantillonage quantitative étage de la faune suprabenthique. *Journal de la Recherche Oceanographique*, **14**, 65–67.

Dauvin, J-C., Sorbe, J-C. & Lorgere, J.-C. (1995) Benthic boundary layer macrofauna from the upper continental slope and the Cap Ferret Canyon (Bay of Biscay). *Oceanologica Acta*, **18**, 113–122.

Davis, F.M. (1925) Quantitative studies on the fauna of the sea bottom. No. 2. Results of investigations in the southern North Sea, 1921–1924. *Fishery Investigations, Ministry of Agriculture, Fisheries and Food, Series 2*, No. 8(4), 50 pp.

Davis, F.M. (1958) *An Account of the Fishing Gear of England and Wales*, 4th edn. *Fisheries Investigations, London, Series 2*, **S** (4), 50 pp.

Day, G.F. (1978) The Day grab – a simple sea-bed sampler. *Report of the Institute of Oceanographic Sciences*, No. 52.

Degraer, S., Moulaert, I., Van Hoey, G. & Vincx, M. (2007) Sieving alive or after fixation: effects of sieving procedure on macrobenthic diversity, density and community structure. *Helgoland Marine Research*, **61**, 143–152.

https://telegram.me/Geologybooks

De Jonge, V.N., & Bouwman, L.A. (1977) A simple density separation technique for quantitative isolation of meiobenthos using the colloidal silica Ludox^{TM}. *Marine Biology*, **42**, 143–148.

Desprez, M. & Ducrotoy, P.P. (1987) Nouvelle méthode de prélèvement des coques (*Cerastoderma edule*) et de la faune associée. *Les Nouvelles du GEMEL, Saint-Valery-sur-Somme*, **4**, 5.

Dickie, L.M. (1955) Fluctuations in abundance of the giant scallop *Placopecten magellanicus* (Gmelin) in the Digby area of the Bay of Fundy. *Journal of the Fisheries Research Board of Canada*, **12**, 797–857.

Dickinson, J.J. & Carey, A.G., Jr (1975) A comparison of two benthic infaunal samplers. *Limnology and Oceanography*, **20**, 900–902.

Diener, D. & Rimer, J.P. (1993) New way benthic sampling. *Proceedings of the Marine Technology Society Conference*, Washington, DC, pp 70–75.

Dillon, W.P. (1964) Flotation technique for separating faecal pellets and small marine organisms from sand. *Limnology and Oceanography*, **9**, 601–602.

Drake, C.M. & Elliott, J.M. (1982) A comparative study of three air-lift samplers used for sampling benthic macro-invertebrates in rivers. *Freshwater Biology*, **12**, 511–533.

Drake, C.M. & Elliott, J.M. (1983) A new quantitative air-lift sampler for collecting macroinvertebrates on stony bottoms in deep rivers. *Freshwater Biology*, **13**, 545–559.

Driscoll, A.L. (1964) Relationship of mesh opening to fauna1 counts in a quantitative benthic study of Hadley Harbor. *Biological Bulletin, Marine Biological Laboratory Woods Hole*, **127**, 368.

Dukerschein, J., Gent, R. & Sauer, J. (1996) Recovery of macro-invertebrates by screening in the field: a comparison between coarse (1.18 mm) and fine (0.60 mm) mesh sieves. *Journal of Freshwater Ecology*, **11**, 61–65.

Dybern, B.I., Ackefors, H. & Elmgren, R. (1976) Recommendations on methods for marine biological studies in the Baltic Sea. *The Baltic Marine Biologists*, **1**, 98 pp.

Dyer, M.F., Fry, W.G., Fry, P.D. & Cranmer, G.J. (1982) A series of North Sea benthos surveys with trawl and headline camera. *Journal of the Marine Biological Association of' the United Kingdom*, **62**, 297–313.

Dyer, M.F., Pope, J.G., Fry, P.D., Law, R.J. & Portmann, J.E. (1983) Changes in fish and benthos catches off the Danish coast in September 1981. *Journal of the Marine Biological Association of the United Kingdom*, **63**, 767–775.

Eagle, R.A., Norton, M.G., Nunny, R.S. & Rolfe, M.S. (1978) The field assessment of effects of dumping wastes at sea: 2. Methods. *Fisheries Research Technical Report, Lowestoft*, **47**, 24 pp.

Ecoscope (2000) *A Species and Habitats Monitoring Handbook*, *Volume 2: Habitat monitoring. Research, Survey and Monitoring Review No. [XX]*. Scottish Natural Heritage, Edinburgh.

Edwards, R. & Steele, J.H. (1968) The ecology of O-group plaice and common dabs at Loch Ewe. I. Population and food. *Journal of Experimental Marine Biology and Ecology*, **2**, 215–238.

Eleftheriou, A. & McIntyre, A.D. (1976) The intertidal fauna of sandy beaches – a survey of the Scottish coast. *Scottish Fisheries Research Report*, No. 6, 61 pp.

Eleftheriou, A. (1979) The ecology and biology of the shallow hyperbenthos in a sandy bay. PhD thesis, Aberdeen University, Aberdeen, 241 pp.

https://telegram.me/Geologybooks

Eleftheriou, A. & Robertson, M.R. (1988) The intertidal fauna of sandy beaches – A survey of the east Scottish coast. *Scottish Fisheries Research Report*, No. 38, 52 pp.

Eleftheriou, A. & Robertson, M.R. (1992) The effects of experimental scallop dredging on the fauna and physical environment of a shallow sandy community. *Netherlands Journal of Sea Research (Proceedings, 26th European Marine Biology Symposium. Biological Effects Of Disturbances On Estuarine And Coastal Marine Environments)*, **30**, 289–299.

Elizalde, M., Dauvin, J.-C. & Sorbe, J.-C. (1991) Suprabenthic mysids from the southern margin of the Cap-Ferret trench (Bay of Biscay): Bathymetric distribution and near-bottom swimming activity. *Annales de l'Institut Oceanographique, Paris. Nouvelle Serie.* **67**(2), 129–144.

Elliott, J.M. & Drake, C.M. (1981) A comparative study of seven grabs used for sampling benthic macroinvertebrates in rivers. *Freshwater Biology*, **11**, 99–120.

Elliott, J.M., Tullett, P.A. & Elliott, J.A. (1993) A new bibliography of samplers for freshwater benthic invertebrates. Occasional Publication No. 30. Freshwater Biological Association, 92 pp.

Ellis, D.V. & Jones, A.A. (1980) The Ponar grab as a marine pollution monitoring sampler. *Canadian Research*, June/July, 23–25.

Emery, K.O. (1961) A simple method of measuring beach profiles. *Limnology and Oceanography*, **6**, 90–93.

Emery, K.O., Merrill, A.S. & Trumbull, V.A. (1965) Geology and biology of the sea floor as deduced from simultaneous photographs and samples. *Limnology and Oceanography*, **10**, 1–21. Collected Reprints, Woods Hole Oceanographic Institute, 1965(1), No. 1508.

Emig, C.C. (1977) Un nouvel aspirateur sous-marins, a air comprimé. *Marine Biology*, **43**, 379–380.

Emig, C.C. & Lienhart, R. (1971) Principe de l'aspirateur sous-marin automatique pour sédiments meubles. *Vie et Milieu (Suppl.)*, **22**, 573–578.

Ervin, J.L. & Haines, T.A. (1972) Using light to collect and separate zooplankton. *Progressive Fish Culturist*, **34**, 171–174.

Espinosa, L.R. & Clark, W.E. (1972) A polypropylene light trap for aquatic invertebrates. *California Fish and Game*, **58**, 149–152.

FAO (1972) *Catalogue of Fishing Gear Designs*, Fishing News (Books), London, 155 pp.

Farris, R.A. & Crezee, M. (1976) An improved Reineck box for sampling coarse sand. *Internationale Revue der Gesamten Hydrobiologie*, **61**, 703–705.

Fast, A.W. (1970) An evaluation of the efficiency of zoobenthos separation by sugar flotation. *Progressive Fish Culturist*, **32**, 212–216.

Finnish IBP-PM Group (1969) Quantitative sampling equipment for the littoral benthos. *lnternationale Revue der Gesamten Hydrobiologie*, **54**, 185–193.

Flannagan, J.F. (1970) Efficiencies of various grabs and corers in sampling freshwater benthos. *Journal of the Fisheries Research Board of Canada*, **27**, 1691–1700.

Forster, G.R. (1953) A new dredge for collecting burrowing animals. *Journal of the Marine Biological Association of the United Kingdom*, **32**, 193–198.

Fossa, J.H., Larsson, J. & Buhl-Jensen, L. (1988) A pneumatic, bottom-activated, opening and closing device for epibenthic sledges. *Sarsia*, **73**(4), 299–302.

Franklin, W.R. & Anderson, D.V. (1961) A bottom sediment sampler. *Limnology and Oceanography*, **6**(2), 233–234.

https://telegram.me/Geologybooks

Frolander, H.F. & Pratt, I. (1962) A bottom skimmer. *Limnology and Oceanography*, **7**, 104–106.

Gage, J.D. (1975) A comparison of the deep sea epibenthic sledge and anchor-box dredge samplers with the van Veen grab and hand coring by divers. *Deep-Sea Research*, **22**, 693–702.

Galbraith, R.D., Rice, A. & Strange, E.S. (2004) An introduction to commercial fishing gear and methods used in Scotland. *Scottish Fisheries Information Pamphlet*, No. 25, 43 pp.

Gallardo, V.A. (1965) Observations on the biting profiles of three 0.1 m^2 bottom-samplers. *Ophelia*, **2**, 319–322.

Gaunt, D.I. & Wilson, J.B. (1975) Acoustic monitoring of dredge behaviour on the sea floor. *Deep-Sea Research*, **22**, 91–97.

Gerdes, D. (1990) Antarctic trials of the multi-box corer, a new device for benthos sampling. *Polar Record*, **26**(156), 35–38.

Gibson, R.N. (1967) The use of the anaesthetic quinaldine in fish ecology. *Journal of Animal Ecology*, **36**, 295–301.

Gilat, E. (1964) The macrobenthonic invertebrate communities on the Mediterranean continental shelf of Israel. *Bulletin de I'lnstitut Océanographique, Monaco*, **62**(1290), 46 pp.

Gilat, E. (1968) Methods of study in marine benthonic ecology. *Colloque Comité du Benthos, Comité International pour l'Exploration de la Mer Mediterranée, Marseille, Norember 1963*, pp. 7–13.

Glover, A.G., Smith, C.R., Mincks, S.L., Sumida, Y.G. & Thurber, A.R. (2008) Macrofaunal abundance and composition on the West Antarctic Peninsula continental shelf: evidence for a sediment 'food bank' and similarities to deep-sea habitats. *Deep-Sea Research II*, **55**, 2491–2501.

Gonor, J.J. & Kemp, P.F. (1978) Procedures for quantitative ecological assessments in intertidal environments. *Ecological Research Series*, US Environmental Protection Agency, Office of Research and Development, EPA-600/3-78-087, Corvallis, OR, 103 pp.

Gordon, D.C., Jr, Kenchington, E.L.R., Wilkinson, K.D., McKeown, D.L., Steeves, G., Chin-Yee, M., Bentham, K., Vass, P., Muschenheim, K., Milligan, T., Bourbonnais, C. & MacIsaac, K. (2000) *Canadian Imaging and Sampling Technology of Studying Marine Benthic Habitat and Biological Communities*. International Council for the Exploration of the Sea, CM, 2000/T:07.

Grange, K.R. & Anderson, P.W. (1976) A soft-sediment sampler for the collection of biological specimens. *Records New Zealand Oceanographic Institute*, **3**, 9–13.

Gray, J.S., McIntyre, A.D. & Stirn, J. (1991) Manual of methods in aquatic environment research part II. Biological assessment of marine pollution with particular reference to benthos. *FAO Fisheries Technical Paper*, No. 324, 49 pp.

Grizzle, R.E. & Stegner, W.E. (1985) A new quantitative grab for sampling benthos. *Hydrobiologia*, **126**(1), 91–95.

Groenewold, S. (1999) Effects of beam-trawl fishery on the food consumption of scavenging epibenthic invertebrates and demersal fish in the southern North Sea, PhD thesis, University of Hamburg, Hamburg, 146 pp.

Groenewold, S. & Fonds, M. (2000) Effects of benthic scavengers of discards and damaged benthos produced by the beam-trawl fishery in the southern North Sea. *Journal of Marine Science*, **57**, 1395.

https://telegram.me/Geologybooks

Grussendorf, M.J. (1981) A bushing-coring device for collecting deep-burrowing infaunal bivalves in intertidal sand. *Fishery Bulletin, National Marine Fisheries Service, NOAA, Seattle, USA*, **79**, 383–385.

Gunderson, D.R. & Ellis, I.E. (1986) Development of a plumb staff beam trawl for sampling demersal fauna. *Fisheries Research*, **4**, 35–41.

Gunter, G. (1957) Dredges and trawls. *Memoirs of the Geological Society of America*, **67**(1), 73–78.

Haahtela, I. (1969) Methods for sampling scavenging benthic Crustacea especially the isopod *Mesidotea entomon(L.)* in the Baltic. *Annales Zoologici Fennici*, **15**, 182–185.

Haahtela, I. (1974) The marine element in the fauna of the Bothnian Bay. *Hydrobiological Bulletin,* **8**, 232–241.

Håkanson, L. (1986) Modifications of the Ekman sampler. *Internationale Revue gesamten Hydrobiologie*, **71**, 719–721.

Hamilton, A.C. (1969) A method of separating invertebrates from sediments using longwave ultraviolet light and fluorescent dyes. *Journal of the Fisheries Research Board of Canada*, **26**, 1667–1672.

Hardy, A.C. (1959) *The Open Sea: Its Natural History. Part II. Fish and Fisheries. New Naturalist Series* No. 37, Collins, London, 322 pp.

Hartman, O. (1955) Quantitative survey of the benthos of San Pedro Basin, Southern California. Part I. Preliminary results. *Allan Hancock Pacific Expeditions*, No. 19(1), 185 pp.

Heip, C., Brey, T., Creutzberg, F., Dittmer, J., Dorjes, J., Duineveld, G., Kingston, P., Mair, H., Rachor, E., Rumohr, H., Thielemanns, L. & Vanosmael, C. (1985) Report on the intercalibration exercise on sampling for macrobenthos, *International Council for the Exploration of the Sea*, *CVM* 1985/L:19.

Hemmings, C.C. (1973) Direct observations on the behaviour of fish in relation to fishing gear. *Helgolander Wissenschaftliche Meeresuntersuchungen*, **24**, 348–360.

Hessler, R.R. & Jumars, P.A. (1974) Abyssal community analysis from replicate box cores in the central North Pacific. *Deep-Sea Research*, **21**, 185–209.

Hesthagen, I.H. (1978) The replicability of sampling the hyperbenthic region by means of Beyer's 50 cm epibenthic closing net. *Meeresforschung Report*, **26**(1–2), 1–10.

Higgins, R.C. (1972) Comparative efficiencies of the Smith-McIntyre and Baird grabs in collecting *Echinocardium cordatum* (Pennant) (Echinoidea: Spatangoida) from a muddy substrate. *Records New Zealand Oceanographic Institute*, **1**, 135–140.

High, W.L., Ellis, I.E., Schroeder, W.W. & Loverich, G. (1973) Evaluation of the undersea habitats – Tektite II. Hydro-Lab, and Edalhad – for scientific saturation diving programs. *Helgolander Wissenschaft Meeresuntersuchungen*, **24**, 16–44.

Hiscock, K. (1996) Marine Conservation Review; rationale and methods. Unpublished Report by the Joint Nature Conservation Committee, Peterborough.

Holme, N.A. (1949) A new bottom-sampler. *Journal of the Marine Biological Association of the United Kingdom*, **28**, 323–332.

Holme, N.A. (1953) The biomass of the bottom fauna in the English Channel off Plymouth. *Journal of the Marine Biological Association of the United Kingdom*, **32**, 1–49.

Holme, N.A. (1959) A hopper for use when sieving bottom samples at sea. *Journal of the Marine Biological Association of the United Kingdom*, **38**, 525–529.

Holme, N.A. (1961) The bottom fauna of the English Channel. *Journal of the Marine Biological Association of the United Kingdom*, **41**, 397–461.

https://telegram.me/Geologybooks

Holme, N.A. (1964) Methods of sampling the benthos. *Advances in Marine Biology*, **2**, 171–260.

Holme, N.A. & Barrett, R.L. (1977) A sledge with television and photographic cameras for quantitative investigation of the epifauna on the continental shelf. *Journal of the Marine Biological Association of the United Kingdom*, **57**, 391–403.

Holme, N.A. & McIntyre, A.D. (eds) (1984) *Methods for the Study of Marine Benthos*, IPB Handbook No. 16, 2nd edn, Blackwell Publishing Ltd., Oxford, 387 pp.

Hopkins, T.L. (1964) A survey of marine bottom samplers. *Progress in Oceanography*, **2**, 215–256.

Howmiller, R.P. (1971) A comparison of the effectiveness of Ekman and Ponar grabs. *Transactions of the American Fisheries Society*, **100**(3), 560–564.

Huberdeau, L. & Brunel, P. (1982) Efficacité et selectivite faunistique comparee de quatre appareils de prélèvements endo-, epi- et suprabenthiques sur deux types de fonds. *Marine Biology*, **69**, 331–343.

Hudson, P.L. (1970) Quantitative sampling with three benthic dredges. *Transactions of the American Fisheries Society*, **99**, 603–607.

Hunter, W. & Simpson, A.E. (1976) A benthic grab designed for easy operation and durability. *Journal of the Marine Biological Association of the United Kingdom*, **56**, 951–957.

Ingham, A.E. (ed.) (1975) *Sea Surveying*, John Wiley & Sons, Chichester, text, 306 pp. illustrations, 233 pp.

Ivanov, A.I. (1965) Underwater observations of the functioning of sampling equipment for benthos collections (Petersen, Okean dredges). *Okeanologiia*, **5**(5), 917–923 (in Russian); *Oceanology*, **5**, 119–124 (English translation).

Janssen, G., Kleef, H., Mulder, S. & Tydeman, P. (2008) Pilot assessment of depth related distribution of macrofauna in surf zone along Dutch coast and its impications for coastal management. *Marine Ecology*, **29**(suppl 1), 186–194.

Jensen, K. (1981) Comparison of two bottom samplers for benthos monitoring. *Environmental Technology Letters*, **2**, 81–84.

Johansen, A.C. (1927) Preliminary experiments with Knudsen's bottom sampler for hard bottom. *Meddelelser fra Kommissionen for Havundersogelser, Serie fiskeri*, **8**(4), 6 pp.

Jonasson, A. & Olausson, E. (1966) New devices for sediment sampling. *Marine Geology*, **4**, 365–372.

Jonasson, P.M. (1955) The efficiency of sieving techniques for sampling freshwater bottom fauna. *Oikos*, **6**, 183–207.

Jones, W.E. (1980) Field teaching methods in shore ecology. In: *The Shore Environment. Volume 1: Methods* (eds J.H. Price, D.E.G. Irvine & W.F. Farnham), Systematics Association Special Volume 17(a), Chapter 2, pp. 19–44, Academic Press, London, 321 + 41 pp.

Jones, W.E., Bennel, S., Beveridge, C., McConnell, B., Mack-Smith, S., Mitchell, J. & Fletcher, A. (1980) Methods of data collection and processing in rocky intertidal monitoring. In: *The Shore Environment. Volume 1: Methods* (eds J.H. Price, D.E.G. Irvine & W. F. Farnham) Systematics Association Special Volume 17(a), Academic Press, London, pp 137–170.

Jumars, P.A. (1975) Methods for measurement of community structure in deep-sea macrobenthos. *Marine Biology*, **30**, 245–252.

https://telegram.me/Geologybooks

Kain, J.M. (1958) Observations on the littoral algae of the Isle of Wight. *Journal of the Marine Biological Association of the United Kingdom*, **31**, 769–780.

Kaiser, M.J., Rogers, S.I. & McCandless, D.T. (1994) Improving quantitative surveys of epibenthic communities using a modified 2-m beam trawl. *Marine Ecology Progress Series*, **106**(1–2), 131–138.

Kajak, Z. (1971) Benthos of standing water. In: *A Manual on Methods for the Assessment of Secondary Productivity in Fresh Waters* (eds W.T. Edmondson & G.G. Winberg), IBP Handbook No. 17, pp. 25–65, Blackwell Scientific Publications, Oxford, 358 pp.

Kajak, Z., Dusoge, K. & Prejs, A. (1968) Application of the flotation technique to assessment of absolute numbers of benthos. *Ekologia Polska, Seria A*, **29**, 607–619.

Kanneworff, P. (1979) Density of shrimp *(Pandalus borealis)* in Greenland waters observed by means of photography. *Rapports et Procès-verbaux des Réunions Conseil International pour l'Exploration de la Mer*, **175**, 134–138.

Kanneworff, E. & Nicolaisen, W. (1973) 'The Haps'. A frame-supported bottom corer. *Ophelia*, **10**, 119–128.

Kanneworff, E. & Nicolaisen, W. (1983) A simple, hand-operated quantitative, bottom sampler. *Ophelia*, **22**(2), 253–255.

Kaplan, E.H., Welker, J.R. & Krause, M.G. (1974) A shallow-water system for sampling macrobenthic infauna. *Limnology and Oceanography*, **19**, 346–350.

Kawamura, G. & Bagarinao, T. (1980) Fishing methods and gears in Panay Island, Philippines. *Memoirs of Faculty of Fisheries Kagoshima University*, **29**, 81–121.

Kenchington, E.L.R., Gilkinson, K.D., MacIsaac, K.G., Bournonnais-Boyce, C., Kenchington, T.J., Smith, S.J. & Gordon, D.C., Jr (2006) Effects of experimental otter trawling on benthic assemblages on Western Bank, northwest Atlantic Ocean. *Journal of Sea Research*, **56**, 249–270.

Kenny, A.J. & Rees, H.L. (1994) The effects of marine gravel extraction on the macrobenthos: early post-dredging recolonisation. *Marine Pollution Bulletin*, **28**, 1–19.

Kermabon, A., Blavier, P., Cortis, V. & Delauze, H. (1966) The 'Sphincter' corer: a wide-diamter corer with water-tight core-catcher. *Marine Geology*, **4**, 149–162.

Kingston, P. (1988) Limitations on offshore environmental monitoring imposed by sea-bed sampler design. *Advances in Underwater Technology*, **16/31**, 273–281.

Kissam, P. (1956) *Surveying*, 2nd edn, McGraw-Hill, New York, 482 pp.

Knudsen, M. (1927) A bottom sampler for hard bottom. *Meddelelserfra Komissionen for Havundersogelser, ser Fisk*, **8**(3), 4 pp.

Kostylev, V.E., Elandsson, J., Ming, M.Y. & Williams, G.A. (2005) The relative importance of habitat complexity and surface area in assessing biodiversity: fractal application on rocky shores. *Ecological Complexity*, **2**, 272–286.

Koulouri, P.T., Dounas, C.G. & Eleftheriou, A. (2003) A new apparatus for the direct measurement of otter trawling effects on the epibenthic and hyperbenthic macrofauna. *Journal of Marine Biological Association of the United Kingdom*, **83**, 1363–1368.

Kushlan, J.A. (1981) Sampling characteristics of enclosure fish traps. *Transactions of the American Fisheries Society*, **110**, 557–562.

Kutty, M.K. & Desai, B.N. (1968) A comparison of the efficiency of the bottom samplers used in benthic studies off Cochin. *Marine Biology*, **1**, 168–171.

Lackey, R.T. & May, B.E. (1971) Use of sugar flotation and dye to sort benthic samples. *Transactions of the American Fisheries Society*, **100**, 794–797.

https://telegram.me/Geologybooks

Lagler, K.F. (1978) Capture, sampling and examination of fishes. In: *Methods for Assessment of Fish Production in Fresh Waters* (ed. Bagenal, T.), IBP Handbook No. 3, 3rd edn, Chapter 2, pp. 6–47, Blackwell Scientific Publications, Oxford, 365 pp.

Lamotte, M. & Bourliere, F. (1971) *Problèmes d'Ecologie: I'échantillonnage des peuplements animaux des milieux aquatiques*, Comité francais du Programme Biologique International, Masson et Cie, Paris, 294 pp.

Lampadariou, N., Karakassis, I. & Pearson, T.H. (2005) Cost/benefit analysis of a benthic monitoring programme of organic benthic enrichment using different sampling and analysis methods. *Marine Pollution Bulletin*, **50**, 1606–1618.

Larsen, P.F. (1974) A remotely operated shallow water benthic suction sampler. *Chesapeake Science*, **15**, 176–178.

Lassig, J. (1965) An improvement to the van Veen bottom grab. *Journal du Conseil Permanent International pour l'Exploration de la Mer*, **29**, 352–353.

Laubier, L., Martinais, J. & Reyss, D. (1971) Opérations de dragages en mer profonde. Optimisation du traict et détermination des trajectoires grace aux techniques ultrsonores. *Rapport Scientifiques et Techniques, Centre National Pour l'Exploitation des Océans*, **3**, 23 pp (French and English texts).

Lauff, G.M., Cummins, K.W., Eriksen, C.H. & Parker, M. (1961) A method for sorting bottom fauna samples by elutriation. *Limnology and Oceanography*, **6**, 462–466.

Lewis, F.G., III & Stoner, A.W. (1981) An examination of methods for sampling macrobenthos in seagrass meadows. *Bulletin of Marine Science*, **31**, 116–124.

Lewis, J.R. (1964). *The Ecology of Rocky Shores*, English University Press, London, 323 pp.

Lie, U. & Pamatmat, M.M. (1965) Digging characteristics and sampling efficiency of the 0.1 m^2 van Veen grab. *Limnology and Oceanography*, **10**, 379–384.

Lincoln, R.J. & Sheals, J.G. (1979) *Invertebrate Animals. Collection and Preservation*, British Museum (Natural History), Cambridge University Press, Cambridge, 150 pp.

Lindeboom, H.J. & de Groot, S.J. (1998). *The effects of different types of fisheries on the North Sea and the Irish Sea benthic ecosystems*. NIOZ Rapport, 1998-1991/RIVO-DLO Report C003/98, 404 pp.

Lisitsin, A.I. & Udintsev, G.B. (1955) A new type of grab. *Trudy Vsesoyuznogo gidrobiologicheskogo obshchestva*, **6**, 217–222 (in Russian).

Little, F.J. & Mullins, B. (1964) Diving plate modification of Blake (beam) trawl for deep-sea sampling. *Limnology and Oceanography*, **9**, 148–150.

Long, B.G. & Wang, Y.G. (1994) Method for comparing the capture efficiency of benthic sampling devices. *Marine Biology*, **121**, 397–399.

Longhurst, A.R. (1964) A review of the present situation in benthic synecology. *Bulletin de l'Institut Oceanographique de Monaco*, **63**(1317), 54 pp.

Macer, T.C (1967) A new bottom-plankton sampler. *Journal du Conseil Permanent International pour l'Exploration de la Mer*, **31**, 158–163.

MacGinitie, G.E. (1935) Ecological aspects of a California marine estuary. *American Midland Naturalist*, **16**, 629–765.

MacGinitie, G.E. (1939) Littoral marine communities. *American Midland Naturalist*, **21**, 28–55.

Maitland, P.S. (1969) A simple corer for sampling sand and finer sediments in shallow water. *Limnology and Oceanography*, **14**, 151–156.

https://telegram.me/Geologybooks

Marsh, L.M., Bradbury, R.H. & Reichert, R.E. (1984) Determination of the physical parameters of coral distributions using line transects intersect data. *Coral Reefs*, **2**(4), 175–180.

Martel, A & Chia, F.-S. (1991) Drifting and dispersal of small bivalves and gastropods with direct development. *Journal of Experimental Marine Biology and Ecology*, **150**(1), 131–147.

Mason, J., Chapman, C.J. & Kinnear, J.A.M. (1979) Population abundance and dredge efficiency studies on the scallop, *Pecten maximus* (L.). *Rapports et proces-verbaux des réunions. Conseil International pour l'Exploration de la Mer*, **175**, 91–96.

Masse, H. (1967) Emploi d'une suceuse hydraulique transformée pour les prélèvements dans les substrats meubles infralittoraux. *Helgolander wissenschaftliche Meeresuntersuchungen*, **15**, 500–505.

Masse, H., Plante, R. & Reys, J.P. (1977) Etude comparative de l'efficacite de deux bennes et d'une suceuse en fonction de la nature du fond. In: *Biology of Benthic Organisms* (eds B.F. Keegan, P. O'Ceidigh & P.J.S. Boaden). 11th European Symposium on Marine Biology. Galway, October 1976. Pergamon Press, Oxford, pp 433–441.

Mattheisen, G.C. (1960) Intertidal zonation in populations of *Mya arenaria*. *Limnology and Oceanography*, **5**, 381–388.

McConnaughey, R.A., Mier, K. & Dew, C.B. (2000) An examination of chronic trawling effect on soft-bottom benthos of the Eastern Bering Sea. *Journal of Marine Science*, **57**, 1377–1388.

McIntyre, A.D. (1956) The use of trawl, grab and camera in estimating marine benthos. *Journal of the Marine Biological Association of the United Kingdom*, **35**, 419–429.

McIntyre, A.D. (1961) Quantitative differences in the fauna of boreal mud associations. *Journal of'the Marine Biological Association of the United Kingdom*, **41**, 599–616.

McIntyre, A.D. & Eleftheriou, A. (1968) The bottom fauna of a flatfish nursery ground. *Journal of the Marine Biological Association of the United Kingdom*, **48**, 113–142.

McLachlan, A. & Brown, A. (2006) *The Ecology of Sandy Shores*, Academic Press, New York, 373 pp.

McManus, D.A. (1965) A large-diameter coring device. *Deep-Sea Research*, **12**, 227–232.

McNeely, R.L. & Pereyra, W.T. (1961) A simple screening device for the separation of benthic samples at sea. *Journal du Conseil Permanent International pour l'Exploration de la Mer*, **26**, 259–262.

Menzies, R.J. (1962) The isopods of abyssal depths in the Atlantic Ocean. In: *Abyssal Crustacea* (eds J.L. Barnard, R.J. Menzies & M.C. Bacescu), Vema Research Series 1, pp. 79–206, Columbia University Press, New York, 233 pp.

Menzies, R.J. (1972) Current deep benthic sampling techniques from surface vessels. In: *Barobiology and the Experimental Biology of the Deep Sea* (ed. R.W. Brauer), pp. 164–169, University of North Carolina, Chapel Hill, NC, 428 pp.

Menzies, R.J. & Rowe, G.T. (1968) The LUBS, a large undisturbed bottom sampler. *Limnology and Oceanography*, **13**, 708–714 (Contribution from Florida State University, 224).

Menzies, R.J., Smith, L. & Emery, K.O. (1963) A combined underwater camera and bottom grab: a new tool for investigation of deep-sea benthos. *Internationale Revue der Gesamten Hydrobiologie*, **48**, 529–545.

https://telegram.me/Geologybooks

Menzies, R.J., George, R.Y. & Rowe, G.T. (1973) *Abyssal Environment and Ecology of the World Oceans*, John Wiley & Sons, New York, 488 pp.

Morello, E.B., Froglia, C., Atkinson, R.J.A. & Moore, P.G. (2005) Impacts of hydraulic dredging on a macrobenthic community of the Adriatic Sea, Italy. *Canadian Journal of Fisheries and Aquatic Science*, **62**, 2076–2087.

Morgans, J.F.C. (1965) A simple method for determining levels along seashore transects. *Tuakara*, **13**, 3.

Morton, B. & Lee, C.N.W. (2012) The composition and spatial distribution of scavenging hyperbenthos in the Cape d'Aguilar Marine Reserve, Hong Kong. *Journal of Marine Biological Association*, **92**(1), 39–47.

Motoh, H. (1980) Fishing gear for prawn and shrimp used in the Philippines today. *Technical Report Aquaculture Department, Southeast Asian Fisheries Development Centre*, No. 5, 43 pp.

Mulder, M. & van Arkel, M.A. (1980) An improved system for quantitative sampling of benthos in shallow water using the flushing technique. *Netherlands Journal of Sea Research*, **14**, 119–122.

Myers, AC. (1979) Summer and winter burrows of a mantis shrimp, *Squillaempusa*, in Narragansett Bay, Rhode Island (USA). *Estuarine and Coastal Marine Science*, **8**, 87–98.

Myers, E.H. (1942) Rate at which Foraminifera are contributed to marine sediments. *Journal of Sedimentary Petrology*, **12**, 92–95 (Collected Reprints, Woods Hole Oceanographic Institution, 314).

Nalepa, T.F. & Robertson, A. (1981) Screen mesh size affects estimates of macro- and meiobenthos abundance and biomass in the Great Lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, **38**, 1027–1034.

Nalwalk, A.J., Hersey, J.B., Reitzel, J.S. & Edgerton, H.E. (1962) Improved techniques of deep-sea rock dredging. *Deep-Sea Research*, **8**, 301–302 (Collected Reprints, Woods Hole Oceanographic Institution, 1216).

Nelson-Smith, A. (1979) Monitoring the effect of oil pollution on rocky sea shores. In: *Monitoring the Marine Environment* (ed. D. Nichols), Institute of Biology Symposia No. 24, pp. 25–53, Institute of Biology, London, 205 pp.

Neumann, H., Ehrich, S. & Kroncke, I. (2008) Spatial variability of epifaunal communities in the North Sea in relation to the sampling effort. *Helgoland Marine Research*, **62**, 215–225.

Ockelmann, K.W. (1964) An improved detritus-sledge for collecting meiobenthos. *Ophelia*, **1**, 217–222.

Oele, E. (1978) Sand and gravel from shallow seas. *Geologie en Mijnbouw*, **57**, 45–54.

Oug, E. (1977) Faunal Distribution close to the sediment of a shallow marine environment. *Sarsia*, **63**(2), 115–121.

Owen, D.M., Sanders, H.L. & Hessler, R.R. (1967) Bottom photography as a tool for estimating benthic populations. In: *Deep-Sea Photography* (ed. J.B., Hersey), The Johns Hopkins Oceanographic Studies 3, Chapter 21, Johns Hopkins Press, Baltimore, MD, pp 229–234.

Pamatmat, M.M. (1968) Ecology and metabolism of a benthic community on an intertidal sandflat. *Internationale Revue der Gesamten Hydrohiologie*, **53**, 211–298 (Department of Oceanography, University of Washington, Contribution 427).

https://telegram.me/Geologybooks

Paterson, C.G. & Fernando, C.H. (1971) A comparison of a simple corer and an Ekman grab for sampling shallow water benthos. *Journal of the Fisheries Board of Canada*, **28**, 365–368.

Pauly, D. (1973) Uber ein Gerat zur Vorsortierung von Benthosproben. *Berichte der Deutschen Wissenschaftlichen Kommission fur Meeresforschung*, **22**, 458–460.

Pavithran, S., Ingole, B., Nanajkar, M. & Goltekar, R. (2009) Importance of the sieve size in deep-sea macrobenthic studies. *Marine Biology Research*, **5**(4), 391–398.

Pearcy, W.C. (1972) Distribution and die1 changes in the behavior of pink shrimp, *Pandalus jordani*, off Oregon. *Proceedings of the National Shellfisheries Association*, **62**, 15–20.

Pearson, M. (1978) A lightweight sediment sampler for remote field use. *Journal of Sedimentary Petrology*, **48**(2) 653–656.

Pearson, T.H., Josefson, A.B. & Rosenberg, R. (1985) Petersen's benthic stations revisited. Is the Kattegat becoming eutrophic? *Journal of Experimental Marine Biology and Ecology*, **92**(2–3), 157–206.

Pedrick, R.A. (1974) Nonmetallic elutriation and sieving device for benthic macrofauna. *Limnology and Oceanography*, **19**, 535–538.

Pemberton, G.S., Risk, M.J. & Buckley, D.E. (1976) Supershrimp: deep bioturbation in the Strait of Canso, Nova Scotia. *Science*, **192**, 790–791.

Peters, R.D., Timmins, N.T., Calvert, S.E. & Morris, R.J. (1980) *The IOS Box Corer: Its Design, Development, Operation and Sampling*, Issue 106, Institute of Oceanographic Sciences (Unpublished Manuscript), 7 pp.

Petersen, C.G.J. & Boysen Jensen, P. (1911) Valuation of the sea. I. Animal life of the sea bottom, its food and quantity. *Report from the Danish Biological Station*, No. 20, 81 pp.

Phillips, B.F. & Scolaro, A.B. (1980) An electrofishing apparatus for sampling sublittoral benthic marine habitats. *Journal of Experimental Marine Biology and Ecology*, **47**, 69–75.

Picard, J. (1965) Recherches qualitatives sur les biosciences marines des substrats dragables de la région marseillaise. *Recueil des Travaux de la Station Marine d'Endoume. Bull*, **36**(Fasc. 52), 1–160.

Pickett, G. (1973) The impact of mechanical harvesting on the Thames estuary cockle fishery. *Laboratory Leaflet, MA FF*, **29**, 21 pp.

Pihl-Baden, S. & Pihl, L. (1984) Abundance, biomass and production of mobile epibenthic fauna in *Zostera marina* (L.) meadows, western Sweden. *Ophelia*, **23**, 65–90.

Polte, P.A., Schanz, A. & Asmus, H. (2005) The contribution of seagrass beds (*Zostera noltii*) to the function of tidal flats as a juvenile habitat for dominant, R.C. mobile epibenthos in the Wadden Sea. *Marine Biology*, **147**, 813–822.

Powers, C.F. & Robertson, A. (1967) Design and evaluation of an all-purpose benthos sampler. *Great Lakes Research Division, Special Report*, **30**, 126–131.

Price, J.H., Irvine, D.E.G. & Farnham, W.F. (eds) (1980) *The Shore Environment. Volume 1. Methods*, Systematics Association Special Volume 17(a), Academic Press, London, 321 + 41 pp.

Pringle, J.D. (1984) Efficiency estimates for various quadrat sizes used in benthic sampling. *Canadian Journal of Fisheries and Aquatic Sciences*, **41**(10), 1485–1489.

Probert, P.K. (1984) A comparison of macrofaunal samples taken by box-corer and anchor-box dredge. *NZOI Records*, **4**(13), 150–156.

https://telegram.me/Geologybooks

Proudfoot, P.K., Elliott, M., Dyer, M.F., Barnett, B.F., Allen, J.H., Proctor, N.L., Cutts, N., Nikitik, C., Turner, G., Breen, J., Hemmingway, K.L. & Mackie, T. (2000) Collection and processing of macrobenthic samples from soft sediments: a best practice view. *Proceedings of the Humber Benthic Field Methods Workshop 1997*, University of Hull, Hull, 47 pp.

Pugh, J.C. (1975) *Surveying for Field Scientists*, Methuen, London, 230 pp.

Pullen, E.J., Mock, C.R. & Ringo, R.D. (1968) A net for sampling the intertidal zone of an estuary. *Limnology and Oceanography*, **13**, 200–202.

Rees, H.L., Moore, D.C., Pearson, T.H., Elliott, M., Service, M., Pomfret, J. & Johnson, D. (1990) Procedures for the monitoring of marine benthic communities at UK sewage sludge disposal sites. *Scottish Fisheries Information Pamphlet*, No. 18, Department of Agriculture and Fisheries for Scotland, Aberdeen, 79 pp.

Reineck, H.E. (1958) Kastengreifer und Lotrohre 'Schnepfe', Gerate zur Entnahme ungestorter, orientierter Meeresgrundproben. *Senckenbergiana lethaea*, **39**, 42–48, 54–56.

Reineck, H.E. (1963) Der Kastengreifer. *Natur und Museum*, **93**, 102–108.

Reineck, H.E. (1967) Ein Kolbenlot mit Plastik-Rohren. *Senckenbergiana lethaea*, **48**, 285–289.

Reish, D.J. (1959a) A discussion of the importance of screen size in washing quantitative marine bottom samples. *Ecology*, **40**, 307–309.

Reish, D.J. (1959b) Modification of the Hayward orange peel bucket for bottom sampling. *Ecology*, **40**, 502–503.

Reys, J.-P. (1964) Les prélèvements quantitatifs du bcnthos de substrats meubles. *Terre et la Vie, 1, Recueil des Travaux de la Station Marine d' Endoume*, **46**(18) 94–105.

Reys, J.-P. & Salvat, B. (1971) L'échantillonage de la macrofaune des sédiments meubles marins. In: *Problemes. d'Ecologie: l'echantillonage des peuplements animaux des milieux aquatiques* (eds M. Lamotte & F. Bourliere), pp. 185–242, Comité francais du Programme Biologique International. Masson et Cie, Paris, 294 pp.

Rice, A.L., Aldred, R.G., Darlington, E. & Wild, R.A. (1982) A quantitative estimation of the deep-sea megabenthos; a new approach to an old problem. *Oceanologica Acta*, **5**, 63–72.

Richards, S.W. & Riley, G.A. (1967) The benthic epifauna of Long Island Sound. *Bulletin of the Bingham Oceanographic Collection*, **19**, 89–135.

Riddle, M.J. (1989) Bite profiles of some benthic grab samplers. *Estuarine, Coastal and Shelf Science*, **29**(3), 285–292.

Riedl, R. (1961) Etudes des fonds vaseux de l'Adriatique. Méthodes et résultats. *Recueil des Travaux de la Station Marine d'Endoume*, **23**, 161–169.

Riley, J.D. & Corlett, J. (1965) The numbers of O-group plaice in Port Erin Bay. *Report of the Marine Biological Station, Port Erin*, **78**, 51–56.

Robinson, J.E., Newell, R.C., Seiderer, L.J. & Simpson, N.M. (2005) Impacts of aggregate dredging on sediment composition and associated benthic fauna at an offshore dredge site in the southern North Sea. *Marine Environmental Research*, **60**, 51–68.

Rothlisberg, P.C. & Pearcy, W.G. (1977) An epibenthic sampler used to study the ontogeny of *Pandalus jordani* (Decapoda, Caridea). *Fishery Bulletin*, **74**, 990–997.

Rowe, G.T. & Clifford, CH. (1973) Modification of the Birge-Ekman box corer for use with SCUBA or deep submergence research vessels. *Limnology and Oceanography*, **18**, 172–175.

https://telegram.me/Geologybooks

Rowell, T.W., Schwinghamer, P., Chin-Yee, M., Gilkinson, K., Gordon, D.C., Jr, Hartgers, E., Hawryluk, M., McKeown, D.L., Prena, J., Reimer, D.P., Sonnichsen, G., Steeres, G., Vass, W.P., Vine, R & Woo, P. (1997) Grand banks otter trawling experiment: III sampling equipment, experimental design and methodology. *Canadian Technical Report of Fisheries and Aquatic Sciences*, No. 2190, 36 pp.

Rumohr, H. (1999) Soft bottom macrofauna: collection, treatment and quality assurance of samples. *ICES Techniques in Marine Environmental Sciences*, No. 27, 19 pp.

Rumohr, H. (2009). Soft bottom macrofauna: Collection, treatment, and quality assurance of samples. *ICES Techniques in Marine Environmental Sciences*, No. 43, 20 pp.

Russell, B.C., Talbot, F.H., Anderson, G.R.V. & Goldman, B. (1978) Collection and sampling of reef fishes. In: *Coral Reefs: Research Methods* (eds D.R. Stoddart & R.E. Johannes), *Monographics on Oceanographic Methodology No. 5*, pp. 329–345, UNESCO, Paris, 581 pp.

Sanders, H.L. (1956) Oceanography of Long Island Sound, 1952–1954. X. The biology of marine bottom communities. *Bulletins of the Bingham Oceanographic Collection*, **15**, 345–414.

Sanders, H.L., Hessler, R.R. & Hampson, G.R. (1965) An introduction to the study of deep-sea benthic fauna1 assemblages along the Gay Head-Bermuda transect. *Deep-Sea Research*, **12**, 845–867 (*Collected Reprints, Woods Hole Oceanographic Institution*, 1666).

Schlacher, T.A. & Wooldridge, T.H. (1996) How sieve mesh size affects sample estimates of estuarine benthic macrofauna. *Journal of Experimental Marine Biology and Ecology*, **201**(1–2), 159–171.

Sellmer, G.P. (1956) A method for the separation of small bivalve molluscs from sediments. *Ecology*, **37**, 206.

Shand, C. & Priestley, R. (1999) A towed sledge for benthic surveys. *Fisheries Research Services, Scottish Fisheries Information Pamphlet,* No. 22, 8 pp.

Sirenko, B.I., Markhaseva, E.L., Buzhinskaya, G.N., Golikov, A.A., Menshutkina, T.V., Petryashov, V.V., Semenova, T.N., Stepanjants, S.D., & Vassilenko, S.V. (1996) Preliminary data on suprabenthic invertebrates collected during the RV Polarstern cruise in the Laptev Sea. *Polar Biology*, **16**(5), 345–352.

Sjolund, T. & Purasjoki, K.J. (1979) A new mechanism for the van Veen grab. *Finnish Marine Research*, **246**, 143–146.

Sly, P.G. (1969) Bottom sediment sampling. *Proceedings of the 12th Conference on Great Lakes Research*, pp. 883–898 (Collected Reprints. Canada Center for Inland Waters, 2–23).

Smith, K.L. & Howard, J.D. (1972) Comparison of a grab sampler and large volume corer. *Limnology and Oceanography*, **17**, 142–145.

Smith, W. & McIntyre, A.D. (1954) A spring-loaded bottom sampler. *Journal of the Marine Biological Association of the United Kingdom*, **33**, 257–264.

Sorbe, J.C. (1983) Description d'un traineau destiné a l'échantillonnage quantitatif étage de la faune suprabenthique neritique. *Annales de l'Institut Oceanographique de Paris* (NS), **59**(2), 117–126.

Southward, A.J. (1965) *Life on the Sea-Shore*, Heinemann, London, 153 pp.

Steedman, H.F. (ed.) (1976) Zooplankton fixation and preservation. In: *Monographs on Oceanographic Methodology No. 4*, UNESCO, Paris, 350 pp.

https://telegram.me/Geologybooks

Stephen, W.J. (1977) A one-man profiling method for beach studies. *Journal of Sedimentary Petrology*, **47**, 860–863.

Steven, G.A. (1952) *Nets. How to Make, Mend and Preserve Them*, Routledge and Kegan Paul, London, 128 pp.

Stoddart, D.R. & Johannes, R.E. (eds) (1978) Coral reefs: research methods. In: *Monographs on Oceanographic Methodology*, No. 5, UNESCO, Paris, 581 pp.

Stoner, A.W., Greening, H.S., Ryan, J.D. & Livingston, R.J. (1983) Comparison of macrobenthos collected with cores and suction sampler in vegetated and unvegetated marine habitats. *Estuaries*, **6**(1), 76–82.

Sylvand, B. (1995) La Baie des Veys Littoral Occidental de la Baie de Seine, Manche) 1972–1993: structure et évolution à long-terme d'un ecosysteme benthique intertidal de substrat meuble sous influence estuarienne, PhD Thesis, University of Caen, Caen, 397 pp.

Thamdrup, H.M. (1938) Der van Veen-Bodengreifer. Vergleichsversuche uber die Leistungsfahigkeit des van Veen- und des Petersen-Bodengreifers. *Journal du Conseil Permanent International pour l'Exploration de la Mer*, **13**, 206–212.

Thayer, G.W., Williams, R.B., Price, T.J. & Colby, D.R. (1975) A large corer for quantitatively sampling benthos in shallow water. *Limnology and Oceanography*, **20**, 474–480.

Thomas, M.L.H. (1960) A modified anchor dredge for collecting burrowing animals. *Journal of the Fisheries Research Board of Canada*, **17**, 591–594.

Thomas, M.L.H. & Jelley, E. (1972) Benthos trapped leaving the bottom in Bideford River, Prince Edward Island. *Journal of the Fisheries Research Board of Canada*, **29**, 1234–1237.

Thorson, G.E. (1957) Sampling the benthos. *Memoirs of the Geological Society of America*, **67**(1), 61–73.

Thouzeau, G. & Hily, C. (1986) A-QUA.R.E.V.E.: une technique nouvelle d'échantillonnage quantitatif de la macrofaune epibenthique des fonds meubles. *Oceanologica Acta*, **9**(4), 507–513.

Thouzeau, G. & Lehay, D. (1988) Variabilité spatio-temporelle de la distribution de la croissance et de la survie des juveniles de *Pecten maximus* (issus des pontes 1985, en baie de Saint-Brieux. *Oceanologica Acta*, **11**(3), 267–283.

Thouzeau, G. & Vine, R. (1991) L'échantillonnage du megabenthgos: Technique dévelopée sur les Georges Bank (Atlantique nord-ouest). *Comptes Rendues de l'Academie des Sciences de Paris, Serie III*, **312**(12), 607–613.

True, M.A., Reys, J.-P. & Delauze, H. (1968) Progress in sampling the benthos: the benthic suction sampler. *Deep-Sea Research*, **15**, 239–242.

Tyler, P. & Shackley, S.E. (1978) Comparative efficiency of the Day and Smith-McIntyre grabs. *Estuarine and Coastal Marine Science*, **6**, 439–445.

UKOOA (2001) *Marine Environmental Surveys Database on the UKCS*. UK Benthos, Aberdeen.

Ursin, E. (1954) Efficiency of marine bottom samplers of the van Veen and Petersen types. *Meddeleleser Fra Danmarks Fiskeri-og Havundersogelser*, N.S. **1**(7), 8 pp.

Ursin, E. (1956) Efficiency of marine bottom samplers with special reference to the Knudsen sampler. *Meddeleleser Fra Danmarks Fiskeri-og Havundersogelser*, N.S. 1(14), 6.

Uzmann, J.R., Cooper, R.A., Theroux, R.B. & Wigley, R.L. (1977) Synoptic comparison of three sampling techniques for estimating abundance and distribution of selected

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megafauna: submersible vs camera sled vs otter trawl. *Marine Fisheries Review,* **39**(12), 11–19.
van Arkel, M.A. & Mulder, M. (1975) A device for quantitative sampling of benthic organisms in shallow water by means of a hushing technique. *Netherlands Journal of Sea Research*, **9**, 365–370.
van Cleve, R., Ting, R.Y. & Kent, J.C. (1966) A new device for sampling marine demersal animals for ecological study. *Limnology and Oceanography*, **11**, 438–443.
van Veen, J. (1933) Onderzoek naar het zandtransport von rivieren. *De Ingenieur*, **48**, 151–159.
von Brandt, A. (1972) *Fish Catching Methods of the World*, Fishing News (Books), West Byfleet, 240 pp.
von Brandt, A. (1978) Bibliography for fishermen's training. *FA0 Fisheries Technical Paper*, **184**, 176 pp.
Watkin, E.E. (1941) Observations on the night tidal migrant Crustacea of Kames Bay. *Journal of the Marine Biological Association of the United Kingdom*, **26**(8), 1–96.
Whitehouse, J.W. & Lewis, B.G. (1966) The separation of benthos from stream samples by flotation with carbon tetrachloride. *Limnology and Oceanography*, **11**, 124–126.
Whitlatch, R.B. & Osman, R.W. (1998) A new device for studying benthic invertebrate recruitment. *Limnology and Oceanography*, **43**(3), 516–523.
Wickstead, J. (1953) A new apparatus for the collection of bottom plankton. *Journal of the Marine Biological Association of the United Kingdom*, **32**, 347–355.
Wigley, R.L. (1967) Comparative efficiencies of van Veen and Smith-McIntyre grab samplers as revealed by motion pictures. *Ecology*, **48**, 168–169.
Wigley, R.L. & Emery, K.O. (1967) Benthic animals, particularly Hyalinoecia (Annelida) and Ophiomusium (Echinodermata), in sea-bottom photographs from the continental slope. In: *Deep-Sea Photography* (ed. J.B. Hersey), The Johns Hopkins Oceanographic Studies 3, Chapter 22, pp. 235–349. Johns Hopkins Press, Baltimore, MD, 310 pp.
Wigley, R.L. & Theroux, R.B. (1970) Sea-bottom photographs and macrobenthos collections from the continental shelf off Massachusetts. *United States Fish and Wildlife Service, Special Scientific Report-Fisheries*, No. 613, 12 pp.
Wildish, D.J. (1978) Sublittoral macro-infaunal grab sampling reproducibility and cost. *Technical Report, Fisheries and Marine Service, Canada*, No. 770, 4 pp.
Williams, D.D. & Williams, N.E. (1974) A counterstaining technique for use in sorting benthic samples. *Limnology and Oceanography*, **19**, 152–154.
Wolff, T. (1961) Animal life from a single abyssal trawling. *Galathea Reports*, **5**, 129–162.
Word, J.Q. (1976) Biological comparison of grab sampling devices. Southern California Coastal Water Research Project. Annual Report for the Year Ending 30 June 1976, pp. 189–194.
Worswick, J.M. & Barbour, M.T. (1974) An elutriation apparatus for macro-invertebrates. *Limnology and Oceanography*, **19**, 538–540.
Yocum, W.L. & Tessar, E.J. (1979) Sled for sampling benthic fish larvae. *Progressive Fish-Culturist*, **42**(2), 118–119.
Zinn, D.J. (1969) An inclinometer for measuring beach slopes. *Marine Biology*, **2**, 132–134.
Zismann, L. (1969) A light-trap for sampling aquatic organisms. *Israel Journal of Zoology*, **18**, 343–348.

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Chapter 6
Meiofauna Techniques

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Abstract

Meiofaunal organisms are mobile multicellular animals that are smaller than macrofauna and larger than microfauna. The size boundaries of meiofauna are generally based on the standardised mesh apertures of sieves with 500 μm (or 1000 μm) as upper and 63 μm (or 42 μm) as lower limits. Meiofauna are ubiquitous, inhabiting most marine substrata, often in high densities. Meiofauna are highly diverse, and several phyla are only known to occur as meiofauna. Owing to their small size and high densities, specialised techniques are required to collect, preserve and examine meiofauna. These are described, along with approaches to determine biomass of these small animals. Their small size also makes them useful candidates for manipulative experiments, and culturing of individual species and approaches to experiments on whole communities are briefly discussed.

Keywords meiofauna, nematodes, copepods, benthos, sample collection, sample processing, sample fixation, enumeration, microscopy, biomass determination, culturing, microcosms, mesocosms

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6.1 Introduction

Meiofaunal organisms are mobile metazoans that are smaller than macrofauna and larger than microfauna. The size boundaries of meiofauna are based on the standardised mesh apertures of sieves with 500 μm (or 1000 μm) as upper and 63 μm (or 42 μm) as lower limits. All fauna passing through the coarser mesh and retained on the finer one may be considered to be meiofauna. Note that this definition does not include protozoa (which are not metazoans). For the purposes of this chapter, we refer to a mesh aperture of 63 μm, although if a smaller mesh is used to extract the fauna the same mesh or smaller should be used for all subsequent procedures. Though such a division of the fauna on the basis of size could be considered to be somewhat arbitrary, studies on the size spectra of benthic animals

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(Schwinghamer, 1981b; Warwick, 1984, 1989; Warwick *et al.*, 2006) show that the division is real, as species in benthic communities have a genuine bimodal size distribution. Furthermore, these different-sized components of the fauna are also ecologically distinct, in that the meiofauna tend to be separated from macrofauna in terms of their reproduction (all meiofauna are *in situ* breeders), dispersal (no meiofaunal organisms have a specific dispersal phase) and life histories (most meiofaunal juveniles resemble the adults). Despite this, meiofaunal research as a discipline began relatively late, with the term meiofauna first being used as recently as 1942 (Mare, 1942). For decades, meiofaunal research was seen as something separate from general 'benthic ecology', with its emphasis on macrofauna. This was partly a result of the perceived difficulties of conducting meiofaunal research, which apparently required specialised equipment for sample collection and taxonomic expertise beyond the scope of most laboratories. Happily, this situation is changing. Great efforts have been made to simplify, and to some extent standardise, meiofaunal research techniques for routine benthic analyses. An increased research effort worldwide has seen the adoption of some equipment and techniques as acceptable, and the rejection of others. Simplified pictorial keys have been developed, which allow even non-specialists to attempt the identification of many major taxa to the level of putative species. As a result, meiofaunal research has become what it always should have been, namely an integral part of benthic research, rather than an esoteric pastime for a few specialists.

One sign of the growth and maturation of meiofaunal research has been the appearance of books dedicated to the subject. Two books of particular note are those by Higgins and Thiel (1988) and Giere (2009). Both books present comprehensive treatments of methods used in meiofaunal research. Our intention is not to repeat all that is included therein. Rather, we aim to present methods currently commonly used for meiofaunal research, although where specialised or more up-to-date techniques are available we shall refer to these in passing as well.

6.2 Sample collection

Any instrument or method suitable for sampling macrofauna will, in principle, also be suitable for the smaller organisms. The main difference between sampling for macrofauna and meiofauna is that, because of the much higher numerical density of the latter, smaller samples are usually adequate. These samples can be obtained by subsampling from a larger volume samples, but because this can introduce errors or inaccuracies, there are advantages in collecting a sample that can be examined entire.

Intertidal sediments

Quantitative sampling

In sediments, coring is the best quantitative sampling technique. Corers have a known cross-sectional area and may be made from tubes or pipes of any rigid

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material. Transparent (e.g. Perspex) or translucent materials have the advantage that the sediment sample can be viewed in the tube. In some deposits, where stones or shells are present, or when very deep cores are required in single lengths, the tube may need to be hammered into the bottom and in such cases a metal corer is probably required. The choices of core diameter and sampling depth, and, therefore, the sample volume, depend on the requirements of a particular study. In many sandy intertidal habitats, a diameter of 2–4 cm has been found to give samples that can be sorted entire, and cores of about 1 cm diameter are suitable for estuarine muds where densities are usually high. Short cores are usually adequate on muddy grounds, since most of the animals live in the upper layers, and there is little life below 6–8 cm (Rees, 1940; McIntyre, 1968). On sand, however, with interstitial species extending to great depths, cores of 20–30 cm may be required to collect the bulk of the fauna. Animals are known to exist in considerable numbers below 50 cm (Renaud-Debyser, 1963), and even below 1 m in exposed beaches (McLachlan *et al.*, 1979), where the species are likely to be different from those in the surface layers. Corers should have smooth internal and external surfaces to reduce friction and the lower edge should be bevelled to aid penetration of the sediment. To enable removal of intact sediment cores, an air- or watertight closure such as a rubber stopper is required at the upper end. In well-consolidated sediments such as fine mud a bottom closure is not generally required, provided that the diameter of the core is not excessive. In less well-consolidated sediments such as coarse sand it may be necessary to close the bottom of the core-tube prior to withdrawing it from the sediment. This is generally achieved by inserting the core, closing it at the top, and then digging away the sediment next to the core tube until a stopper can be put in place and the core tube removed from the sediment.

Care should be taken to avoid compaction of sediments. As the core is forced into the sediment, friction between the sediment and the walls of the corer may compress the sediment within the tube, altering the volume of sediment sampled and potentially biasing the reconstruction of vertical profiles. Another potential problem is that animals may be drawn down from upper to lower levels as the corer is inserted into the sediment. Compaction and draw-down of material may be minimised by using a core with smooth surfaces, or by using a corer with a larger internal diameter. Applying suction to the top of the corer as it is inserted may also reduce compaction. For long core tubes in intertidal sand, for example, a gentle suction may be applied by mouth as the corer is inserted. More conveniently, and more commonly, a corer with a piston may be employed. A small but simple hand-held piston corer may be made by cutting the needle end from a plastic disposable syringe (e.g. Chandler & Fleeger, 1983) and bevelling the cut end with a file. To sample with such a device the piston should initially be near the lower end of the tube (Fig. 6.1). The tube of the corer should then be pushed slowly into the sediment to the required depth, or even a little further. It is helpful if markings indicating the required depth are made on the outside of the tube. The piston provides suction, which prevents core compression during sampling and retains the sample within the tube as it is withdrawn. If the corer is inserted beyond the required sample

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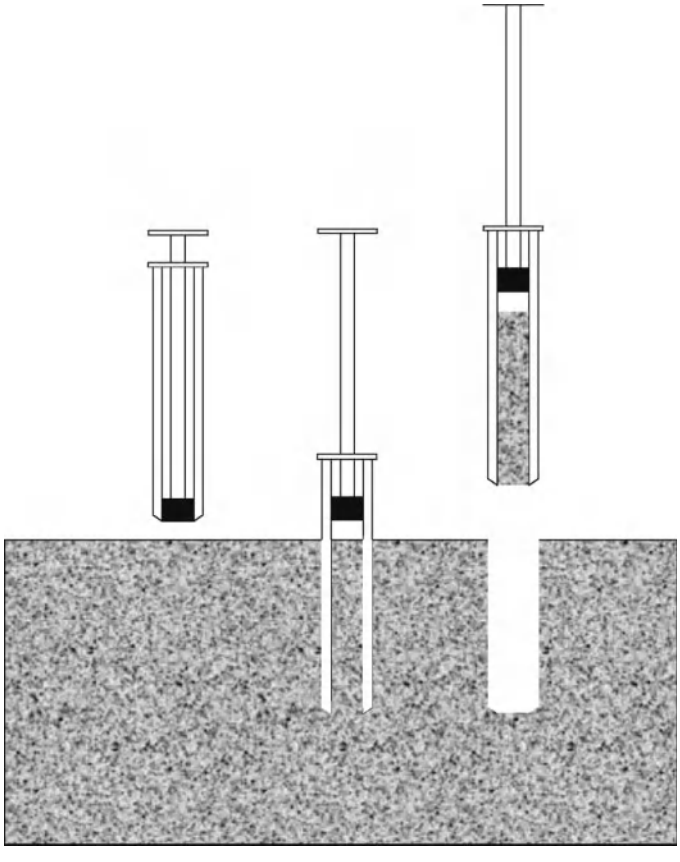

Fig. 6.1 Technique for collecting a sediment core using a piston corer such as a sawn-off disposable syringe. The piston is placed near to the sediment surface and held steady as the core tube is pushed into the sediment. Suction from the piston holds the sediment in place as the core is withdrawn.

depth, the piston can be used to extrude the excess sediment, which may then be removed with a knife or similar implement. The piston may then be used to extrude the sample into a suitable container for fixation.

In removing the sample from a standard core tube, it is best to allow the core to slide down into the collecting vessel, since this causes less disturbance than pushing it out with a piston. Fenchel (1967) describes a method by which the top stopper of the core tube is replaced by a bored cork fitted with a short glass tube attached to a length of rubber tubing closed by a clamp. Opening and closing the clamp allows the core to slide down inside the tube stepwise. Alternatively, more control may be obtained by compressing and releasing the top rubber bung with the fingers. If the deposit is of dry sand and the column tends to break rather than slide evenly, a small quantity of filtered seawater carefully added to the top helps to keep the column intact. In samples from muddy ground, the lower part

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of the core may form a plug of clay, and the sample must then be pushed out from below with a piston. This method is less satisfactory, since it compresses the core and may cause water and particles to mix from one layer to another, although this may be ameliorated by using potter's clay to seal cores from below (Cleven, 1999).

The vertical distribution of meiofauna in the sediment may be of interest. In sediments such as coarse sand, where core compression is unlikely to be a problem, cores may be extruded from below using an appropriately sized piston and sliced off in appropriate layers. In sediments where the surface layers are poorly consolidated, such as soft muds, it may not be possible to extrude cores without loss. Cores may be allowed to slip downwards out of the core tube in controlled steps by carefully manipulating the top closure, allowing them to be cut into appropriate intervals without causing additional compaction. Markings at appropriate intervals on the piston or on the core tube help to ensure that cores are cut in the right places. As meiofauna are known to migrate in standing sediments, cores should be processed as soon after collection as possible. For very deep vertical profiles specialised corers (e.g. Renaud-Debyser, 1957) may be necessary. Alternatively, a pit can be dug and core samples taken from the vertical face (Pollock, 1970). The majority of studies of the vertical distribution of meiofauna have examined variation in community structure between slices 1 and 2 cm thick. Very small-scale profiles may be more appropriate if interactions between species (such as competition) are of interest. Cores may be sectioned in slices as thin as 1 mm using a modified core-tube attached to a micrometer (Fig. 6.2). Such a device was used by Joint *et al.* (1982) to examine interactions between diatoms, meiofauna and bacteria in the surface layers of an intertidal sand flat, by Warwick and Gee (1984) to compare fine-scale vertical distributions in sands and muds and by Fleeger and Gee (1986) in an experimental evaluation of interspecific competition in harpacticoid copepods.

Qualitative sampling

If specific animals are required in large numbers, or if only particular groups are to be studied, it may be useful to deal with quite large volumes of deposit. For qualitative sampling on intertidal sediments, a general impression of the meiofauna can be obtained by simply digging a trench and allowing water to accumulate in it. Actively-swimming animals are then scooped from the water with a fine plankton net, and the more sedentary or adhesive forms are sampled by collecting sand grains from the trench. A refined version of this procedure, the 'méthode des sondages' is often used on tideless beaches to concentrate animals from the level of the water table (Delamare-Deboutteville, 1960). Alternatively, sand can be added to filtered seawater (preferably containing anaesthetic such as 6%, i.e. 73.2 g litre^{-1}, $MgCl_2$) in a bucket, stirred, and the supernatant poured through a fine sieve.

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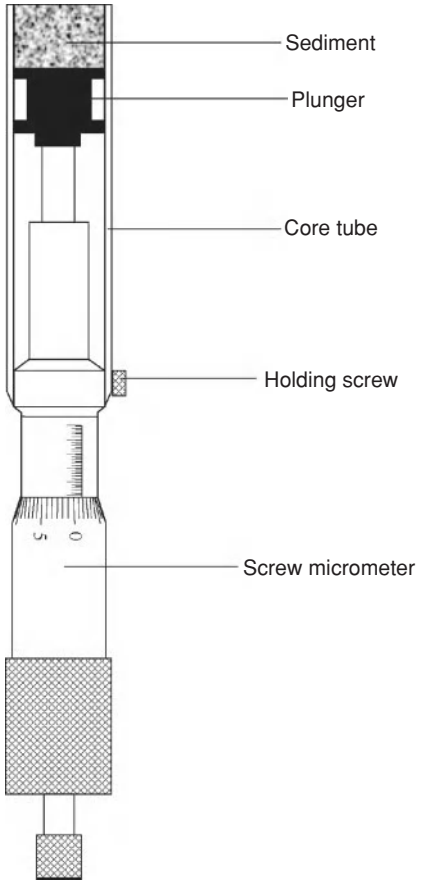

Fig. 6.2 Sampler constructed from a sawn-off disposable syringe mounted on a micrometer screw. Turning the screw raises the plunger, and the distance by which it is raised can be read off the vernier scale. Sediment sections are sliced off the top of the core.

Subtidal sediments

Quantitative sampling

When sampling sub-littorally, a basic choice must be made between getting the investigator to the seabed, and bringing some of the seabed to the surface, prior to sampling. A variety of methods is available for the collection of meiofauna from shallow sub-littoral environments, including direct collection using SCUBA, and remote sampling using a range of samplers such as grabs and coring devices (Fleeger *et al.*, 1988; Blomqvist, 1991; Kramer *et al.*, 1994; Somerfield & Clarke, 1997).

In shallow water, where SCUBA divers can work, some of the intertidal techniques used can give highly satisfactory results. Divers usually obtain superior samples because they are able to position the samplers with care and to insert the

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core slowly (McIntyre, 1971). Also, by being able to observe the sampling activity the investigator may gain insights into the nature of the site and the details of the sampling. In areas beyond the practical reach of SCUBA, cores may be taken using submersibles or remotely controlled vehicles (Thiel & Hessler, 1974; Thistle, 1978). A variety of remote samplers have been constructed for sampling in shallow water by attaching a core tube to the end of a pole. Associated with the core tube is a closure mechanism, located usually only at the top of the tube, which is either operated automatically as the core tube is pushed into the sediment (e.g. Frithsen *et al.*, 1973) or is operated remotely from the surface. These corers are light, simple to operate, but limited to water depths of 4 m or so.

Although a range of samplers suitable for use from small boats have been designed, few of them have been adopted for general use. Good design features for a remote meiofaunal sampler include a controlled arrival at the sediment surface and open flow-through tubes to reduce water disturbance and bow-wave effects, slow penetration of the sediment and tubes of sufficient size to reduce core compression and trip and closure mechanisms that do not interfere with water flow through the tube or disturb the sediment before or during penetration. A bow-wave is formed when water flows around rather than through the sampler as it is lowered. As the device reaches the sediment surface, this wave causes a flow, which washes surface material and meiofauna out of the area that is to be sampled, introducing bias. Sediments with an easily resuspended surface or a flocculent layer are the most difficult to sample.

Deliberate corers (Fig. 6.3) are wire-lowered devices that consist of a supporting frame and a moveable sampling head that carries one or more core tubes. The frame is lowered until it settles on the seabed with the core tubes a short distance (30 cm or so) above the sediment surface. The sampling head is automatically released and slowly lowers the core tubes into the sediment, at a slow speed controlled by some sort of damping device such as a piston. Once the core tubes have penetrated the sediment an automatic trip device triggers the closure mechanisms at the top of each core (generally a spring-loaded lid with a rubber seal). As the wire is hauled the cores are withdrawn from the sediment. In sediments through which water can flow, such as coarse sand, the suction induced by the top closure alone will not be sufficient to retain the cores. Therefore, a further device is required to close the bottom of each core tube. Various types have been designed, but those that are external to the core tubes are preferable.

One of the most commonly used deliberate corer designs is that of Craib (1965), which carries a single core tube, approximately 5 cm in diameter, which is closed at its lower end by a rubber ball on a lever that swings the ball into the lower end of the tube as it is withdrawn from the sediment. Samples of 15 cm length are produced, and good samples have been reported from sediments ranging from hard sand to soft mud. The apparatus weighs 44 kg and can be used from a small boat. Its main disadvantage is that, since it must stand upright for a short period while the tube is penetrating, there may be difficulties in bad weather, on uneven

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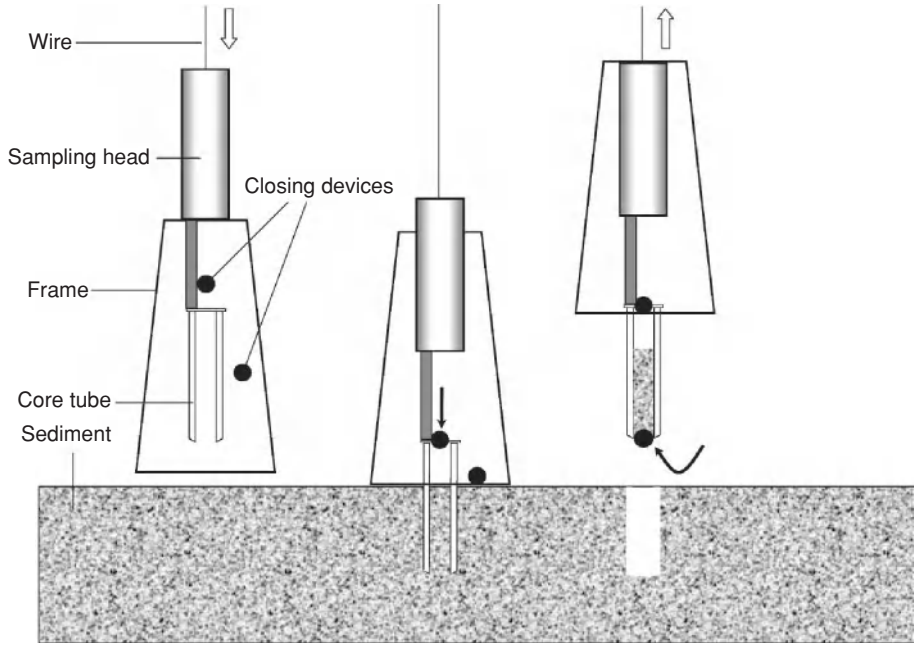

Fig. 6.3 Schematic illustrating the operation of a deliberate corer. The frame is lowered until it settles on the seabed with the open core tubes a short distance above the sediment surface. The moveable sampling head is automatically released and slowly lowers the core tubes into the sediment, at a slow speed controlled by a damping device. Once the core tubes have penetrated the sediment an automatic trip device triggers the closure mechanisms at the top of each core. As the wire is hauled the cores are withdrawn from the sediment and a further device closes the bottom of each core.

bottoms or in deep water. A much larger device, the Barnett–Watson multiple corer (Barnett *et al.*, 1984), takes up to 12 cores simultaneously. This sampler requires a large vessel for its operation, but is suitable for sampling in very deep water. Each core tube is closed by a lower lid mounted on a long lever that falls onto the sediment after triggering and swings into the closing position as the tubes rise above the sediment surface. Intermediate-sized multiple corers are now widely available. These are broadly based on the design of Barnett *et al.* (1984) but are generally smaller and take fewer (generally four) cores, often closed by a spring-loaded sliding plate. Modern designs are flexible, in that tubes of a range of different sizes may be mounted. Additional instrumentation may also be carried, along with video cameras and lights. Such devices do not require large vessels for their operation, but may still take samples in deep water. Smaller deliberate corers may struggle to penetrate sandy sediments, in which case additional weights may need to be added to the frame.

Box corers are, as their name suggests, large deliberate corers that take a square core. The closure mechanism is a large spade that is levered under the lower end of the core as it is lifted, there being no seal applied to the top of the core (although the

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surface of the core is protected from water movement by sealed doors). Although widely used for the retrieval of 'undisturbed' sediment samples, there is evidence for bow-wave induced bias in the operation of such samplers (Blomqvist, 1991; Bett *et al.*, 1994), and it is probable that they do not routinely take samples with a quality equal to smaller deliberate corers such as the Craib corer (Fleeger *et al.*, 1988).

Although grabs are widely used for quantitative sampling (see Chapter 5), many workers have severe reservations about the quality of such samples (e.g. Elmgren, 1973; Heip *et al.*, 1977; Blomqvist, 1991). Grabs are usually constructed with two jaws that are forced together to enclose a sample of sediment. The grab must be such that it can be opened from the top, allowing vertical cores to be taken by hand. Cores should be taken only when it has been possible to keep the grab upright with little disturbance to the surface sediment of the sample. Jaw penetration and closure disturb and compress the sediment while the relatively solid construction of the majority of types may induce a bow-wave that disturbs the sediment surface prior to sampling (see Chapter 5). Even in grab types with open space inside them, such as the van Veen grab, disturbance may be strong.

The normal method of collecting a subsample of sediment from a box core or a grab for meiofaunal work is to take cores using a hand-held corer such as described for the collection of intertidal sediments. Box cores and grabs are designed for sampling macrofauna, and, therefore, collect sediment from an area that is large in relation to the small-scale patchiness of meiofaunal organisms. For this reason it is advisable to collect at least three such subsamples and to pool them into a single sample, the contents of which are then more representative of the contents of the sampler as a whole, rather than one small part of them.

Much has been written about the relative merits of different sampling methods for meiofauna. The general conclusion is that different samplers provide samples of differing quality. Cores collected by hand are generally considered to be the best, both intertidally and sub-littorally. If samples cannot be collected directly then a sampling method that collects a contained core directly from the seabed is the next best. Least preferred are sampling methods that attempt to retrieve a quantity of undisturbed sediment from which subsamples may be collected using a hand corer on the surface, such as subsampling from box corers or grabs. Of the latter, box corers are generally considered to be preferable, and it has been said that grab sampling should be avoided, if possible, for quantitative meiofaunal sampling (Fleeger *et al.*, 1988). However, it should be noted that dogma should not be allowed to dictate the sampling methods used for a particular piece of work. There may be cases where subsampling from a grab is a sensible option, depending on the prevailing conditions, the type of sediment to be sampled, the ancillary equipment available on the ship and, most importantly, the particular scientific question that the investigator is addressing (Somerfield *et al.*, 1995). Differences between samplers are often attributed to bow-wave effects (e.g. Blomqvist, 1991; Bett *et al.*, 1994). Bow-wave effects are undoubtedly real but it should be remembered that a host of

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other factors can also influence the quality of samples during and after retrieval from the seabed (Somerfield & Clarke, 1997).

Qualitative sampling

The epibenthic meiofauna, and species living in the surface centimetre of flocculent bottom material, can be collected non-quantitatively in large numbers for taxonomic and other purposes with epibenthic sledges. This type of apparatus can also be used in a semi-quantitative way to investigate the seasonal appearance of the so-called temporary meiofauna – the juvenile stages of the macrofauna, which are often patchily distributed and usually restricted to the superficial deposits. These sledges have wide runners designed to disturb and skim off the sediment surface, which is then collected in a fine net (Mortensen, 1925; Ockelmann, 1964). For collecting kinorhynchs, Higgins (1964) fitted a rake-like apparatus at the leading edge behind which was a plane-blade that, like a carpenter's plane, removed the upper layer of substratum. Newell (1971) described a small box dredge specifically designed for the collection of meiofauna.

Secondary substrata

Quantitative sampling

Substrata other than sediments have meiofauna living on them. Examples include plants such as algae or seagrasses, sessile animals such as hydroids, bryozoans, sponges, barnacles and corals, hard substrata such as rock and even motile animals such as crustaceans, echinoderms and molluscs. It is almost impossible to sample such substrata quantitatively. It may be possible to detach a portion of the substratum from a known area, or to collect a standard volume of the substratum, but in either case the degree to which samples may be regarded as quantitative is questionable. Such problems may be exacerbated by the fact that many meiofaunal organisms have quite specific microhabitat requirements, so may not be distributed evenly throughout the substratum (Somerfield & Jeal, 1996). To overcome these problems, one approach is to use Artificial Substratum Units (ASUs). These may be manufactured to a standardised design and deployed according to a standardised protocol in such a way that they are directly comparable. Generally, they are constructed to mimic particular naturally occurring secondary substrata, such as seagrass blades (Bell & Hicks, 1991), algae (Edgar, 1991), pneumatophores (Gwyther & Fairweather, 2002) or secondary substrata with a network of interstices resembling sponges, holdfasts or algal tufts such as are ubiquitous in shallow-water areas with hard substrata (Gee & Warwick, 1996). The latter may usefully be mimicked using nylon-mesh pan-scourers (Schoener, 1974) and, after an appropriate period of deployment, support communities of meiofauna that are comparable to those in adjacent substrata (Cummings & Ruber, 1987; Gee & Warwick, 1996).

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When secondary substrata (natural or artificial) are retrieved underwater, it is necessary to contain the animals within the structure as it is collected, by surrounding the substratum with a plastic bag prior to detaching it for example. Relatively little quantitative work has been undertaken on the meiofauna of aufwuchs or hard substrata. Suction samplers (e.g. Tanner *et al.*, 1977) may be useful, although most samples are collected by scraping a known area.

Qualitative sampling

The majority of methods for sampling secondary substrata are qualitative in nature. Any sampling method that collects secondary substrata may be used, although those that minimise the extent to which the substrata are subjected to strong water flows are to be preferred. Examples of collecting methods include collection by hand, collection by epibenthic or rock dredges, sledges and trawls or collection by dragging a plankton net through an area of secondary substrata such as a seagrass bed.

6.3 Fixation and preservation

Preservation and extraction techniques depend on the degree to which taxa are to be identified. Certain groups, sometimes referred to as 'hard' meiofauna, such as nematodes, copepods, ostracods and kinorhynchs, remain identifiable after rough preservation in the sediment using 4% formaldehyde. However, 'soft' meiofauna (gastrotrichs, turbellarians, etc.) are difficult to recognise after this treatment, and, for these, live extraction and examination are essential (Table 6.1).

Fixation of meiofauna is normally done by bulk fixation of samples. Although alternatives such as microwave fixation or gluteraldehyde (Pfannkuche & Thiel, 1988) have been suggested, bulk fixation usually involves the use of formalin. However, the fixation method used must be appropriate both for the taxonomic group of interest (Table 6.2) and for the purpose of the study. For standard analyses of 'hard' taxa, samples should be fixed with 10% formalin as soon as possible after collection, and stored in 4% formalin. Formalin to be used as a fixative should be buffered at a minimum pH of 8.2 and diluted to a 10% solution using filtered seawater. For large bulk samples including sediments it may be preferable to add 40% stock solution directly to the samples, and then to make up the volume of the sample with filtered seawater to achieve the required concentration. Invert the sealed container several times to ensure that the fixative solution and the sample are adequately mixed. Formalin fixation of samples should last a week or more, but samples may be preserved for longer in the fixative if required. Despite its widespread use, formalin is dangerous, and care should be taken to ensure that adequate health and safety precautions are taken to minimise the risk of inhalation

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Table 6.1 Special methods of collection and extraction for particular meiofaunal taxa. For further details, see Higgins and Thiel (1988).

Cnidaria	Extract alive. Usual decantation procedures.
Turbellaria	Extract alive. Handle with fine paintbrush or pipette. Decantation often works without anaesthetic. If sediment sample is allowed to stagnate, may concentrate on sediment surface. Seawater ice technique effective.
Gnathostomulida	As for Turbellaria.
Nemertea	Extract alive. Usual decantation procedures.
Nematoda	Standard methods of collection and extraction (alive or preserved).
Gastrotricha	Extract alive using relaxation-decantation or seawater ice. Handle with fine micropipette rapidly (they are frustratingly adhesive to surfaces).
Rotifera	Extract alive using relaxation-decantation or seawater ice.
Loricifera	Standard methods of collection and extraction (alive or preserved).
Priapulida	Standard methods of collection and extraction (alive or preserved).
Kinorhyncha	Standard methods of collection and extraction (alive or preserved) are appropriate, but an effective way of extracting large quantities from offshore muds is to pass a stream of fine air bubbles (from an aquarium airstone) through a suspension of the sediment, which brings them to the surface film. This does not work for intertidal species (their cuticle is not hydrophobic).
Polychaeta	Standard methods of collection and extraction (alive or preserved), but alive preferable (many species fragile).
Oligochaeta	Standard methods of collection and extraction (alive or preserved).
Tardigrada	Standard methods of collection and extraction (alive or preserved). Narcotisation of live material essential prior to decantation (cling strongly to sand grains): brief fresh water shock is effective.
Ostracoda	Standard methods of collection and extraction (alive or preserved).
Mystacocarida	Standard methods of collection and extraction (alive or preserved). Narcotisation not necessary for live extraction (do not adhere to sand grains).
Copepoda and other microcrustaceans	Standard methods of collection and extraction (alive or preserved).
Halacaridae	Usual methods of collection. Best extracted by decantation of preserved material (resistant to anaesthetics or fresh water shock).
Bryozoa, Gastropoda, Holothuroidea, Tunicata	These taxa have a few meiofaunal representatives present in low abundance. Best extracted in large non-quantitative samples by live elutriation after narcotisation.

of fumes or contact with the solution. Sediment samples are usually brought back to the laboratory for extraction.

6.4 Sample processing

Extraction

In order to examine and count meiofauna, the animals must be extracted from the sediment. Extraction methods vary according to the type of sediment and depend on whether extraction is to be qualitative – to obtain representative specimens – or

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Table 6.2 Special methods of preparation and examination of material for particular meiofaunal taxa. For further details see Higgins and Thiel (1988).

Cnidaria	Examine alive. Contract with normal preservation: narcotise before fixation, then shock fix in hot strong formalin.
Turbellaria	Examine alive. Anaesthetise, restrict to small drop of liquid, then fix with fast-acting fixative (Bouin's, gluteraldehyde). Serial anatomical reconstruction necessary for descriptions: embed in wax or resin.
Gnathostomulida	As for Turbellaria.
Nemertea	Examine alive. Taxonomic descriptions require histological work, as for Turbellaria.
Nematoda	Fix in 10% formalin (4% formaldehyde). Transfer by slow evaporation to anhydrous glycerol for whole mounts (note pigmentation, e.g. 'eyespots', first, as this may disappear). Mount on normal glass slides or Cobb slides (see main text).
Gastrotricha	Examine alive. Fix after relaxation in $MgCl_2$ with Bouin's fluid. Embedding and serial sectioning necessary for some taxonomic work.
Rotifera	Fix in 5–10% formalin. Slow evaporation into pure glycerol for mounting as for nematodes.
Loricifera	Fix in 2% gluteraldehyde of 6–8% formalin. Transfer by slow evaporation to anhydrous glycerol for whole mounts. Mount on normal glass slides or Cobb slides (see main text).
Priapulida	Examine alive or fixed. Transfer by slow evaporation to anhydrous glycerol for whole mounts.
Kinorhyncha	Fix in 6–10% formalin. Mounting in a modified Hoyer's solution is recommended, but slow evaporation into glycerol is also adequate. Mount on normal glass slides or Cobb slides (see main text).
Polychaeta	Examination of live specimens advantageous. Fix in 10% formalin. After at least 24 h, store in 70–80% alcohol. Dissection of single segments of fixed specimens with a pair of parapodia often necessary for identification.
Oligochaeta	Fix in 10% formalin, then transfer to 70–80% alcohol. Whole mounts in glycerol usually sufficient for identification.
Tardigrada	Fix and preserve in 3–7% formalin. Mount by slow evaporation into anhydrous glycerol.
Ostracoda	Fix in 10% formalin. For identification, specimens dissected with microneedles or sharpened tungsten wire. Shell removed and kept dry in a covered slide. Appendages dissected out and mounted in polyvinyl lactophenol.
Mystacocarida	No special fixation technique. Store in ethanol after fixation.
Copepoda and other microcrustaceans	Fix in 4–5% formalin. Transfer to 70% alcohol for long-term storage. Specimens can sometimes be identified from whole mounts in glycerol sufficiently thick that the specimen can be rolled by pushing the coverslip. However, dissection and mounting of limbs is often essential: this requires skill and patience, and is best learned from a practising expert rather than from books.
Halacaridae	Fix and store in 70% alcohol. For critical examination, must be cleared: pierce body with tungsten needle and squeeze out body contents in a drop of lactic acid (gentle warming helps the clearing process). Permanent mounts can be made in glycerine on Cobb slides.
Bryozoa, Gastropoda, Holothuroidea, Tunicata	Examine live. Details of specialised treatment of preserved material can be found in Higgins and Thiel (1988).

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quantitative, i.e. to extract every organism possible for detailed counts. Extraction can be done on either fresh or preserved samples. If the vertical distribution of the fauna is to be studied, it is essential that the sample should be divided into appropriate sections immediately on collection, since changes within the sample (e.g. in packing, water content and temperature) can produce rapid alterations in the vertical distribution of the fauna.

For qualitative extraction, the sample can be allowed to stand in seawater in the laboratory and the deposit examined at intervals when organisms that come to the surface (often aggregated away from the light) can be pipetted off. Stirring the sample, or bubbling air through it, brings certain types of animals (such as most kinorhynchs and some small crustaceans, which have hydrophobic cuticles) to the surface film of the water, from where they can be scooped off using a wire loop or blotting paper. A bicycle pump attached to an aeration block by flexible tubing can be used in the field to bubble samples. For deep-living fauna, the sample may be shaken and decanted, preferably using an anaesthetic as described below.

Techniques for quantitative extraction fall into two broad categories. Those like decantation, elutriation and flotation, which rely on the different rates of sedimentation of organisms and sediment particles, are suitable for both living and preserved material. Techniques that employ an environmental gradient drive living animals out of the sediment.

Sediment

Preserved material

Fixed sediment samples contain a mixture of formalin and sediment components such as silt, clay, sand grains and organic detritus, in addition to the fauna. Although the following processes are often referred to as ‘extraction of the fauna’, in reality what we are endeavouring to do is to extract various sediment components, so as to end up with the fauna from the original sample and as little else as possible. Although these techniques are generally adequate for preserved samples, if possible, occasional live samples should be examined to help with interpretation of the preserved material, and to allow identification of the more delicate forms. Some taxa with dense shells (bivalve larvae, ostracods) are not adequately extracted by these techniques that rely on the differential settlement of animals and sediment components in water or other fluids.

Prior to processing samples, it is important to check that the laboratory fresh water supply does not contain meiofauna. To do this, run tap water through a 63 μm sieve for 5–10 minutes and check the contents of the sieve (if any) under a binocular microscope. If meiofauna is present, it is necessary to make some arrangement to have it removed. Many of the following techniques involve washing and concentrating meiofauna on sieves with fresh water. Attaching a flexible tube to the fresh water tap is highly recommended, as it greatly increases one’s control

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over the direction and strength of the flow. Meiofauna are small, so do not use a strong jet of water, and splashing must be avoided.

A problem with extracting meiofauna from some fine sediments is the presence of cohesive lumps of clay and faecal pellets, which are difficult to sieve out and which may conceal animals. With preserved samples, pre-treatment to improve the sieving efficiency is possible. Thiel *et al.* (1975) used ultrasonic treatment, by means of either an ultrasonic bath or a probe, which effectively broke up sediment aggregations and improved sieving efficiency without undue damage to the meiofauna. Barnett (1980) found that freezing in a domestic freezer for 24 hours, followed by thawing, broke down resistant sediments into small particles, which could then be further fragmented with the water-softening agent 'Calgon'. A solution made by adding approximately 100 g of Calgon to 500 ml of the sample in formalin was effective, the mixture being stored for 24 hours with intermittent shaking.

An initial washing of the sample with fresh water on a 63 μm sieve removes the finer sediment components, silt and clay, and much of the formalin. Take care not to overload the sieve, larger muddier samples may need to be sieved in smaller amounts. Puddling may help if the sieve becomes clogged. Continue washing until the water passing through the sieve is relatively clear. For some fine sediments, especially if the initial samples are small, this is sufficient to reduce the sample to a quantity that can be extracted with Ludox™.

Some types of samples may contain significant quantities of larger material such as leaf or paper fragments. It may be helpful in such circumstances to remove this material by washing the sample through a 1 mm sieve nested on top of the 63 μm sieve. Extreme care is necessary not to clog the smaller mesh as it is not in direct view. The contents of the 1 mm sieve should be checked under a binocular microscope to ensure that any meiofauna has been washed out of it.

If, after initial washing, the sample contains appreciable quantities of sand, this can be removed by a decantation extraction. Wash the remaining sample into a 1 litre wide-mouth stoppered measuring cylinder (no more than 150 ml of sediment at a time) and fill the cylinder to above the 1 litre mark with fresh water. Put in the stopper and invert the cylinder 5–10 times to distribute the sediment evenly throughout the volume. Allow to stand briefly, so that dense particles (mainly sand) drop out, then carefully pour the supernatant onto a 63 μm sieve. Repeat 3–6 times. If the next process is to be a flotation extraction with Ludox (see below), do not be too concerned if some fine sand appears on the 63 μm sieve. It is good practice to check an aliquot of the sediment remaining in the cylinder for meiofauna before discarding it, particularly if the sample is one of the first to be processed from a new survey, to assess the efficiency of the extraction.

The key to flotation extraction (or density separation, as it is sometimes called) is to suspend the fauna and sediment in a dense fluid that has a specific gravity very close to that of the animals. Therefore, the animals are neutrally buoyant, and remain in suspension, but sediment components are negatively buoyant and

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slowly sink. Various media have been used – sugar solutions (Anderson, 1959; Heip *et al.*, 1974; Higgins, 1977), magnesium sulphate (Lydell, 1936), sodium chloride (Lyman, 1943), zinc chloride (Sellmer, 1956) and carbon tetrachloride (Dillon, 1964). The disadvantage of most of them, such as NaCl or sucrose, is that they have a very high osmotic potential and, therefore, can damage some of the fauna. These media have now been largely replaced by the use of the colloidal silica polymer 'Ludox' produced by Du Pont, primarily developed for use in iron foundries, which has been found to have properties that make it ideal for the extraction of meiofauna. It is available in a range of grades, but Ludox is widely used.

As supplied, Ludox has a specific gravity of 1.40. The specific gravity needed to extract meiofauna is approximately 1.13, so the stock solution must be diluted. Adding two parts fresh Ludox to three parts fresh water will give a solution of approximately the correct density, but the density should be measured with a hydrometer. For whole sediment samples some workers prefer to make up a slightly denser solution (specific gravity 1.18) so that the interstitial water in the sediment further dilutes the Ludox to the desired specific gravity. Do not use seawater directly with Ludox, as this can cause the suspended silica to precipitate, rendering the sample useless.

As previously mentioned, Ludox is a colloidal silica solution, and as silica dust is harmful, care must be taken with its use. Sieves, glassware and other equipment such as washbottles, which have been used for Ludox, should be soaked in dilute NaOH solution and rinsed in hot water. It is a wise precaution to wear rubber gloves when handling Ludox. The disadvantage of Ludox is that, at the present time, it is generally possible to purchase it only in large quantities (300 kg drums), although some chemical suppliers have been known to supply it in smaller amounts.

A number of centrifugation techniques involving Ludox have been used (e.g. De Jonge & Bouwman, 1977; Pfannkuche & Thiel, 1988), but the following method has been found to be simple and effective. After decantation, or after initial washing, if the sample consists of a small amount of material, carefully wash the extracted portion of the sediment to one side of the sieve, then wash it into a tall-form beaker using Ludox. Add at least 10 times the sample volume of Ludox, made up to specific gravity 1.13. Stir vigorously to distribute the sample evenly throughout the volume and leave to settle for at least 40 minutes. Carefully pour the supernatant through a 63 μm sieve into a jug. Return the Ludox to the sample and repeat the process 2–4 times. Wash the extracted fauna thoroughly with fresh water. If the sample is not to be worked on immediately, preserve it with 4% formalin (or 70% alcohol with 5% glycerol) in a suitable container, such as a glass tube with an airtight plastic closure.

Live material: coarse sediment

Where the sediment particles are heavier than the animals living in the interstices, decantation or elutriation techniques may be used, as previously described for

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preserved material, and these may be simple or elaborate. Seawater, rather than fresh water, should be used. It is important to use filtered seawater to prevent contamination of the sample with organisms that might be present in, for example, the deck water supply of a research vessel. Best results are obtained if the whole sample is first treated with an anaesthetic, since many interstitial animals tend to attach to sand grains when motion occurs. A solution of magnesium chloride isotonic with seawater is widely used – 75.25 g $MgCl_2.6H_2O$ dissolved in 1 litre distilled water is approximately isotonic with seawater of 35 psu (since this salt is very hygroscopic, a solution is made up more accurately by heating the salt to constant weight at 500–600°C, 35.24 g of the anhydrous salt being used to make 1 litre of solution). This is sufficient to relax the fauna without adverse effects after several minutes' treatment, but the addition of 10% alcohol is also satisfactory.

Elutriation is a more sophisticated, and perhaps more efficient, version of the decanting procedure introduced by Boisseau (1957) for meiofauna work. Hockin (1981) described a simple system for it, constructed from readily available 'Quickfit' laboratory glassware. Tiemann and Betz (1979) used a long, narrow, elutriation vessel, 40 cm in height and 8 cm at its widest part. They emphasise the value of this shape in reducing turbulence and achieving better animal/sediment separation. Elutriation in warm water, described by Uhlig *et al.* (1973), has improved efficiency for nematodes that stretch out under these conditions, and for ostracods that open their shells thus increasing water resistance.

Behavioural methods for extraction of living animals were originally developed for terrestrial work, and make use of the activity of the organisms in response to changes imposed by the operator on their physical conditions. A technique was developed by Uhlig (1966, 1968) specifically for marine work. Sediment is placed in a tube on a nylon gauze base that just dips into filtered seawater in a collection dish. Crushed seawater ice is added to a layer of cotton wool on top of the sediment and, as the ice melts, organisms move down into the collecting dish. The method has certain limitations and can be applied only to sandy sediments, which have a capillary structure. Most of the smaller forms leave the deposit and concentrate in the collecting dish but nematodes and some of the larger animals are not extracted quantitatively, and the sediment should later be elutriated or decanted to obtain a complete extraction. However, the technique is well suited to 'soft' meiofauna (and also to ciliates and flagellates).

Live material: fine sediment

For quantitative extraction of living material from fine muds and silts, hand sorting is generally required. Divide the sample into two size fractions, and remove the finest material and preservative, using a fine sieve. A mesh of 63 μm is appropriate since this is usually accepted as defining the upper limit of the silt–clay fraction of the sediment, but even finer meshes (30 or 40 μm) are often used to ensure that most of the fauna is retained in the sieve residue. Most of the silt–clay fraction of

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the deposit, along with the smallest meiofauna – mainly juvenile stages – passes through the sieve. The residue is hand-sorted under a stereoscopic microscope, attention always being paid to the surface film of the overlying water as some groups, such as Kinorhyncha, tend to be caught by surface tension. Normal hand sorting from fine material passing through the sieve is difficult, and an appropriate subsampling technique can be employed. The volume thus subsampled should be related to the size of the sorting dish so that the settled subsample covers the bottom of the dish with a layer only a few grains thick. When viewed by light from below, living animals can easily be seen either directly as they move or by the track they leave. Counts from the subsample are adjusted to give an estimate for the total volume of water, and this is added to the sieve residue count to obtain a value for the original sample. The section of the sample awaiting examination must be kept under suitable conditions of temperature and restricted light so that the animals remain in good condition. The whole procedure is time-consuming, and restricts the number of samples that can be dealt with, but it does permit an accurate count of the fauna in fine muddy deposits.

With many organic muds, large quantities of light debris present in the sieve residue severely hamper the sorting of meiofauna. Density separation techniques, used to separate preserved animals from such debris, have not been widely employed for living meiofauna because the flotation media are usually toxic, or cause distortion of the organisms. However, Schwinghamer (1981a) described a method for the extraction of living organisms from such sediments using centrifugation in a buffered mixture of sorbitol and the silica sol 'Percoll' (Pharmacia Fine Chemicals AB, Uppsala, Sweden), which is non-toxic. It may be possible to detoxify the Ludox by dialysis (De Jonge & Bouwman, 1977), and use it with living specimens.

Secondary substrata

Meiofauna may be extracted from secondary substrata by agitating the substrata vigorously in a bucket of seawater, removing the substrata and then pouring the contents of the bucket through a sieve. A narcotising agent is recommended, as many meiofaunal organisms are adapted to grip their substrata tightly. Structurally complex substrata such as algal holdfasts, or ASUs of the nylon-mesh pan-scourer type, should be broken up prior to extracting the meiofauna. If, as is often the case, quantities of coarse or fine sediment or detritus are washed out of the substrata along with the meiofauna, then these can be removed using the appropriate extraction techniques described above for sediments.

6.5 Storage and preservation

For the preservation of specimens, once they are extracted, a concentration of 4% formalin is adequate. To minimise the risks associated with formalin, a solution of

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70% ethanol (or industrial methylated spirit) to which 5% glycerol has been added may be preferred. Unless the sample containers are particularly well sealed, the alcohol tends to evaporate slowly. The glycerol does not evaporate and, therefore, prevents specimens from drying out. It also serves to prevent some organisms such as harpacticoids from becoming brittle. Samples should be checked regularly and topped up with spirit as necessary.

6.6 Sample splitting

Typically, sediment samples contain large numbers of meiofaunal organisms, and it would be impossible to count and identify all of them. However, meiobenthic communities are patchy at small spatial scales, so simply taking smaller samples is not the answer. This is why we take larger samples and then routinely identify a proportion, extracted at random from the whole sample, which we refer to as a subsample. As a general rule, a subsample that contains at least 200 specimens is adequate for standard community analyses. It is helpful to keep the subsample size (defined as a fraction of the whole sample) constant within a particular study. Subsampling can be undertaken before extraction of the fauna, but it is generally more efficient to split the sieve residue of completely extracted samples of the meiofauna.

Elmgren (1973) built a special sample divider, consisting of a plexiglass cylinder with its bottom divided into eight equal chambers. A preserved sample is poured into the sample divider, a little detergent added (to prevent copepods and ostracods adhering to the water surface), the volume made up to 1 litre and a tightly fitting lid applied. The sample divider is then inverted and vigorously shaken for a short while. The sample is given about one hour to settle to the bottom, during which time a few twists of the sample divider cause material sedimenting onto the dividing walls to fall down into the chambers. The water is slowly drained off through a tap, until it reaches the level of the dividing walls, the rubber stoppers in the bottoms of the chambers are removed, and the subsamples drained into eight small containers. A gentle jet of water washes out remaining sediment. Jensen (1982) described a modified version, with a removable mixing chamber and a central cone-shaped splitter.

Rather more simple techniques have also proved robust and reliable. An extremely simple but effective subsampler can be constructed as follows. Bend the handle of a kitchen ladle so that it hangs with the bowl horizontal. Fill the bowl with water and measure the volume 20 times. Find a container with a flat bottom and vertical sides made from a reasonably strong translucent plastic, with a volume about 30 times that of the ladle. Sawing the top off a chemical reagent container can often make such a container. Measure a volume of water equivalent to 20 times the volume of the ladle into the plastic container, place it on a horizontal surface and allow it to settle. Mark the level of the meniscus with a permanent marker at various points around the container. Carefully remove one ladleful of water, and

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mark the position of the meniscus again. Repeat this process to give a series of marks on the container denoting volumes equivalent to various numbers of ladles. It is used as follows: if, after extraction, the sample has been stored in formalin or alcohol, wash the sample with fresh water on a 63 μm sieve. Wash the extracted sample into the plastic container with fresh water and add water until the total volume is equivalent to a known number of ladle volumes. The key to efficient subsampling with this apparatus is in agitating the contents of the container in such a way as to distribute the sample throughout the volume homogeneously. Simply stirring the sample with a circular motion concentrates the meiofauna. The best method is to use the ladle to agitate the sample vigorously with a vertical motion for 20–30 seconds, then to remove carefully a single ladleful to a 63 μm sieve. Carefully rinse the ladle, collecting the washings on the sieve. If further subsamples are required the remaining volume must be agitated prior to each removal. Once subsamples have been removed, the remaining sample should be returned to formalin or alcohol, and the size of subsample removed should be recorded on the container. Note that minor errors in the volume extracted add up, so using this method to remove more than about a quarter of the sample (five ladle volumes) begins to become unreliable.

Other simple subsampling techniques rely on similar principles. The sample can be washed into a tall-form beaker, made up to a known volume with tap water, vigorously agitated into an even suspension and a subsample rapidly withdrawn using a sampler of known volume such as a Stempel or Hensen pipette (available from Hydro-Bios, Kiel, West Germany). It is convenient to adjust the volume in the beaker so that a 1/10th or 1/20th subsample is withdrawn each time. Alternatively, the sample may be washed into a measuring cylinder, made up to a known volume with tap water, agitated by vigorous bubbling induced by blowing through a graduated pipette (with a fairly wide mouth) and then quickly sucking up a measured volume of suspension. This latter technique is not advisable with material that has been in contact with formalin (or other chemicals such as gluteraldehyde for that matter).

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6.7 Examination and counting

Sorting

The sorting of meiofaunal samples is time-consuming and labour-intensive. It is necessary to sort samples only if one is interested in identifying components of the meiofauna that cannot routinely be identified in whole mounts, such as harpacticoid copepods. Picking out is done using fine stainless steel forceps for larger, more robust material or with a fine glass pipette, fine tungsten needle, sharpened quill or similar instrument for smaller more delicate organisms. However, it may be useful to examine extracted samples, in water, under a binocular microscope with

about 250 × magnification, in order to check that animals are present, to count individuals of major groups (e.g. nematodes and copepods), or to pick out larger pieces of detritus. A small petri dish with lines scored on the bottom is ideal for the purpose. The spacing between the lines should be slightly less than the field of view of the microscope. The use of a moveable stereo-microscope on an extended arm is also an option. The petri dish can be fixed in position (with a piece of graph paper beneath it) and the animals are not disturbed during the scanning of the dish bottom and the surface film (Uhlig *et al.*, 1973).

To aid the detection of animals during sorting samples may be stained. Rose Bengal has commonly been used for this purpose. It may be added to 10% formalin during sample fixation. 10 ml of a 1% solution, made by dissolving 1 g of the stain in 1 litre of 10% formalin, added to each litre of fixative creates a sufficient stain after several days. Samples that have already been extracted may be temporarily immersed in a 1% solution for 15 minutes, although some animals with impenetrable cuticles, such as kinorhynchs and some halacarids, will not take up the stain unless they are immersed for 48 hours or more.

Preparation for microscopy

Details of the special requirements of all major meiofaunal groups are given in Table 6.2. The soft meiofauna can usually only be identified with certainty when alive, but it may be helpful to reduce or eliminate mobility by the use of a compression chamber such as the rotary microcompressor described by Spoon (1978) or the special chamber (available from Hydro-Bios) described by Uhlig and Heimberg (1981), which can also be used with inverted microscopes. With such an instrument it is possible gradually to reduce the gap between the glass base-plate and coverslip (or between two coverslips), and thus to squeeze the animal until it can no longer move. Alternatively, animals can be placed in a drop of seawater under a standard coverslip, and the water may then be gradually removed using filter paper until the required degree of compression is achieved. Adding an anaesthetic to the water may also be helpful.

For hard meiofauna, it is possible to make permanent slides, which can be examined at leisure and retained as a reference collection. There are two approaches to mounting samples of hard meiofauna. For taxonomic work, or for the routine examination of taxa that require dissection, specimens must be picked out and mounted either singly or in small numbers on individual slides, either with or without dissection. For the routine identification of other taxa, bulk mounts have proved to be extremely efficient. A bulk mount is made by mounting a whole sample or subsample on one or more slides. Although there is a range of available mounting media that have been recommended for different taxa, the majority of hard meiofauna can be mounted in anhydrous glycerol. The transfer to glycerol needs to be controlled to prevent the collapse of specimens, and this is usually achieved using modifications of the evaporation technique described by Seinhorst (1959).

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If individual specimens have been picked out into a smaller container, such as a watch glass, carefully pipette off as much fluid as possible. Add a mixture of dilute ethanol and glycerol. The exact recipe for this mixture is not important, but it should contain approximately 5% glycerol and 10–30% ethanol. This can be mixed up and stored in a labelled washbottle. For bulk mounts, wash subsamples (or whole samples if subsampling has not been carried out) on a small 63 μm sieve and then wash the material into a cavity block with the same mixture. The watch glass or cavity block is then placed in a desiccator to evaporate off the ethanol and water, leaving the specimens or samples in pure glycerine. This process takes several days, but if more rapid transfer is required the watch or cavity block may be placed on a warm hotplate (20–30°C) or in an oven at 50°C, when evaporation is completed in about 24 hours. If the material is to be left for longer than a day, the cavity block should be partially covered to exclude dust. Although it has been washed several times, formalin-fixed material often produces formalin fumes at this stage, and so it is a good idea to carry out the evaporation in a fume cupboard.

Once evaporation is complete the specimens or samples are ready for mounting. For taxonomic work and for some identifications, it is sometimes advantageous to mount specimens in such a way that they may be viewed from both top and bottom. This may be achieved by mounting specimens between two coverglasses in a special holder such as a Cobb mount. This consists of a metal frame the same size as a normal microscope slide, which holds the double coverglass mount securely in place by means of a pair of plastic or cardboard spacers. Specimens may be viewed from either surface simply by turning the preparation over.

More usually, specimens (and samples) are mounted on standard glass microscope slides. Preparations are made by mounting the specimens in a drop of anhydrous glycerol and, after making sure that they are arranged correctly, carefully covering them with a coverglass of an appropriate size. For temporary mounts, the coverglass may be propped up with small pieces of lens tissue or broken coverglass to prevent flattening of the specimens. Permanent mounts may be prepared by supporting the coverglass with fine glass beads (ballotini) or rods of appropriate diameter, and sealing the edges with an appropriate sealant for fluid mounts such as ‘Glyceel’, ‘Brunseal Clear’ or nail varnish. If oil-immersion lenses are to be used it is important that the sealant compound is resistant to the solvents used to remove the oil, such as xylene.

A useful method for making permanent or semi-permanent slides that is routinely employed in a number of laboratories is the wax ring technique. This involves placing on each slide a wax ring with the same shape as, but slightly smaller than, the coverglass intended for use. For bulk mounts, relatively large slides and coverglasses may be appropriate. Wax rings are made by dipping a specially made metal template into molten wax, and then applying it to each slide so as to leave a smooth ring of solid wax on each. The material to be mounted is then placed in anhydrous glycerol within the ring and the coverglass is placed on top. The wax ring

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is then carefully melted, slowly lowering the coverglass, by placing the preparation on a hotplate. On removal from the heat it resolidifies, sealing the preparation and supporting the edges of the coverglass at the same time, although one or two coats of an appropriate sealant are usually applied in addition. Bulk mounts made by this process have proved to be useful after 10 years or more, and standard and Cobb mounts made using wax rings are routinely used for taxonomic preparations of marine nematodes.

Counting

Starting at one corner, bulk mounts should be scanned in a systematic way, so that the investigator can be sure that coverage is complete. The use of a mechanical stage with vernier scales on the *X* and *Y* axes is highly recommended. Scanning is normally done using the $10\times$ objective, giving a magnification of $100\times$. If it is possible to set up the microscope and stage in such a way that one field of view of the $10\times$ objective coincides with a major division of the vernier scale, this will greatly facilitate the process. Conventionally organisms are counted and identified only when the anterior end lies within the field of view. If the slides are examined in a systematic fashion it is then possible to record the position of specimens, for reference purposes or for later closer examination, by reading the coordinates from the vernier scales. If a specimen has come to lie at an awkward angle, or with an important feature hidden under a piece of detritus, applying gentle pressure to the coverglass with a firm pointed implement, such as the point of a pencil, should move it sufficiently to allow identification. Once the slides have been examined they should be stored flat, for example in cardboard slide trays.

Measurement

Identification often depends on making measurements, which can be achieved from camera-lucida drawings, the scale of which is calibrated with a stage micrometer. The lengths of curved structures can be determined by running an opisthometer (map measurer) along the drawing, and then running it back to zero along a scale drawn from the stage micrometer. Alternatively, a digitising tablet may be used to measure lengths from drawings or directly through the camera-lucida. By attaching an electronic camera to the microscope, use may be made of sophisticated image analysis software to measure lengths and areas.

6.8 Biomass determination

The small size of most meiofauna poses methodological constraints on direct biomass measurements of individuals. Generally, estimates of individual meiofaunal biomass are not obtained from direct weight or carbon determinations, but

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from volume estimates. Biomass (wet weight) can be derived from volume by reference to a specific gravity of 1.13 (Wieser, 1960). This nematode-based value is commonly used for other meiofaunal taxa too. For 'hard' taxa, volume estimates are made from measurements of the body length (L) and maximum width (W) of individual specimens. These are converted to volume (V) using the formula

$$V = LW^2C$$

where the value of C relates to the shape of the body (see also Chapter 8). The actual value of C will vary according to the units in which measurements are recorded, and to the shape of each individual animal. As volume estimates made in this way are always somewhat approximate, values of C for individual animals are rarely, if ever, calculated. Instead, approximate values for 'average' animals of various shapes are generally employed (Table 6.3). Volumes of individual animals may also be calculated from scale drawings or photomicrographs. For 'soft' meiofauna volumes may be calculated by gently squashing specimens to a uniform thickness under a coverslip, and then measuring the area of the specimen.

Table 6.3 Approximate conversion factors (C) for calculating body volumes (in nl) of different taxa using the equation $V = LW^2C$, where L is length and W is maximum body width (both in mm). Values for copepods vary widely according to body form (see Warwick and Gee, 1984).

Taxon	Conversion factor (C)		
Acari	399		
Cnidaria	385		
Gastrotricha	550		
Isopoda	230		
Kinorhyncha	295		
Nematoda	530		
Oligochaeta	530		
Ostracoda	450		
Polychaeta	530		
Tanaidacea	400		
Tardigrada	614		
Turbellaria	550		
Copepoda		Body shape	Cross-section
'Cylindrical'	750	Parallel-sided	Round
'Semi-cylindrical'	560	Narrows evenly towards posterior	Round
'Semi-cylindrical compressed'	630	Narrows evenly towards posterior	Laterally flattened
'Semi-cylindrical depressed'	490	Narrows evenly towards posterior	Dorso-ventrally flattened
'Fusiform'	485	Widest near centre of body	Round
'Pyriform'	400	Narrows markedly behind cephalothorax	Round
'Pyriform depressed'	260	Narrows markedly behind cephalothorax	Dorso-ventrally flattened
'Scutelliform'	230	Broad, almost round	Dorso-ventrally flattened

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The thickness of the preparation can be determined using the calibration on the fine-focus knob of a good microscope. A microcompression chamber (Uhlig & Heimberg, 1981) can be a useful tool for this type of work.

If direct measurements of carbon or biomass are to be obtained, care should be taken to account for the effects of fixation/preservation (see Chapter 5). Such direct weight measurements of meiofauna usually require the pooling of a few to many individuals, depending on their size. Electrobalances sensitive to $\pm$ 0.1 μg are the most useful device for such measurements. Animals have to be pre-dried to constant weight before measurement. To achieve this, samples should be rinsed on a GF/C glass fibre filter, oven-dried for 24 hours at 60°C and stored in a desiccator prior to weighing. Ash-free dry weight is obtained by ashing samples in a furnace at 500°C for six hours after drying.

Wet : dry weight ratios have not been determined for most meiofaunal groups. Estimates for dry weight of nematodes vary between 20% and 25% of wet weight (Wieser, 1960; Myers, 1967; Gerlach, 1971), and this value is commonly applied to other meiofaunal taxa as well. Carbon accounts for approximately 40% of dry weight (Feller & Warwick, 1988). The carbon content of meiofaunal wet weight is in the same order as for other invertebrates, the most frequently cited values being 10.6% (Sikora *et al.*, 1977) and 12.4% (Jensen, 1984).

6.9 Cultivation of marine and brackish-water meiobenthos

A detailed account of cultivation techniques for marine and brackish-water meiobenthos is well beyond the scope of this chapter, but it is clear that cultivation may significantly improve our understanding of meiofaunal energetics. Only a limited number of species, most of which belong to the Harpacticoida and Nematoda, have been cultivated in the laboratory. Species amenable to culture are usually opportunistic, have short generation times and high reproductive capacities. Continuous cultures have been established for less than 30 marine and brackish-water nematode species. Of these, 16 belong to a single family (Monhysteridae) typical of detritus-enriched habitats (Moens & Vincx, 1998). Novel cultivation approaches offering suitable conditions for the rearing of a broader range of species would be invaluable, but only limited progress in the development of cultivation techniques has been made over the past two decades, and reviews of cultivation attempts and successes published in the 1970s and 1980s still offer up-to-date information. Among others, the papers by Hicks and Coull (1983) and Chandler (1986) for harpacticoid copepods, by Kinne (1977b) and Moens and Vincx (1998) for nematodes and by Tietjen (1988) for meiofauna in general, are recommended. Several papers in Kinne's (1977a) book on cultivation techniques of marine organisms also provide highly relevant information.

Most culture systems so far have been small scale and closed. Examples include petri dishes filled with agar, and conical flasks containing liquid medium. However,

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some harpacticoid copepods have been successfully established in recirculating, continuous, mass cultures (Fukusho, 1980; Bin Sun & Fleeger, 1995).

6.10 Experimental techniques

Community attributes can be correlated with natural and anthropogenic variables in the field. With careful sampling designs, strong evidence can be accumulated as to which environmental variables appear to affect community structure most. Such studies, however, cannot prove cause and effect. For this, experiments are required in which the effects of an individual factor on community structure are investigated while other factors are held constant or controlled. Field manipulative experiments include, for example, caging experiments to exclude or include predators, or the controlled addition of contaminants to experimental plots. Sediments within the cages or plots are sampled using the appropriate techniques and are treated just like any other samples.

Owing to their small size and life-history characteristics, several meiofaunal taxa, but particularly nematodes and copepods, are useful groups with which to conduct ecological experiments in the laboratory. Two types of experimental set-up are common. In the first, organisms are kept in stock culture, and added to experimental treatments as required. This type of approach is generally used to study species in isolation or specific combinations of species. As mentioned above, only certain groups of species are amenable to culture and thus such experiments are of limited use for the study of the majority of ecological questions, although they are very useful for addressing specific hypotheses.

In a more realistic and ecologically relevant set-up, whole meiofaunal communities may be collected in the field and then added to experimental chambers, either within the sediment in which they were collected or else having been extracted alive from their original sediment. Austen *et al.* (1994) describe a simple 'microcosm' system consisting of 570 ml glass bottles, each stoppered with a rubber bung pierced by two holes, through one of which an airstone diffuser can aerate the liquid within the bottle. Each bottle contains 80–200 g of sediment (depending on sediment type) with meiofauna and 1-μm-filtered seawater adjusted to an appropriate salinity. Experiments are generally run in the dark, initially at a temperature equivalent to field temperatures on the day of collection, and then raised by 1–2°C per day to a final temperature of 20°C. The experimental temperature is chosen to be higher than field temperatures, stimulating and optimising conditions for growth and reproduction. Experiments are generally run for two months, at the end of which the sediment in each bottle is fixed, removed and treated as a single sample. The system is cheap to build and operate. It does have some drawbacks. The sediment structure is disturbed while assembling the experimental units. Copepods and meiofaunal groups other than nematodes do not thrive in the system. While many nematode species survive in the system, some groups (deposit-feeders) do better than others (microphytic

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grazers, predators). Thus, the 'naturalness' of the communities within the system is debatable. Despite this, many species do thrive, and presumably interact, feed and reproduce, so it is true to say that meiofaunal communities are maintained in the system. Such a set-up allows comparisons between treatments so that, although nematode communities are different from field communities, differences between groups of samples can be attributed to experimental treatments. This, and similar, systems have been used for a variety of experiments on the effects of contaminants and dredgings disposal (e.g. Austen & Somerfield, 1997; Schratzberger *et al.*, 2000) and on the effects of physical disturbance, organic enrichment and predation (e.g. Schratzberger & Warwick, 1999a, 1999b).

Larger systems in which meiofaunal communities may be maintained and manipulated are often referred to as 'mesocosms'. Experimental units generally consist of large buckets or boxes containing tens to hundreds of litres of sediment. Various mesocosm designs exist, but the commonest consist of large basins through which natural seawater is circulated, into which the experimental units are placed. Treatments may be allocated to individual containers, or combined within containers, and at the end of the experimental period sediments within the experimental units are sampled using the appropriate techniques and are treated just like any other samples. Mesocosm experiments have the potential advantages that disturbance can be reduced while transferring sediments from the field to the experimental units, the conditions in which meiofauna are maintained are comparatively natural and the experimental units are large enough to allow interactions between meiofauna and macrofauna to be studied (e.g. Austen *et al.*, 1998; Dashfield *et al.*, 2008).

References

Anderson, R.O. (1959) A modified flotation technique for sorting bottom fauna samples. *Limnology and Oceanography*, **4**, 223–225.

Austen, M.C. & Somerfield, P.J. (1997) A community level sediment bioassay applied to an estuarine heavy metal gradient. *Marine Environmental Research*, **43**, 315–328.

Austen, M.C., McEvoy, A.J. & Warwick, R.M. (1994) The specificity of meiobenthic community responses to different pollutants: results from the microcosm experiments. *Marine Pollution Bulletin*, **28**, 557–563.

Austen, M.C., Widdicombe, S. & Villano, N. (1998) Effect of biological disturbance on diversity and structure of a nematode meiobenthic community. *Marine Ecology – Progress Series*, **174**, 233–246.

Barnett, B.E. (1980) A physico-chemical method for the extraction of marine and estuarine benthos from clays and resistant muds. *Journal of the Marine Biological Association of the United Kingdom*, **60**, 255.

Barnett, P.R.O., Watson, J. & Connelly, D. (1984) A multiple corer for taking virtually undisturbed samples from shelf, bathyal and abyssal sediments. *Oceanologica Acta*, **7**, 399–408.

Bell, S.S. & Hicks, G.R.F. (1991) Marine landscapes and faunal recruitment: a field test with seagrasses and copepods. *Marine Ecology – Progress Series*, **73**, 61–68.

https://telegram.me/Geologybooks

Bett, B.J., Vanreusel, A., Vincx, M., Soltwedel, T., Pfannkuche, O., Lambshead, P.J.D., Gooday, A.J., Ferrero, T. & Dinet, A. (1994) Sampler bias in the quantitative study of deep-sea meiobenthos. *Marine Ecology – Progress Series*, **104**, 197–203.

Bin Sun, J.W. & Fleeger, J.W. (1995) Sustained mass culture of *Amphiascoides atopus*, a marine harpacticoid copepod, in a recirculating system. *Aquaculture*, **136**, 313–321.

Blomqvist, S. (1991) Quantitative sampling of soft-bottom sediments: problems and solutions. *Marine Ecology – Progress Series*, **72**, 295–304.

Boisseau, J-P. (1957) Technique pour l'étude quantitative de la faune interstitielle des sables. *Comptes Rendus du Congrés des Sociétés Savantes de Paris et des Départements*, **1957**, 117–119.

Chandler, G.T. (1986) High density culture of meiobenthic harpacticoid copepods within a muddy sediment substrate. *Canadian Journal of Fisheries and Aquatic Sciences*, **43**, 53–59.

Chandler, G.T. & Fleeger, J.W. (1983) Meiofaunal colonization of azoic estuarine sediment in Louisiana: mechanisms of dispersal. *Journal of Experimental Marine Biology and Ecology*, **69**, 175–188.

Cleven, E.-J. (1999) An improved method of taking cores in sandy sediments. *Archiv für Hyrdobiologie*, **147**, 65–72.

Craib, J.S. (1965) A sampler for taking short undisturbed marine cores. *Journal du Conseil Permanent International pour l'Exploration de la Mer*, **30**, 34–39.

Cummings, E. & Ruber, E. (1987) Copepod colonization of natural and artificial substrates in a salt marsh pool. *Estuarine, Coastal and Shelf Science*, **25**, 637–645.

Dashfield, S.L., Somerfield, P.J., Widdicombe, S., Austen, M.C. & Nimmo, M. (2008) Impacts of ocean acidification and burrowing urchins on within-sediment pH profiles and subtidal nematode communities. *Journal of Experimental Marine Biology and Ecology*, **365**, 46–52.

Delamare-Deboutteville, C. (1960) *Biologie Des Eaux Souterraines Littorales et Continentales*. Hermann, Paris.

De Jonge, V.N. & Bouwman, L.A. (1977) A simple density separation technique for quantitative isolation of meiobenthos, using colloidal silica Ludox-TM. *Marine Biology*, **42**, 143–148.

Dillon, W.P. (1964) Flotation technique for separating fecal pellets and small marine organisms from sand. *Limnology and Oceanography*, **9**, 601–602.

Edgar, G.J. (1991) Artificial algae as habitats for mobile epifauna: factors affecting colonization in a Japanese *Sargassum* bed. *Hydrobiologia*, **226**, 111–118.

Elmgren, R. (1973) Methods of sampling sublittoral soft bottom meiofauna. *Oikos, Supplement*, **15**, 112–120.

Feller, R.J. & Warwick, R.M. (1988) Energetics. In: *Introduction to the Study of Meiofauna.* (ed. R.P. Higgins & H. Thiel), Smithsonian Institution Press, Washington, DC, pp 181–196.

Fenchel, T. (1967) The ecology of marine microbenthos. I. The quantitative importance of ciliates as compared with metazoans in various types of sediments. *Ophelia*, **4**, 121–137.

Fleeger, J.W. & Gee, J.M. (1986) Does interference competition determine the vertical distribution of meiobenthic copepods. *Journal of Experimental Marine Biology and Ecology*, **95**, 173–181.

https://telegram.me/Geologybooks

Fleeger, J.W., Thistle, D. & Thiel, H. (1988) Sampling equipment. In: *Introduction to the Study of Meiofauna* (ed. R.P. Higgins & H. Thiel), Smithsonian Institution Press, Washington, DC, pp 115–125.

Frithsen, J.B., Rudnick, D.T. & Elmgren, R. (1973) A new flow-through corer for the quantitative sampling of surface sediments. *Hydrobiologia*, **99**, 75–79.

Fukusho, K. (1980) Mass production of a copepod *Tigriopus japonicus* in combination culture with a rotifer, *Brachionus olicatilis*, fed ω-yeast as a food source. *Bulletin of the Japanese Society of Scientific Fisheries*, **46**, 625–629.

Gee, J.M. & Warwick, R.M. (1996) A study of global biodiversity patterns in the marine motile fauna of hard substrata. *Journal of the Marine Biological Association of the United Kingdom*, **76**, 177–184.

Gerlach, S.A. (1971) On the importance of marine meiofauna for benthos communities. *Oecologia (Berlin)*, **6**, 176–190.

Giere, O. (2009) *Meiobenthology: The microscopic motile fauna of Aquatic sediments*. (2nd edition). Springer Verlag, Berlin, 527 pp.

Gwyther, J. & Fairweather, P.G. (2002) Colonisation by epibionts and meiofauna of real and mimic pneumatophores in a cool temperate mangrove habitat. *Marine Ecology – Progress Series*, **229**, 137–149.

Heip, C., Smol, N. & Hautekiet, W. (1974) A rapid method for extracting meiobenthic nematodes and copepods from mud and detritus. *Marine Biology*, **28**, 79–81.

Heip, C., Willems, K.A. & Goossens, A. (1977) Vertical distribution of meiofauna and the efficiency of the van Veen Grab on sandy bottoms in Lake Grevelingen (The Netherlands). *Hydrobiological Bulletin (Amsterdam)*, **11**, 35–45.

Hicks, G.R.F. & Coull, B.C. (1983) The ecology of marine meiobenthic harpacticoid copepods. *Oceanography and Marine Biology: An Annual Review*, **21**, 67–175.

Higgins, R.P. (1964) Three new kinorhynchs from the North Carolina Coast. *Bulletin of Marine Science of the Gulf and Caribbean*, **14**, 479–493.

Higgins, R.P. (1977) Two new species of *Echinoderes* (Kinorhyncha) from South Carolina. *Transactions of the American Microscopical Society*, **96**, 340–354.

Higgins, R.P. & Thiel, H. (eds) (1988) *Introduction to the Study of Meiofauna*. Smithsonian Institution Press, Washington, DC.

Hockin, D.C. (1981) A simple elutriator for extracting meiofauna from sediment matrices. *Marine Ecology – Progress Series*, **4**, 241–242.

Jensen, P. (1982) A new meiofauna sample splitter. *Annales Zoologici Fennici*, **19**, 233–236.

Jensen, P. (1984) Measuring carbon content in nematodes. *Helgoländer Meeresuntersuchungen*, **38**, 83–86.

Joint, I.R., Gee, J.M. & Warwick, R.M. (1982) Determination of fine-scale vertical distribution of microbes and meiofauna in an intertidal sediment. *Marine Biology*, **72**, 157–164.

Kinne, O. (1977a) *Marine Ecology, Volume III, Cultivation, Part 2*. John Wiley & Sons, Chichester.

Kinne, O. (1977b) Cultivation of animals: research cultivation, (7) Nematoda. In: *Marine Ecology, Volume III, Cultivation, Part 2*. (ed. O. Kinne), John Wiley & Sons, Chichester, pp 691–709.

Kramer, K.J.M., Brockmann, U.H. & Warwick, R.M. (1994) *Tidal Estuaries: Manual of Sampling and Analytical Procedures*. A.A. Balkema, Rotterdam.

https://telegram.me/Geologybooks

Lydell, W.R.S. (1936) A new apparatus for separating insects and other arthropods from soil. *Annals of Applied Biology*, **23**, 862–879.

Lyman, F.E. (1943) A pre-impoundment bottom fauna study of Watts Bar Reservoir area (Tennessee). *Transactions of the American Fisheries Society*, **72**, 52–62.

Mare, M.F. (1942) A study of a marine benthic community with special reference to the micro-organisms. *Journal of the Marine Biological Association of the United Kingdom*, **25**, 517–554.

McIntyre, A.D. (1968) The meiofauna and microfauna of some tropical beaches. *Journal of Zoology*, **156**, 377–392.

McIntyre, A.D. (1971) Deficiency of gravity corers for sampling meiobenthos and sediments. *Nature*, **231**, 60.

McLachlan, A., Dye, A.H. & Van Der Ryst, P. (1979) Vertical gradients in the fauna and oxidation of two exposed sandy beaches. *South African Journal of Zoology*, **14**, 43–47.

Moens, T. & Vincx, M. (1998) On the cultivation of free-living marine and estuarine nematodes. *Helgoländer Meeresunters*, **52**, 115–139.

Mortensen, R.H. (1925) An apparatus for catching the micro-fauna of the sea bottom. *Videnskabelige Meddelelser fra Dansk naturhistorik Forening i Kjøbenhavn*, **80**, 445–451.

Myers, R.F. (1967) Osmoregulation in *Panagrellus reduvivus* and *Aphelenchus avenae*. *Nematologica*, **12**, 579–586.

Newell, I.M. (1971) Problems in the study of subtidal Halacaridae (Acari). In: *Proceedings of the First International Conference on Meiofauna*, *Smithsonian Contributions to Zoology* (ed. N.C. Hulings), Issue 76, Smithsonian Institution Press, Washington, DC. pp 103–107.

Ockelmann, K.W. (1964) An improved detritus-sledge for collecting meiobenthos. *Ophelia*, **1**, 217–222.

Pfannkuche, O. & Thiel, H. (1988) Sample processing. In: *Introduction to the Study of Meiofauna* (ed. R.P. Higgins & H. Thiel), pp. 134–145. Smithsonian Institution Press, Washington, DC.

Pollock, L.W. (1970) Distribution and dynamics of interstitial Tardigrada at Woods Hole, Massachusetts, USA. *Ophelia*, **7**, 145–166.

Rees, C.B. (1940) A preliminary study of the ecology of a mud-flat. *Journal of the Marine Biological Association of the United Kingdom*, **24**, 185–199.

Renaud-Debyser, J. (1957) Description d'un carrotier adapté aux prelèvements des sables de plate. *Revue de l'Institut Français du Pétrole*, **12**, 501–502.

Renaud-Debyser, J. (1963) Recherches Ecologiques sur la fauna interstitielle des sables (Bassin d'Arcachon, Ile de Bimini, Bahamas). *Vie et Milieu*, *Supplement*, **15**, 1–157.

Schoener, A. (1974) Experimental zoogeography: colonization of marine mini-islands. *American Naturalist*, **108**, 715–738.

Schratzberger, M. & Warwick, R.M. (1999a) Differential effects of various types of disturbances on the structure of nematode assemblages: an experimental approach. *Marine Ecology – Progress Series*, **181**, 227–236.

Schratzberger, M. & Warwick, R.M. (1999b) Impact of predation and sediment disturbance by *Carcinus maenas* (L.) on free-living nematode community structure. *Journal of Experimental Marine Biology and Ecology*, **235**, 255–271.

https://telegram.me/Geologybooks

Schratzberger, M., Rees, H.L. & Boyd, S.E. (2000) Effects of simulated deposition of dredged material on structure of nematode assemblages-the role of contamination. *Marine Biology*, **137**, 613–622.

Schwinghamer, P. (1981a) Extraction of living meiofauna from marine sediments by centrifugation in a silica sol-sorbitol mixture. *Canadian Journal of Fisheries and Aquatic Sciences*, **38**, 476–478.

Schwinghamer, P. (1981b) Characteristic size distributions of integral marine communities. *Canadian Journal of Fisheries and Aquatic Sciences*, **38**, 1255–1263.

Seinhorst, J.W. (1959) A rapid method for the transfer of nematodes from fixative to anhydrous glycerine. *Nematologica*, **4**, 67–69.

Sellmer, G.P. (1956) A method for the separation of small bivalve molluscs from sediments. *Ecology*, **37**, 206.

Sikora, J.P., Sikora, W.B., Erkenbrecher, C.W. & Coull, B.C. (1977) Significance of ATP, carbon and caloric content of meiobentic nematodes in partitioning benthic biomass. *Marine Biology*, **44**, 7–14.

Somerfield, P.J. & Clarke, K.R. (1997) A comparison of some methods commonly used for the collection of sublittoral sediments and their associated fauna. *Marine Environmental Research*, **43**, 145–156.

Somerfield, P.J. & Jeal, F. (1996) The distribution of Halacaridae (Acari: Prostigmata) among macroalgae on sheltered rocky shores. *Journal of the Marine Biological Association of the United Kingdom*, **76**, 251–254.

Somerfield, P.J., Rees, H.L. & Warwick, R.M. (1995) Interrelationships in community structure between shallow-water marine meiofauna and macrofauna in relation to dredgings disposal. *Marine Ecology – Progress Series*, **127**, 103–112.

Spoon, D.M. (1978) A new rotary microcompressor. *Transactions of the American Microscopical Society*, **97**, 412–416.

Tanner, C., Hawkes, M.W., Lebednik, P.A. & Duffield, E. (1977) Hand-operated suction sampler for collection of subtidal organisms. *Journal of the Fisheries Research Board of Canada*, **34**, 1031–1034.

Thiel, H. & Hessler, R. (1974) Ferngesteuertes Unterwasserfahrzeug erforscht Tiefseeboden. *Umschau in Wissenschaft und Technik*, **74**, 451–453.

Thiel, H. Von Thistle, D. & Wilson, G.D. (1975) Ultrasonic treatment of sediment samples for more efficient sorting of meiofauna. *Limnology and Oceanography*, **20**, 472–473.

Thistle, D. (1978) Harpacticoid dispersion patterns: implications for deep-sea diversity maintainence. *Journal of Marine Research*, **36**, 377–397.

Tiemann, H. & Betz, K-M. (1979) Elutriation: theoretical considerations and methodological imrpovements. *Marine Ecology*, **1**, 277–281.

Tietjen, J.H. (1988) Culture techniques. In: *Introduction to the Study of Meiofauna* (ed. R.P. Higgins & H. Thiel), Smithsonian Institution Press, Washington, DC, pp 161–168.

Uhlig, G. (1966) Untersuchungen zur Extraktion der vagilen Mikrofauna aus marinen Sedimenten. *Zoologischer Anzeiger, Supplement*, **29**, 151–157.

Uhlig, G. (1968) Quantitative methods in the study of interstitial fauna. *Transactions of the American Microscopical Society*, **87**, 226–232.

Uhlig, G. & Heimberg, S.H.H. (1981) A new versatile compression chamber for examination of living organisms. *Helgoländer Meeresuntersuchungen*, **34**, 251–256.

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Uhlig, G., Thiel, H. & Gray, J.S. (1973) The quantitative separation of meiofauna. A comparison of methods. *Helgoländer Wissenschaftliche Meeresuntersuchungen*, **25**, 173–195.
Warwick, R.M. (1984) Species size distributions in marine benthic communities. *Oecologia (Berlin)*, **61**, 32–41.
Warwick, R.M. (1989) The role of meiofauna in the marine ecosystem: evolutionary considerations. *Zoological Journal of the Linnean Society*, **6**, 229–241.
Warwick, R.M. & Gee, J.M. (1984) Community structure of estuarine meiobenthos. *Marine Ecology – Progress Series*, **18**, 97–111.
Warwick, R.M., Dashfield, S.L. & Somerfield, P.J. (2006) The integral structure of a benthic infaunal assemblage. *Journal of Experimental Marine Biology and Ecology*, **330**, 12–18.
Wieser, W. (1960) Benthic studies in Buzzards Bay. II. The meiofauna. *Limnology and Oceanography*, **5**, 121–137.

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Chapter 7
Deep-Sea Benthic Sampling

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Abstract

Sampling in the deep sea (200–11,000 m) presents unique challenges to scientists because of the distance to the seafloor from the surface and the high hydrostatic pressures found at depth. While there are a myriad of methods and techniques currently available to sample the deep-sea floor, described herein are the most common methods, techniques and tools current available. These range from methods of collecting organisms (e.g. trawls, sleds and traps), sampling sediments (e.g. grabs and corers), imaging the seafloor and fauna (e.g. landers, Autonomous Underwater Vehicles (AUVs) and Remotely Operated Vehicles (ROVs)) and *in situ* experimentation. Also described are generic sampling operations from modern research vessels including subsea tracking and positioning of gear, and future developments.

Keywords deep-sea sampling, underwater technology, high-pressure engineering, sediment retrieval, subsea imaging, *in situ* experimentation, deep-submergence vehicles, sampling methods and tools

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7.1 Introduction

There are many methods that have been used to sample the deep-sea benthos for a wide variety of applications over many years. Most deep-sea benthic sampling is carried out from research vessels. Some instruments, sensors, deployment methods and techniques have evolved rapidly while others have remained relatively unchanged for decades. Many basic principles of sampling are common to both the deep and shallow environments. However, the present chapter confines itself to approaches, from both technical/operational and strategic/scientific perspectives, which have been adapted specifically for use in the deep sea.

From a technical and operational perspective, the deep-sea environment differs from inshore and coastal environments with regard to (i) the extreme hydrostatic

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pressure found at depth; (ii) the greater distances from the surface to seafloor (depth) and (iii) the remoteness from the shore itself.

Dealing with extreme hydrostatic pressure is a very conspicuous problem in the technical design of most instruments. The ambient hydrostatic pressure increases by 1 bar (1 atm or 10^5 Pa) with every 10-m increase in depth. This increase culminates in ~1.1 ton per square centimetre at the deepest ocean depth (11,000 m), a considerable force. However, this force will only be exerted across a pressure differential, i.e. if an instrument has an air cavity, the pressure will act to crush this. To resist this, any instrument that has to be kept in air (i.e. dry), such as a camera or electronics, must be housed in a vessel strong enough to resist the ambient hydrostatic pressure. This is normally done by inserting the components into metal watertight cylinders that have sufficient strength and thickness to withstand the pressure at its intended operational depth. Alternatively, if the instruments do not include any air cavities they can be pressure-compensated (e.g. a lead acid battery). To compensate for the pressure the device is mounted inside a receptacle and flooded with an inert fluid such as mineral oil. It is then sealed from the elements by a watertight flexible membrane. The inert fluid permits electrical contact to resume while the flexible membrane compensates for the volume change with increasing pressure. Other structures such as frames do not suffer from the effects of hydrostatic pressure as long as steps are taken to ensure that water is free to flood all potential air spaces during submergence (no pressure differential). Adapting what may normally be relatively routinely used and simple instruments in coastal zones for use in deep-sea applications can often have considerable cost implications.

Hydrostatic pressure aside, the distance from the surface to the seafloor can create technical and operational challenges. Towing or lowering equipment on thousands of metres of wire, often without any visual reference, requires extremely specialised skills and reliable equipment. This experience and reliability are particularly important as it takes a long time to lower equipment to such depths, and, therefore, failures can waste considerable ship-time (thereby increasing costs). There is limited availability of the vessels suitable for deep-water work; for example, few non-research ships will carry enough wire to lower equipment thousands of metres, or tow, with even longer wires being required.

The distance between deep water and the shore does not necessarily inhibit sampling operations but is a limiting factor in obtaining time on a research vessel. Many smaller ships, research or otherwise, may not be open-ocean worthy owing to exposure to adverse sea states, nor are some capable of the long voyage durations often necessary in deep-sea research. For example, reaching the deep waters of the Mid-Atlantic or Mid-Pacific Ocean still takes one week or more transit time on even the most modern of research vessels.

Given that a suitable vessel has been provided with reliable sampling equipment, and experienced operators, there are still scientific sampling issues specific to deep-sea research. Designing a sampling programme with sufficient replication required to quantitatively sample an area of seafloor is not an exclusive deep-sea concern.

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It is, nevertheless, extremely expensive to gather multiple replicate samples within a relatively small area of seafloor when the number of samples per day is far lower than in shallow waters (Gage & Bett, 2005). This situation can also be heightened by adverse weather conditions, often experienced at sea, which may result in considerable lost time. The ecology of deep-water species (i.e. generally low densities, small body sizes, high species richness and many rare species) requires more and larger samples to be obtained in order to describe the fauna than in shallower waters.

Since the onset of deep-sea benthic sampling there has been a variety of diverse and imaginative instruments and methods used in equally diverse applications, so much so, that it would be unrealistic to describe them all in this chapter. Hereafter we describe instruments and methods that are currently commonplace in sampling the deep-sea benthos. Generally, few instruments are exclusively deployed using any one particular method. Instead, instruments are modified depending on the scientific investigation, the type of vessel used and its capabilities. This ability to rethink how an instrument is delivered to the seafloor can often provide a leap forward in sampling capabilities and discovery. Because of this (and also to avoid repetition and improve clarity), deep-sea benthic methods are explained in two parts: (i) deployment methods, i.e. methods used to deliver a given package to and from the deep-sea floor and (ii) the instruments themselves, which are further categorised by purpose into the following groups: collection of animals, collection of sediment, photography, biogeochemical studies and some examples of unique *in situ* manipulative experiments.

7.2 Sampling from research vessels

Deployment methods

Towing equipment across the seafloor is a traditional method that is still a common practice. This method is used in trawls, epibenthic sleds and camera sleds (Fig. 7.1a). The equipment is paid out from the stern of the ship as the ship moves forward. To avoid towing equipment across unsuitable underwater features such as escarpments, ridges, pipelines, cables, etc., it is good practice to make use of up-to-date charts of the area; in unfamiliar or poorly sampled areas an acoustic survey of the tow path should be performed prior to deployment. The ship's echo-sounder should also be operational throughout the deployment to highlight any changes to the bottom topography and to show up any features that previous surveys might have missed. Towing equipment requires the greatest amount of wire relative to depth than any other method. To maintain full contact with the seafloor a wire-out to depth ratio of up to three may be required. With such a length of wire being towed by the ship, great skill is required by the operator as the equipment may be thousands of metres below and behind the vessel. Communication with the towed

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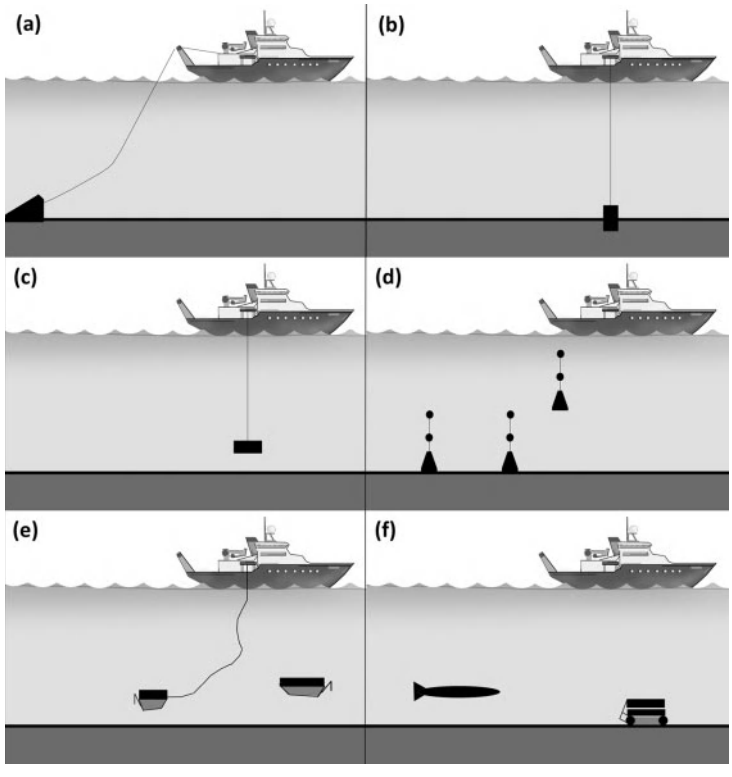

Fig. 7.1 Methods for deployment of deep-sea sampling equipment: (a) towing gear across the seafloor (trawling, camera sleds, epibenthic sleds); (b) lowering gear into seafloor (grabs, box corers, tube corers, video grabs, sediment profilers); (c) lowering gear above seafloor (camera platforms); (d) free-falling gear onto bottom (baited/non-baited camera landers, chamber incubation landers, sediment profiling landers, baited traps); (e) real-time exploration (Remotely Operated Vehicles (ROV), Human Occupied Vehicles (HOV, aka manned submersibles)) and (f) autonomous exploration (Autonomous Underwater Vehicles (AUV), gliders, crawlers).

gear is usually achieved using simple acoustic monitoring that relays pressure and angles to the ship. The operator must interpret these, together with the ship's depth sounding and wire-out displays, to maintain control. Trawls and other towed gear, which require long lengths of wire-out, are deployed from a tapered trawling warp comprising, for example, three ever-increasing thicknesses of wire. This tapered technique is used to reduce excessive strains on the wire itself during deep tows.

Lowering equipment such as sediment grabs/corers or profilers into the seafloor (Fig. 7.1b) requires a ship with wire lengths at least as long as the operational depth. Prior to deploying such equipment, careful site selection must be undertaken to avoid planting the devices on steep slopes or areas of hard ground, which could lead to equipment damage or sample failure. Sediment-penetrating equipment is typically deployed off the vessel from a starboard gantry from the middle of the ship. This reduces the stress and motion of the instrument if the ship is pitching in adverse

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weather conditions. This method requires the ship to maintain position, particularly at the point of contact with the seafloor, a facility that is greatly enhanced in the use of ships with Dynamic Positioning (DP). Accurately monitoring the depth and position of the instrument relative to the ship and desired sample site is done using either simple acoustic pingers or more sophisticated positioning system such as Ultra-Short BaseLine (USBL). Bottom-penetrating gear is usually deployed using a specifically designed coring wire, which, relative to other ships' wires, is heavy duty throughout. This is designed to cope with lowering and hauling heavy loads including the potentially considerable extra force that is caused by suction when gear is pulled out of the seafloor.

Lowering equipment to just above the seafloor is commonly used with camera platforms to perform video and photographic transects (Fig. 7.1c). The instrument is deployed using the starboard gantry. The distance of the instrument from the seafloor (the altitude) is often just a few metres; therefore, to maintain desired altitude with thousands of metres of wire-out requires constant monitoring. More commonly, the wire used has a fibre-optic core, which can relay the video images directly to the ship in real time, which greatly enhances the control of the instrument. During the deployment the ship is either left to drift in the surface currents or to move forward slowly allowing the instrument at the end of the wire to transect the seafloor.

Equipment that free-falls to the seafloor clearly does not require the ship to possess long wires (Fig. 7.1d). Generally, free-fall instruments operate autonomously and sink to the seabed using expendable ballast weights. Once a command is sent, either by on-board timing devices or by acoustic command from the surface, the weights are detached and the instrument rises to the surface with positive buoyancy. Therefore, this method allows multiple packages to operate simultaneously on the seafloor (maximising ship-time efficiency). It also permits instruments to be deployed on a timescale that may be far longer than the voyage itself (e.g. 12 months). Instruments that are unattached to the ship are also independent of any movement from the ship and, therefore, can carry out careful and precise measurements on the seafloor. The risk of equipment loss is higher than if attached to a wire, which means that the ship's officers' skills in manoeuvring the ship must be greater when recovering instruments floating on the surface. Free-fall instruments include baited and non-baited cameras landers, animal traps and landers for biogeochemical studies such as chamber incubation and sediment profiling landers.

It is also possible to explore the deep sea using real-time exploratory vehicles (Fig. 7.1e), either remotely by means of Remotely Operated Vehicles (ROVs) or directly, using manned submersibles (Human Occupied Vehicles; HOV). ROVs are controlled by a team of pilots on the surface vessel via an umbilical cord that allows two-way communications with the vehicle and transmission of all video data in real time. HOVs have many of the same capabilities, except that deployment durations are much shorter as the vehicle is piloted from the inside, which limits the submergence time. HOVs communicate limited information with

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the ship. Both systems have highly flexible capabilities. In the first instance, the video and photographic facilities required to pilot the vehicles also provide in themselves great scope for scientific research. These vehicles also carry a multitude of sampling devices such as grabs, corers, suction samplers and various purpose-built experimental gears. To increase the sampling payload further, 'toolboxes' or 'elevators' can be deployed by free-fall in the vicinity of the ROV/HOV dive; these are loaded with additional samplers that the vehicles can use.

An alternative to using exploratory vehicles controlled in real time is to use pre-programmed Autonomous Underwater Vehicles (AUVs) (Fig. 7.1f). These vehicles are self-powered and, hence, are usually designed to minimise hydrostatic drag (e.g. many are torpedo-shaped). They are often used for long-range transects or grid surveys, as they can cover a much greater area than can be realistically achieved using ROVs and HOVs. Inside the vehicle facing downward are either cameras or optical systems, which generate photo or acoustic maps of the seafloor. The vehicles are equipped with obstacle-avoidance software and, in some cases, the trajectories can, if necessary, be altered by the ship via acoustic communication. At the end of the deployment, the AUV surfaces and locates the surface vessel for recovery. Gliders are extremely low-power AUVs that use changes in buoyancy to dive, flying laterally in the water column with wing-like structures. Upon reaching the target depth, the glider floats vertically to the sea surface where it usually transmits data and may recharge using solar panels before diving again. Such vehicles have already traversed the Atlantic. Alternatively, there are a small number of bottom-crawling AUVs that can be deployed using the free-fall method. Once on the seafloor, the crawler performs a series of tasks (such as chamber incubations) before moving up-current to a new site performing video transects throughout. The crawlers can operate on the seafloor for up to six months and are recovered by expelling ballast weights triggered by acoustic command from the ship.

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Tracking, monitoring and positioning

Deep-sea sampling, which involves large distances between the surface and the seafloor, usually takes considerably more time than sampling in shallow waters. To reduce the risk of failure (and thus valuable ship-time) and increase the accuracy and positioning of any given instrument, it is common practice to use acoustic telemetry to track, monitor and position subsea instruments while submerged.

The most common, as well as perhaps the simplest method, is the use of acoustic beacons that transmit a constantly repeating sound pulse or 'ping' at regular intervals (commonly known as 'pingers'). Pingers are most frequently used when lowering equipment vertically on a wire (e.g. corers) (Fig. 7.2a). The ping is directed towards the seafloor where it is reflected and detected by the ship. The ping is also detected by ship directly from the pinger itself. Therefore, the time-delay between the direct ping and ping 'echo' off the seafloor can be used to calculate the beacon's altitude above the seabed. The acoustic data is streamed to a head-up

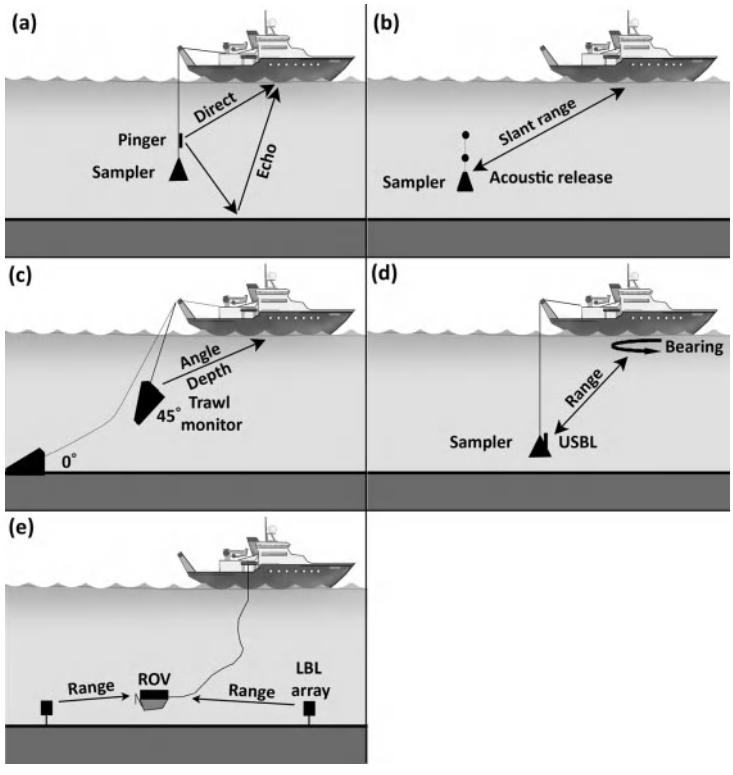

Fig. 7.2 Methods for the positioning and tracking of deep-sea sampling devices. (a) Pingers used to monitor altitude above seafloor. (b) Acoustic release transponders used to monitor the ascent and descent of free-fall vehicles. (c) Trawl monitors used to assess the depth and angle of towed gear. (d) Ultra-Short BaseLine (USBL) beacons used to monitor equipment bearing and range. (e) Long BaseLine (LBL) transponder arrays for accurate positioning within a working area. ROV, Remotely Operated Vehicle.

display on the surface vessel that allows the operator to monitor the descent of a package and thus to determine when it is close to or on the seafloor. The pinger is either coupled directly to the instrument, or a few tens of metres above the package on the main wire to prevent entanglement when the package lands on the seafloor.

Similarly, many acoustic release systems used in free-fall vehicles (landers, traps) have a pinger facility that allows the operator to track the descent or ascent of the instrument (Fig. 7.2b). This facility is used primarily to confirm whether the package is stationary on the seafloor or whether it has been released and is ascending. These acoustic systems also have a slant range facility, which is more commonly used. A slant range is the distance between the instrument and the ship and, therefore, not necessarily dependent on depth or position. However, repeatedly sending the slant range command (e.g. every 60 seconds) from a stationary ship gives the operator a quick indication that the vehicle is indeed ascending, or by moving the ship and checking the slant ranges an improved bottom position can

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be estimated. In the event of a free-fall vehicle not leaving the seafloor, other acoustic diagnostic commands such as battery voltage and orientation (vertical or horizontal) can be used to diagnose the problem.

Simple pingers are not effective when towing equipment (e.g. trawls, epibenthic sleds and camera sleds) and, therefore, other specialised acoustic systems, known as pulse position coded pingers or 'monitors' are used (Fig. 7.2c). Monitors can contain multiple mercury tilt sensors to monitor angle, a calibrated pressure sensor to monitor depth in addition to environmental sensors. Data from these sensors are transmitted to the ship in a series in time-delayed pulses and displayed directly on a head-up display. These appear in a series of traces relative to a reference trace and the distance between the traces can indicate depth, temperature, altitude or environmental parameters. For bottom trawls the tilt switches can show sudden changes in angle when the package is on the bottom, or indicate if it has been accidentally lifted off the bottom.

Other trawl monitoring systems generally used in shallower waters can consist of multiple sensors spaced around the trawl net. These sensor arrays can calculate parameters such as trawl geometry, bottom contact and tow speed, as well as environmental parameters such as current speed, temperature and pressure. These can also perform real-time diagnostics (such as pitch, angle and twisting) to aid in door control. However, pulse position coded monitors are more commonly used in deep-water applications.

Another more sophisticated method for subsea acoustic positioning is USBL (Fig. 7.2d), which consists of a transceiver mounted on a pole protruding from the ship's hull, and a transponder/responder beacon on the submerged instrument package or vehicle (e.g. corer and lander). The position of the beacon is calculated from the range and bearing measured by the transceiver.

The transceiver transmits an acoustic pulse detected by the subsea transponder beacon, which replies with a unique acoustic pulse. The ship-mounted transceiver detects this return pulse and time-delay from the transmission of the initial acoustic pulse until the reply is measured and converted into a range (m). The transceiver contains an array of transducers (typically three or more), which also permits the angle to be calculated via 'phase-differencing'. The calculated angle together with the range provides an accurate subsea position (range, bearing, altitude and heading). USBL beacons can be coupled to vertically lowered gear directly on the equipment itself. Using USBL positioning from a non-DP vessel will provide an accurate position of where the sample was taken, whereas using USBL with a DP vessel has the added advantage of accurately targeting the sampling site, even in very deep waters.

In AUV applications, inverted USBL systems can be used (iUSBL), where the transceiver is mounted directly on the underwater vehicle, and the transponder on the target (the ship). In this application, the processing takes place within the vehicle, which allows it to locate the transponder for operations such as locating the ship and automatic docking.

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A Long BaseLine (LBL) acoustic positioning system comprises a baseline transponder array deployed on the seafloor, which acts as reference points for navigation (Fig. 7.2e). These are generally deployed around the perimeter of a work site where ROVs are in operation. The LBL method provides a high positioning accuracy and stability irrespective of depth with a general accuracy of <1 m. LBL systems are generally used for precision underwater survey work where the accuracy or position stability of ship-based positioning systems (e.g. USBL) is insufficient.

7.3 Collecting animals from the deep-sea floor

Trawling

Trawling is the traditional method for collecting large samples of organisms from the deep sea. Although many different types of trawls are in use globally, there are two basic types of demersal trawl used regularly in deep-sea applications: beam trawls and otter trawls (Table 7.1). Most of the designs originated from coastal fishing, and have either been physically or operationally modified for use in the deep sea, where there are difficulties that do not occur in shallower fishing areas, such as unknown topography and large wire-out to depth ratios.

Trawling does not produce quantitative samples, although recent studies suggest they may be sufficient to monitor long-term trends in the megabenthos (Billett *et al*., 2001). However, samples are qualitatively biased and can provide under-estimates of the true faunal density (Bett *et al*., 2001). The efficiency of the trawl and the quality of the catch are very much dependent on factors such as the experience of the operator, the accuracy of both the sounding and trawl monitoring equipment and the suitability of the type of trawl for the target species.

The most common beam trawl used in sampling the deep sea is the Agassiz trawl (also known as Sigsbee or Blake trawl). It consists of two D-shaped runners joined by 3 m long beams that create a fixed trawl mouth area of between 1.5 and

Table 7.1 Deployment operations of the Agassiz, *Chalut à Perche* and the OTSB 14 trawls for deep-water applications.

Net	Agassiz trawl	*Chalut à Perche*	OTSB 14
Type	Light beam trawl	Heavy beam trawl	Otter trawl
Mouth size	0.5 m × 3.0 m	1.5 m × 6.0 m	2.0 m × 8.5 m
Ballasted	~100 kg	500 kg	n/a
Wire payout speed	~50 m min^{-1}	~50 m min^{-1}	~50 m min^{-1}
Ship's payout speed	~2 knots	~2 knots	4 knots
Wire-out to depth ratio	~1.7 : 1	~2 : 1	~2.5–3 : 1
Bottom speed	1–1.5 knots	1.5–2 knots	2–3.5 knots
Bottom time	20–50 min	1–2 h	Up to 3 h
Hauling speed	50 m min^{-1}	50 m min^{-1}	50 m min^{-1}

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2.1 m^2 depending on the size of the runners and beam. It has a main net mesh size of 20 mm and a cod-end (the part of the trawl where fish are retained) lined with 10 mm shrimp netting. It is used primarily for collecting benthic megafauna and elements of the benthopelagic fauna. More traditional beam trawls, such as the *Chalut à Perche*, include a wooden upper beam designed to keep the trawl the correct way up, have varying mesh sizes and much wider mouth areas (1.5 m × 6 m; 3 m^{-2}). The trawl mouths are often fitted with tickler chains to ensure that the leading edge traverses the sediment surface picking up animals.

The main advantage in Agassiz and beam trawling is that the fixed mouths permit the trawl to be deployed in adverse weather conditions, are relatively easy to deploy, are not susceptible to entanglement due to net collapse and, therefore, can be towed very slowly if on rough or unfamiliar ground. Beam trawls are commonly fitted with weak-links in case the trawl becomes snagged on the bottom. If hauled too vigorously, the weak-links sheer and the strain is transferred to the cod-end. This may lead to the loss of some (or even all of the net and catch) though hopefully some will be salvaged.

Agassiz and other heavier beam trawls are lowered to the seabed at ~50 m min^{-1} while travelling at 2 knots. The bottom time and speed varies depending on the trawl type. For example, an Agassiz trawl is typically towed for 20 to 50 minutes at 1–1.5 knots, whereas the *Chalut à Perche* is towed at 1.5 to 2 knots for one to two hours. The tow speed is dependent on the accuracy of acoustic monitoring between the ship and the net. The time of the tow across the seafloor also varies depending on depth; the trawls generally fish for longer periods when operated deeper, although the condition of the recovered samples will deteriorate with these longer tows. Results from a comparison of beam trawl types at different speeds showed that towing speed has no significant effect of demersal fauna such as fish and crustaceans (Yeh & Ohta, 2002).

Beam trawls have been used in extremely deep waters, even to hadal depths (Kullenberg, 1956; Wolff, 1976). The fixed mouths of beam trawls allow them to be lowered almost vertically from the ship and very slowly dragged across the seafloor. They are ballasted with over 100 kg of weight to assist in maintaining positive bottom contact. This method can be used with a wire-out to depth ratio closer to 1:1; although not quantitative, it has nevertheless been used to collect animals from deep trenches where other trawling techniques would require ~30 km of wire to be paid out. Such an enormous length of wire would have sufficient weight and drag to snap itself when towed.

Beam trawls are ideal for sampling epifauna but are less effective than otter trawls at catching large mobile animals such as fish, due to the relatively small mouth area. Fish are typically caught in shallow waters using twin warped otter trawls. Otter trawls are larger nets with a non-fixed mouth that is held open by two boards attached to the main wire as it traverses the seafloor. The ground wire has a tickler chain and possibly mud rollers with a cod-end incorporated at the rear

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of the net. Otter trawls are advantageous in deep waters where fish populations are relatively low, as they sample a greater volume of water per unit towing time (Stein, 1985). Although twin warp otter trawls have been successful in the deep sea (Gordon & Duncan, 1987), single warped trawling is more common (Stein, 1985). Otter trawling for fish relies heavily on the herding effect whereby fish are herded in front of the trawl mouth and when exhausted fall back into the net. The herding is formed by the warp and other rigging such as otter boards ahead of the trawl mouth. As a result of this, fish catches from twin warps can differ from those caught by single warp (Gordon *et al*., 1996).

Of the single warp otter trawls, the semi-balloon otter trawl or OTSB (also known as OTSB 14 or Marinovitch Trawl) has proved to be the most popular (Merrett & Marshall, 1981; Gordon, 1986). Originally designed as a Mexican shrimp trawl, the OTSB has a fine mesh throughout and has a head rope of up to 14 m, with an effective mouth size of about 2 m $\times$ 8.5 m. Although the OTSB can be fished using twin or single warps, the single warps seems to be the most common method.

Unlike beam trawls, which have fixed mouths, otter trawls must be towed at around 2 knots through the water during payout to prevent net collapse. This means that deployments depend on the ship's power and winch speed capabilities; commonly used settings are 50 m min^{-1} for the winch speed and 4 knots for the ship. As a result of having to tow, the wire-out to depth ratios reach 2.5–3 : 1. Once on the bottom they are towed for up to three hours at 2–3.5 knots, but again, this may vary depending on monitoring capabilities, time, depth and considerations of samples size versus sample condition. OTSBs are then hauled to the surface at 50 m min^{-1}.

OTSBs have been used extensively in deep-water application though primarily for fish. Their main disadvantage is the loss of smaller organisms through the relatively large mesh size and subsequent under-representation of epifauna. As with most sampling devices, in order to investigate an area of deep-sea floor comprehensively, a combination of several methods is recommended where possible.

Deep-sea trawling is a difficult operation and can be both lengthy and laborious. Most research ships are not designed for trawling, which can mean that many people are required to deploy an otter trawl and once in the water it needs to be continually monitored from the ship using acoustic trawl monitors. Failure to land the trawl on the seafloor correctly can result in net entanglement and prevent the net from working. Failure of a deep-water trawl is not only unfortunate because potential samples have not been recovered but paying out such lengths of wire takes a great deal of time. One unsuccessful trawl in the deep sea can waste up to 50% of one day's ship-time, which can be very costly. The time it takes to perform a trawl from start to finish, whether beam or otter trawl, is shown in 7.3. These data assume average weather conditions with a suitable ship and an experienced operator.

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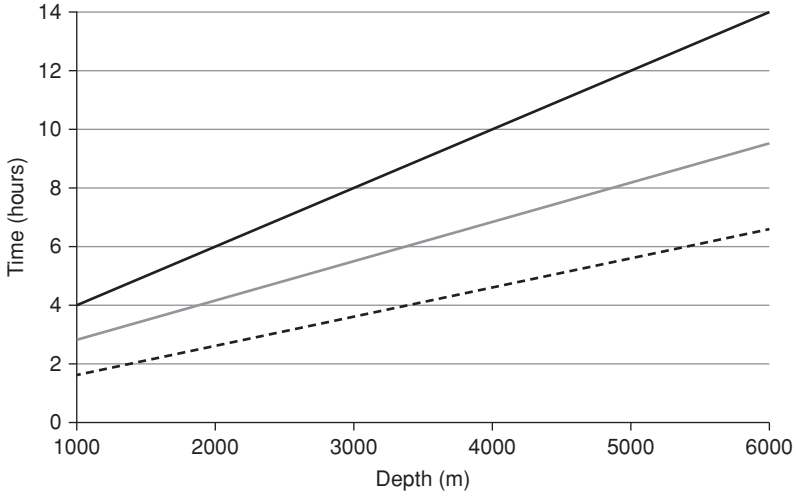

Fig. 7.3 Deployment times (payout, bottom and haul) at 1000 m depth intervals for the Agassiz light beam trawl (dashed), the *Chalut à Perche* heavy beam trawl (grey line) and the OTSB 14 otter trawl (black line). Deployment times shown do not include the launch and recovery times.

Epibenthic sleds

Epibenthic sleds (or sledges) have been used in deep-sea biology for many years (Hessler & Sanders, 1967; Aldred *et al.*, 1976; Rice *et al.*, 1982). These sleds typically comprise a flattened mesh bag mounted in a metal frame attached to wide runners. They are towed across the seafloor in a manner similar to beam trawling to collect smaller benthic organisms than is possible with trawls. The mesh bag (or net) has a fixed steel mouth with blades running along the top and bottom designed to skim off the top layer of sediment that is filtered through the mesh. The large runners prevent the sled from digging into the sediment by dispersing the load on the seafloor over a larger surface area. Most research institutes design and construct their own versions of epibenthic sleds and, therefore, there are no 'standard' designs, although most are fairly similar.

The mouth dimensions are relatively small compared with, for example, Agassiz trawls; they can vary from 0.81 m × 0.3 m (0.24 m^{-2}) to 2.29 m × 0.61 m (1.4 m^{-2}). Similarly, the mesh sizes can vary between designs. The mesh often consists of monofilament nylon with a 1 mm^2 aperture or terylene mesh with a 4.5 mm^2 aperture. A cod-end protrudes behind the sled by a metre or more and is protected by canvas aprons. Where larger mesh apertures are used in the main bag, the cod-end is often then reduced to 1 mm^2 apertures. Samples obtained from sleds require very careful washing on recovery as the sleds also collect large volumes of sediment along with the sample.

The cutting blades around the mouth were originally mounted to slice the top layer of sediment off but often resulted in the bag becoming clogged with sediment.

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Reducing the angle of the blade to run parallel with, or pointing slightly towards the seafloor was found to prevent this (Gage, 1975). To avoid sampling pelagic organisms during hauling, the mouth is often designed to close at a predetermined time, when bottom contact is lost or if there is a change in hydrostatic pressure. Many sleds are symmetrical, which allows them to operate no matter how they land on the seafloor. This permits near vertical lowering, which saves time.

Sleds are typically deployed with a portion of the tow wire in contact with the seafloor. This increases the likelihood of the sled remaining in positive and consistent bottom contact and has been found to increase the catch of small organisms as they are disturbed by the tow wire prior to capture in the sled.

Over the years, epibenthic sleds have slowly evolved, with the introduction of camera systems to image the area immediately in front of the sledge to evaluate its catch performance and variability between hauls (Rice *et al*., 1979). Other designs introduced mechanical odometers to accurately record or relay to the ship (in real time via acoustics) the distance travelled across the seafloor (Rice *et al*., 1982). These more sophisticated sleds include tickler chains, camera systems and multiple mesh bags. Multiple mesh bags have been incorporated into single sleds, which vary in mesh size and height above the bottom in an attempt to sample and distinguish epibenthic from suprabenthic organisms.

Sampling with epibenthic sleds is non-quantitative as catches vary a great deal if towed at different speeds (Gage *et al*., 1980; Harrison, 1988) and have even been shown to vary between hauls at the same depths and locations (Rice *et al*., 1979).

Traps

Collecting benthic organisms from the deep sea using baited traps is an old and well-established method (Paul, 1973; Isaacs & Schwartzlose, 1975). Baited traps are capable of recovering mobile scavengers at bathyal, abyssal and even hadal depths (Shulenberger & Hessler, 1974; Hessler *et al.,* 1978; Thurston, 1979; Stockton, 1982; Wickins, 1983; Blankenship *et al*., 2006). Baited trap designs are extremely diverse and range from simple funnel traps to pressure-retaining hyperbaric chambers. The size (length and tube diameter) and design of these traps vary depending on the target species. They can be deployed in many different ways, from moored arrays to ROV-deployed.

The simplest and most common baited trap is the small invertebrate funnel trap used to recover small scavenging crustaceans, typically amphipods. These traps comprise a tube with two mesh funnels on either end, and bait secured inside the tube (Fig. 7.4a). Scavengers locate the trap by following the odour plume and are funnelled into the trap whereupon they feed on the bait. As the funnel exits are not easily located from the inside, most trapped animals are recovered. This method is extremely simple and has proved to be very efficient in deep-sea applications. The only disadvantage to using this method is that it traps only bait-attracted species. Nevertheless, because there might be some loss of samples because of escapes

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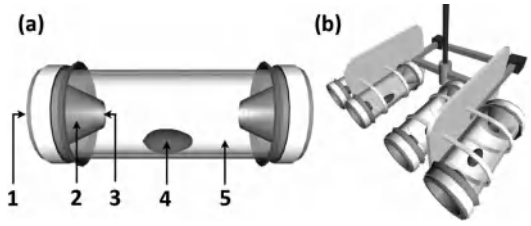

Fig. 7.4 Funnel traps for the collection of scavenging invertebrates. (a) The basic principles and features of the trap (1, trap opening; 2, funnel; 3, funnel entrance; 4, bait; 5, outer tube (transparent for clarity)). (b) An example of tethered trap clusters with current vanes to orientate entrances into the current.

and wash-out during the ascent and recovery of the traps, this method cannot be considered as quantitative. To address the issue of wash-out, some systems have converted closing water samplers (Niskin bottles) into funnel traps (Blankenship *et al*., 2006) or have integrated specific closing mechanism (Hargrave *et al*., 1995).

The simplicity of these traps means that they are readily mounted to baited camera systems (Lampitt *et al*., 1983; Jones *et al*., 2003; Heger *et al*., 2007) and can be delivered directly to and from the seafloor using ROVs or manned submersibles (Lawson *et al*., 1993).

Baited traps arrays have been used to investigate the vertical structure of benthopelagic species (Smith *et al*., 1979a; Ingram & Hessler, 1983; Smith & Baldwin, 1984). These arrays comprise an expendable ballast weight and release mechanism, a long (tens or hundreds of metres) mooring line with flotation. Traps are then secured at intervals on the mooring line. Some systems use twin traps or clusters of traps that are swivelled and orientated into the prevailing current using current vanes to increase the catch size (Ingram & Hessler, 1983; Charmasson & Calmett, 1987; Fig. 7.4b).

The funnel trap principle can be enlarged to capture larger animals such as fish (Isaacs & Schick, 1960; Rowe *et al*., 1986). Again, they are typically deployed using the free-fall principle and comprise a large cuboid or trapezoidal mesh cage. Bait is placed internally to entice the fish inside.

In contrast to the small invertebrate funnel traps that can recover large numbers of specimens not likely to be caught in trawls, fish traps tend to recover extremely low numbers compared to trawling. This is partly a result of the reluctance of deep-sea fish to enter a trap, probably due to a lack of hiding instinct (Jamieson *et al*., 2006). However, the specimens recovered using traps are in much better condition than those trawled. Nevertheless, sometimes it is single specimens that are required. For example, Drazen *et al*. (2001) developed an array of tube traps to investigate the diet of deep-sea fish through stomach content analysis. Due to decompression during recovery, stomach contents are often lost. Each trap captured a fish by pulling it inside the tube via a spring-loaded baited hook, and then closing a trap door behind it. If the stomach contents were regurgitated, the trap retained the

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material. The spring-loaded baited hook eliminated the problem of fish not entering the trap of their own accord and the trap recovered one fish per tube. Another fish-trapping design was developed to measure the oxygen consumption of a deep-sea fish autonomously (Bailey *et al*., 2002). Originally, a standard fish trap principle was used but equipped with closing watertight floors and acrylic walls. As fish did not enter this type of trap easily, the design was eventually changed, based on a different principle (Jamieson *et al*., 2006), that of Jones *et al*. (1998). Fish were enticed into a floored target area using bait and a watertight box was released from above to trap the fish whereupon oxygen measurements commenced.

Both the large and small baited trap methods have been developed further in order to maintain pressure and thus bringing organisms back to the surface at high pressure. Several designs such as those described by MacDonald and Gilchrist (1978; 1982), Yayanos (1981) and Treude *et al*. (2002) are capable of trapping amphipods, recovering them to the surface at pressure to allow hyperbaric tolerance experiments to take place. Even bigger hyperbaric traps have been developed to collect fish at their ambient high pressure (Phleger *et al*., 1979; Wilson & Smith, 1984; Drazen *et al*., 2005). These systems, although large and complex, are capable of maintaining fish at pressure for several days, allowing investigations of, for example, *in situ* respirometry, to be undertaken in the laboratory. These systems are all free-fall vehicles that rely on fish either swimming into the trap or being forced in by spring-loaded hooks; therefore, there is no control on what species is sampled.

Hyperbaric traps have more recently been modified for ROV manipulation, which gives the added advantage of selective sampling (Koyama *et al*., 2002; Shillito *et al*., 2008). Once a specimen is selected, it is sampled using a suction device with an integrated chamber, which when occupied is decoupled and placed inside the hyperbaric trap and closed by the ROV. The trap is then transported to the surface to begin *in vivo* experimentation.

Suction samplers

One of the most efficient means of collecting individual organisms from the deep sea is the use of suction samplers (or *slurp guns*) operated by ROVs or manned submersibles. Suction samplers comprise a nozzle (with a T-handle) connected to a flexible hose that feeds into a carousel of chambers. The ROV or submersible positions the nozzle over the desired organism using the T-handle and the suction pump is started. The organism is sucked through the hose and deposited into the chamber. The carousel then rotates to line up a new chamber ready for the next sample. Alternatively, a fine mesh can be slid between the nozzle and the hose to lift an organism temporarily off the seafloor. The organism is held against the mesh while the suction is applied. The organism is then deposited into a large sampling box (often called a 'Bio-Box') when the suction is turned off. This method can sample relatively robust benthic organisms in very good condition (e.g. Asteroids

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and Holothurians). More fragile animals, such a xenophyophores, will be destroyed during the transit through the hose. This method is greatly advantageous when using ROV and submersibles to sample biological communities living in dense and localised habitats such as cold seeps or hydrothermal vents (Humes, 1988; Bellan-Santini & Thurston, 1996). These vehicles have the capability to locate and position themselves very accurately and the suction sampler allows organisms of a particular species, size or position to be sampled. The overall sample size is relatively small but targeting individual specimens and recovering them in excellent condition is often more advantageous.

The suction samplers have been used for sampling sediments (Vetter & Dayton, 1998), free-swimming or pelagic organisms (Smith, 1982) and have also been incorporated into more complex experiments. For example, Smith and Baldwin (1982) used a suction sampler to capture deep-sea amphipods in a respirometry chamber allowing *in situ* measurements of oxygen consumption. This system is also sufficient for measuring oxygen consumption in fish (Smith & Laver, 1981). The suction method is also used to deposit organisms into hyperbaric chambers, which can be recovered to the surface at high pressure (Shillito *et al*., 2008).

Perhaps the simplest way to collect animals from the deep sea using ROVs is to pick them either directly with the manipulator arm or to use small scoops and baskets. The manipulator arm, which can only be used to collect relatively robust organisms such as holothurians, requires great skill by the operator. More fragile organisms such as echinoids can easily be collected using baskets and scoops. Once the organisms are collected they are either placed inside a purpose-built experimental enclosure or stowed in the ROV collection box. Although the number of animals collected may be relatively low compared to other methods, this method is excellent for collecting pristine samples that would otherwise be badly damaged. Examples of the suction sampler and other direct collection methods using ROVs are shown in Figs. 7.5 and 7.6.

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7.4 Collecting sediment from the deep-sea floor

Grabs

Sediment grabs are mechanical devices that are traditionally lowered down through the water column on a wire and are triggered, on contact with the seafloor, to grab a sediment sample.

Grabs were designed originally for limnology and open-sea oceanography and were used extensively from the beginning of deep-sea research, even as deep as 10,120 m (Thorson, 1957). The last century saw many diverse types of the sediment grab, which are reviewed in Chapter 5.

The most common types in use today are the Petersen, van Veen and Day grabs. Although each mechanism is slightly different, all these designs rely on a trigger

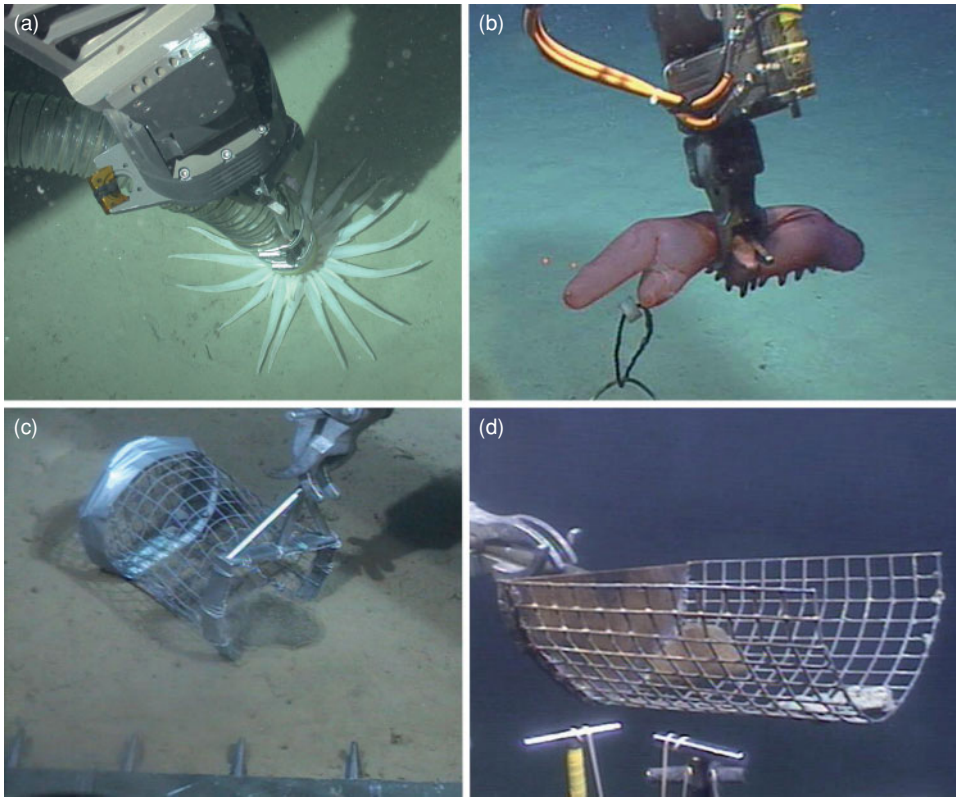

Fig. 7.5 Collecting organisms from the deep-sea benthic using a Remotely Operated Vehicle (ROV). (a) Suction sampling an anemone. (b) Direct collection of holothurians using the manipulator arm. (c, d) Collecting echinoids using the baskets and scoops, respectively. (Images © the National Oceanography Centre, Southampton (a, b), and © Ken Smith, MBARI, the Deep Submergence Group, WHOI, USA (c, d).) (For a colour version of this figure, see Plate 7.1.)

mechanism that closes a pair of jaws on contact with the seafloor, a principle based on the original Petersen grab. The surface area and sample volume vary according to design and for deep-sea use; some designs are often scaled up to collect larger samples. Typically, Petersen grabs sample 930 cm^{-2} (9.9 litres) of sediment. Van Veen grabs vary a good deal in size and are readily available in sizes of 250 cm^{-2} (3.1 litres), 1000 cm^{-2} (15 litres) and 2000 cm^{-2} (50 litres). Day grabs typically collect from 600 cm^{-2} (6 litres) up to 1000 cm^{-2} (15 litres) of sediment.

Although wire-deployed sediment grabs are still used in shallower waters today, their efficiency (or reliability) and effectiveness at quantifying the marine benthos have been under scrutiny since their conception (Wigley, 1967; Smith & Howard, 1972). There are two inherent faults of grab samplers in general, particularly if used for quantitative analysis. First, bow-waves generated by the grab's descent are capable of sweeping surficial sediment particles and light-bodied organisms away from the sample area (Wigley, 1967); second, since the depth of penetration is

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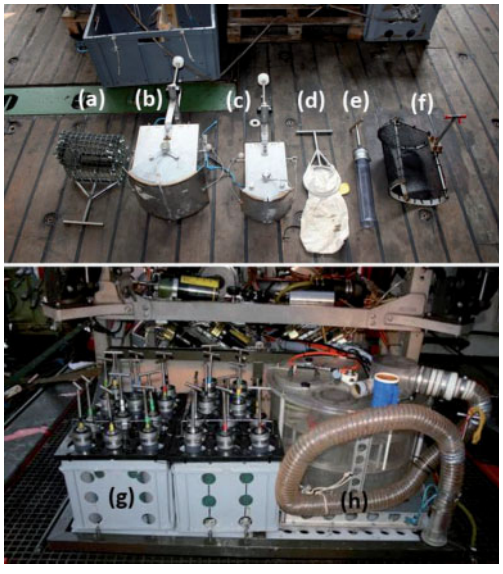

Fig. 7.6 Examples of Remotely Operated Vehicle (ROV)-operated tools used in deep-sea research for collecting organisms and sediment. (a) Epifauna basket, (b) and (c) sediment grabs, (d) sample net, (e) sediment push core, (f) epifauna scoop, (g) 24 push cores mounted on the tool tray and (h) suction sampler. (Images © the National Oceanography Centre, Southampton.) (For a colour version of this figure, see Plate 7.2.)

entirely dependent on substrate type, the result is that it may not sample organisms that burrow below the grab's penetration depth (Smith & Howard, 1972). Another problem with wire-deployed grabs specific to deep-sea use is their overall reliability in sampling procedures. Over long distances from the surface vessel requiring excessive wire-out for such a small and light device, grabs may often be accidentally closed on the way down, may hit the seafloor at undesired angles or suffer severe wash-out during the long ascent from the seafloor.

Methods to reduce the undesired bow-wave include fitting large screen apertures on each jaw to increase the vertical venting of water through the grab's jaws, for example the Smith–McIntyre grab (Smith & McIntyre, 1954; Wigley, 1967), and slowing the descent speed of the grab when close to the seafloor. However, the issues of bow-wave formation, under-penetration and a relatively small sample meant that for deep-sea operation, grabs were superseded first by box corers and then by tube corers. However, it was an early grab design, called the Ekman corer, upon which the box core principle was based.

The Ekman corer was never quite suited for use in the deep sea as it required a metal weight (messenger) to be manually slid down the wire to trigger the closing mechanism. The Ekman grab has, nevertheless, been revived in recent years despite the known inefficiencies of such devices. However, the steadily increasing use of ROVs and manned submersibles has provided ways to target areas of, or

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organisms on, the seafloor precisely. In addition to imaging equipment, suction samplers and tube corers, the original and smaller inshore sediment grab designs have been modified for ROV operations (Fig. 7.6). The Ekman type grab (and similar) has repeatedly been modified to allow ROVs to trigger the closing mechanism (Rowe & Clifford, 1973; Vetter & Dayton, 1998). The use of grabs in this way eliminates the problems of bow-waves and sediment disturbance. Although ROV-operated grabs are not used as quantitative benthic samplers, they are highly efficient collecting tools for relatively large and fragile epifauna. Organisms such as asteroids, echinoderms and even extremely fragile xenophyophores, which are either too big, prone to damage or would otherwise be obliterated by any other means of collection, can be sampled. The grab is typically secured to the ROV tool tray, uncoupled by the ROV and gently placed over the targeted organism. The grab is then gently pushed into the sediment, thus enclosing the organism, the seafloor immediately underneath it and the overlying water. The jaws are triggered by the ROV operator, and the sample is retracted and is carefully secured back on the tool tray. Furthermore, the smaller grab designs such as the Ekman allow multiple grabs to be taken in one dive. Therefore, although the use of grabs in the traditional sense is now a relatively uncommon practice in deep water, grabs have found a niche as precision tools for virtually undisturbed sampling of intact benthic organisms.

The basic principles of wire-deployed sediment grabs have also been used to develop the deep-sea video grab, used primarily in geological applications. Video grabs are lowered off the ship via the starboard gantry with conducting cables that allow two-way communication. Acoustic tracking and visual images from downward-facing cameras are used to identify the bottom. These grabs typically have very large clam shell jaws that are hydraulically closed on command from the surface via the conducting cable. The cable can also power video systems inside and outside the grab; these are streamed up the cable in real time and allow the user to perform video transects and then to target a representative area of seafloor with the grab. Furthermore, this operation provides a means with which to actively seek and carefully recover particular objects or features that would otherwise be missed by, for example, the box corer. The navigation facility also provides access to complex topographical features and localised substrates such as gas hydrates and hydrothermal vents (Greinert *et al*., 2001; Klinkhammer *et al*., 2001). Video-guided grabs provide the user with precise location, selection and determination of quality and quantity of sample collection. Some more sophisticated systems feature a camera within the grab capable of inspecting the sample, thus giving the user the option to discard it and try again.

These video grabs provide much more controlled and much larger samples with greater accuracy than traditional sediment grabs. Video grabs currently in operation include the IFM-GEOMAR TV Grab (TVG), capable of 0.6 m^{-3} samples and the NOCS HyBIS capable of 0.25 m^{-3} samples. Both are capable of 6000 m operations.

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Box corers

Spade corers were developed in the 1970s to address the sampling deficiency of traditional wire-deployed grabs in obtaining quantitative biological samples under a given surface area. Though originally named spade corers, but now commonly known as 'box corers', they quickly replaced grab samplers as the standardised quantitative sediment retrieval method in deep waters. Box corers were designed to tackle the problems of the sediment-disturbing 'scooping' action of grabs and retrieve much larger, deeper and less disturbed samples.

Box corers (Fig. 7.7) are composed of a detachable square metal open-ended box coupled to the end of a central column weighted with lead that is slotted and gimballed within an outer frame. A spade is held horizontally by a spring-loaded bolt at the top of the corer. The top of the box has hinged one-way flaps that either open on descent by the flow of water, or are held open, thus reducing the bow-wave when approaching the seafloor. Box corers can weigh up to 750 kg (e.g. the Haja box corer) and are lowered to the seafloor from a research ship (typically via the starboard side) using high-strength coring wire (e.g. ~16 mm Ø, 18 T breaking load) at ~60 m min^{-1} and monitored using an acoustic pinger coupled to the wire above. By monitoring the depth via the pinger, the descent

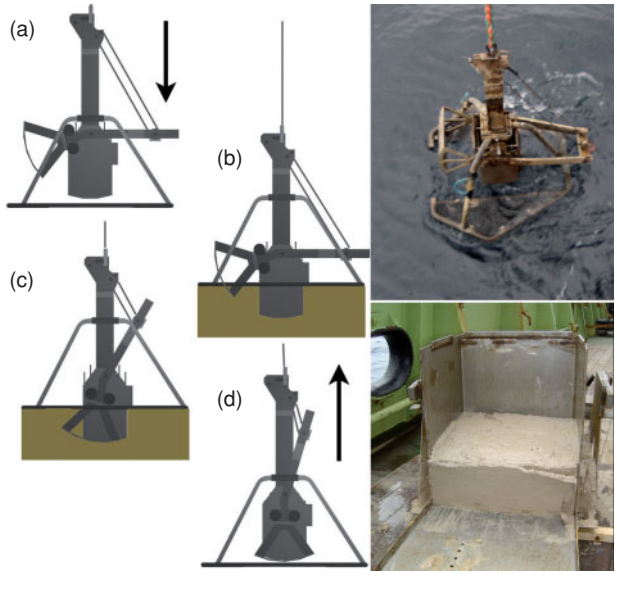

Fig. 7.7 The USNEL Mk II box core operations. (a) The spade is in the cocked horizontal position during descent. (b) The weighted central column drives the box into the sediment while the outer frame rests on the seafloor. (c) The locking pin deactivates and hauling begins to rotate the spade under the box. (d) The corer is hauled to the surface with the block of sediment sealed inside the box. To the right is an image of a box corer being deployed and an example of a sediment sample taken from 4000 m deep. (Images © the University of Aberdeen, UK.) (For a colour version of this figure, see Plate 7.3.)

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speed is decreased to ~10–15 m min^{-1} when approaching the seafloor. Depending on the ship's ability to hold its position, and any potential rolling caused by adverse weather conditions, the speed at which it is eventually lowered into the seafloor may need to be increased to 30 m min^{-1} to ensure positive penetration. However, the chances of obtaining a sample, let alone an undisturbed one, are poor. When the corer reaches the seafloor, the outer frame rests on the sediment surface while the weighted inner column drives the box into the sediment under gravity. Seafloor contact is evident from the ship by a distinct decrease in winch tension. The action of the column sliding through the frame activates the withdrawal of the spring-loaded pin that releases a short length of cable, permitting the spade to swing through ~90° through the seafloor, under the box and encapsulating the sediment inside the box. The vertical penetration of the box into the sediment and the action of the spade taking place outside the sample-enclosed area, means that box corers can take relatively undisturbed samples compared to those taken with grabs. Furthermore, a lead or rubber seal on the inside of the spade seals the box as it is hauled out of the seafloor. Likewise, the hinged one-way flaps on the lid of the box are held in a closed position during ascent. The corer is hauled slowly (~10 m min^{-1}) out of the sediment. A large increase in winch tension, which drops sharply away on a good core, can be observed due to suction until it is completely free of the seafloor. The corer is then hauled to the surface at ~50 m min^{-1}. Once the corer is on deck, the box filled with sediment can be detached from the frame and replaced with an empty box and redeployed while the first is sampled and processed. As a general guide to deployment times, box corers take ~16 minutes per 1000 m to descend and ~20 minutes per 1000 m to haul in.

There have been many iterations of the box core principle since its invention. Each design varies slightly in depth and surface area of the sample taken, and, consequently, the volume as well. Some designs use a symmetrical double-spade action similar to an Ekman grab. The two spades swing down through 45° and meet directly under the box, which is thought to reduce the unilateral force during pullout from the seafloor. This design also creates a very efficient seal and provides a greater mechanical leverage on closing, making it more efficient for coarser sediments.

Most of these designs have retained the square box but some, for example the Haja, can be fitted with cylindrical ones. The box corer principle has been further modified by some users. Jumars (1975) developed the 'Vegematic' subcore system whereby the internal box was subdivided into 10 or more subcores, which can be removed separately upon retrieval. A further development saw the design of the multibox corer (Gerdes, 1990). The multibox corer comprised nine separate, albeit much smaller, boxes. Both the *Vegematic* and multibox core systems were used to further address spatial distributions of organisms on the seafloor. The main designs in operation are the Reineck, GOMEX, USNEL, Haja and various other commercially available models (see Table 7.2).

A comparison of the Reineck box core and the Smith–McIntyre grab was made by Smith and Howard (1972) who found that the box core retrieved a higher

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Table 7.2 Sampling specifications of box cores currently available.

Box corer	Reference	Sample penetration depth (cm)	Sample surface area (cm × cm [m^{-2}])	Sample volume (m^{-3})	Sample shape
Reineck	Bouma and Marshall, 1964	45	20 × 30 (0.04)	0.027	Square
GOMEX	Boland and Rowe, 1991	50	25 × 25 (0.06)	0.031	Square
USNEL Mk I	Hessler and Jumars, 1974	50	50 × 50 (0.25)	0.125	Square
USNEL Mk II	–	50	50 × 50 (0.25)	0.125	Square
Haja K11/18a	www.nioz.nl (accessed 9 November 2012)	55	50 × 50 (0.25)	0.151	Square
Haja K11/18b	www.nioz.nl (accessed 9 November 2012)	55	50 Ø (0.196)	0.108	Round
Haja K16a	www.nioz.nl (accessed 9 November 2012)	55	30 × 20 (0.06)	0.033	Square
Haja K16b	www.nioz.nl (accessed 9 November 2012)	55	30 Ø (0.07)	0.039	Round
Commercially available	–	40	29 × 20.7 (0.06)	0.024	Square
Commercially available	–	40	34.5 × 29 (0.10)	0.04	Square
Commercially available	–	50	50 × 50 (0.25)	0.125	Square
Commercially available	–	60	50 × 50 (0.25)	0.15	Square
Multibox	Gerdes, 1990	45	12 × 20 (0.024)	0.011 (× 9)	Square

macrofaunal abundance and biomass. The box corer was found to collect much older, deeper and different size classes of organisms to the grab. The box corer in general is a superior sampling gear to grabs but they do still suffer loss of the fine surficial layer (Bett *et al*., 1994) and contamination of the overlying water sample (Shirayama & Fukushima, 1995). Box corers can be efficient sampling devices, but their success and usefulness depends on the scientific question being addressed. The ability to retrieve a large, deep block of sediment from the deep-sea floor is still vitally important in many areas of research.

Tube corers

Although the box corer was capable of taking far less disturbed samples compared to sediment grabs, the size, weight and the generic design still cause a degree of bow-wave formation. This became more problematic with the growing interest in the flocculent surficial layer of sediment and the delicate organisms therein, rather than the demand of greater penetration.

This issue led to the development of the Barnett–Watson Multiple Corer, simply known as the 'Multicorer' (Barnett *et al*., 1984). The Multicorer, based on the inshore Craib corer, comprises an array of up to 12 core tubes of 56.5 mmØ

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(25.1 cm^{-2}). The cores are coupled to a central shaft hanging within an outer frame. This central column has a water-filled dashpot. To minimise disturbance, the dashpot hydraulically dampens the descent of the cores once the outer frame has reached the bottom. Likewise, all 12 cores are open during the descent to allow the water to flow freely through them. Upon contact with the seafloor, a closing mechanism deploys rubber hemispheres to seal the top of the core during pullout. This seal provides sufficient suction to pull an undisturbed sediment core from the seabed with enough time for the mechanism to close the bottom end of the cores on leaving the seafloor.

The ship-side operation of the Multicorer is similar to that of the box corer. A pinger, or USBL beacon, is coupled above the corer on the wire (or on the corer itself) and is monitored during its descent at ~50–60 m min^{-1}. On approaching the seafloor the corer is slowed down to ~10–15 m min^{-1} until the winch tension drops, indicating that the corer has reached the bottom. The corer is then left for a short period of time to allow the slow penetration of the cores before a slow pullout and final hauling commences at 50 m min^{-1}.

Upon recovery, the undisturbed cores are removed individually and preserved either whole or extruded and sliced into desired depth horizons.

The Barnett–Watson Multicorer has in recent years been superseded by the Bowers and Connelly Megacorer. The operations of the Megacorer are similar to the Multicorer. The main difference between the two types is the core tube dimensions and closing mechanism. The Megacorer tubes are 10 cmØ, but can be alternatively fitted with smaller-sized tubes (59 mmØ), penetrate 20–40 cm into the sediment and are also hydraulically damped. The shift to a larger core diameter was based on efficiency trials by divers with different core diameters (McIntyre, 1971), but some users find this size of sample too large to cope with and, therefore, a mix of tube sizes may be required. The Megacorer closing mechanism drops a sealed lid on the top of the core as it penetrates the sediment. This action also releases a slicing mechanism that slices the bottom of the core and seals it as soon as it is free of the seafloor.

The Megacorer has a greater projected area in the direction of travel relative to the box corer. The closing mechanisms are also more fragile and, therefore, a slower descent rate of 35–40 m min^{-1} is recommended. This slower speed also accounts for the greater drag on the corer, which prevents the wire being paid out faster than the corer is sinking, which would otherwise lead to entanglement and severe damage. When the Megacorer is ~100 m above bottom, the descent speed is decreased to 10 m min^{-1} until seafloor contact is made. It is left for a short period of time to allow the cores to penetrate before being slowly extracted. With the relatively fragile closing mechanism closed, it can be hauled to the surface at a faster 45–50 m min^{-1}. As a general guide to deployment times, Megacorers take ~25 minutes per 1000 m to descend and ~20 minutes per 1000 m to haul in. Like the Multicorer the Megacorer tubes can be removed individually and processed accordingly.

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Fig. 7.8 The Bowers and Connelly Maxicorer being deployed and a close-up of two successful cores from the deep sea. (Images © the University of Aberdeen, UK.) (For a colour version of this figure, see Plate 7.4.)

Comparisons between box corers and grabs have shown that box corers are of better quality; however, comparisons between box corers and Megacorers have shown the Megacorer to be of better quality still (Gage & Bett, 2005). The USNEL Mk II box corer has been shown to under-estimate abundance by 48–66% in comparison with the Megacorer. This can be attributed once again to the bow-wave formed by the box corer on descent. Therefore, great care must be taken when selecting the correct coring device to address the scientific question. If the surficial sediment layer and organisms therein are important then the Megacorer would be most appropriate. Conversely, if large and deep blocks of sediment are required then perhaps the box corer is more suitable.

There are currently several different types of tube corer commercially available based on both the Barnett–Watson Multicorer and the Bowers and Connelly Megacorer (Fig. 7.8). Each manufacturer has rather confusingly named each model variations of multi-, mega-, maxicorer, etc., which often refers to the number and internal diameter of the core tubes, and in some cases different institutes have the same name for different corers. Therefore, care should be taken when selecting the most appropriate corer. The specifications of current tube corers that are readily available are listed in Table 7.3. Note that some manufacturers state the tube OD and not ID on their specification sheets, which may confuse sampled area sizes.

Corers can now be deployed to much greater depths with the advent of aramid ropes, because their inherent buoyancy means that they will not snap under their own weight. However, this buoyancy can create its own problems when the load comes off the rope on bottom contact. Slack rope can jump off sheaves or pool on deck, while the core sinks into the seabed.

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Table 7.3 Sampling specifications of tube corers currently available.

Tube corer	Number of cores	Core diameter (mm)	Core depth (mm)	Total sample volume (m^{-3})
Barnett–Watson Multicorer	12	56.5	400	0.012
Bowers and Connelly Megacorer	12	100	400	0.038
Commercially available	12	110 or 65	400	0.046/0.016
Commercially available	8	110 or 65	400	0.031/0.011
Commercially available	4	110 or 65	400	0.015/0.005
Commercially available	6	100	370	0.017
Commercially available	4	110	350	0.011
Commercially available	6	110	500	0.024

ROV corers

One disadvantage of using wire-deployed corers concerns spatial accuracy. Although a DP-controlled ship with USBL positioning can position a corer to within 10 m as deep as 5000 m, sampling at even finer resolutions (within centimetres to metres) is not possible. Once the corer is in the water the user has little or no control over exactly where it samples on the seabed. This is, of course, ideal for statistically random sampling of large areas such as abyssal plains. However, the scientific objective often requires a greater degree of precision such as coring next to a whale fall, cold seep bacterial mats, areas where the terrain is too complex to risk wire-deployed corers such as canyons or ridges or areas that are simply too deep to operate wire-deployed packages such as hadal trenches.

However, cores are still obtainable by using ROV-operated push cores (Fig. 7.9). Push cores are small, typically 58 mmØ × 300 mm long and have either a non-return 'flutter' valve or a manual valve at the top with a 'T-handle' that allows the ROV operator to handle it. A typical deep-sea ROV can take tens of push cores on its tool tray. Each core is placed inside a quiver and secured to the tool tray. The ROV can then select a core (typically numbered or colour coded) and lift it out of the quiver by the T-handle and gently insert it into the sediment creating very little sediment disturbance. As it is inserted, the water is vented out through the top valve. If using a manual valve, the ROV then releases the core and turns a closing valve on the top of the core. If using a flutter valve the core is simply pulled out straight away. Flutter valves allow only water to vent out of the core. Once the core is filled with sediment and pulled out, the suction pulls the valve closed. The core is then carefully retrieved from the seafloor and set down onto a bung secured to the inside of the designated quiver. As flutter valve push cores occasionally allow the sample to slide out, great care should be taken when the sample is essential or non-repeatable, e.g. from an area that has been seeded.

This ROV-operated coring method is essential for targeted coring on a small scale but can also provide the means by which to perform transects of, for example, tens to hundreds of metres in a straight line from a known object or habitat. This push-coring method has been used in the deepest point on earth (10,900 m; Kato

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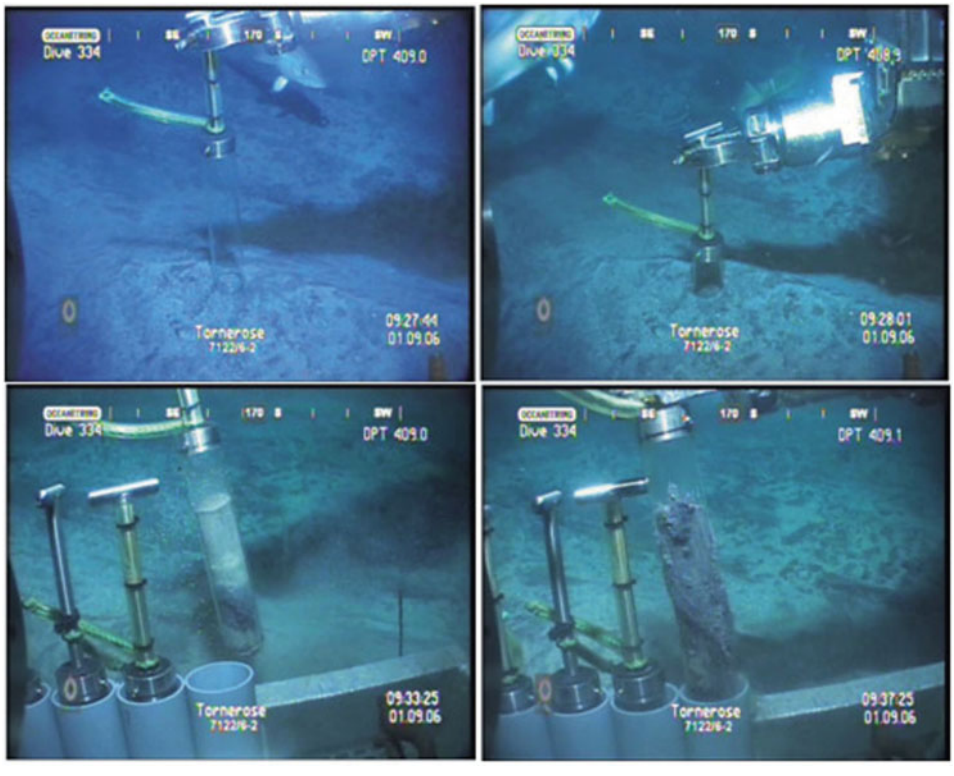

Fig. 7.9 Remotely Operated Vehicle (ROV) push cores in use in the Barents Sea. (Images © SERPENT.) (For a colour version of this figure, see Plate 7.5.)

et al., 1997), in close proximity to and in the vicinity of whale falls (Goffredi *et al*., 2004), and within cold seep bacterial mats (van Dover & Fry, 1994) none of which would otherwise have been possible.

7.5 Imaging the deep-sea floor

In situ imaging provides a non-destructive method for assessing deep-water ecosystems that can cover wide areas. Geo-referenced images can be used at a broad scale for mapping of habitats directly and for ground-truthing acoustic maps. At a fine scale, visual images can reveal organism distributions or habitat associations that are otherwise lost when samples are recovered to the surface. Likewise, *in situ* observations constitute an important tool in the study of the behaviour of deep-sea benthos in a natural setting. This is not usually possible in the laboratory as the extreme changes in hydrostatic pressure and temperature caused by bringing an organism up from the deep sea usually results in mortality or, at least, physiological stress.

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For imaging the seafloor, there are different methods in use responding to different scientific applications, but all have one factor in common: the necessity to sufficiently illuminate and observe a given area of seafloor. The success of the imaging operation is determined by three factors: (i) the sensitivity of the camera, (ii) the intensity of illumination and (iii) the height above the seafloor. The positioning of the lights and camera, and the distance from the target are usually derived from previous experience and a certain amount of testing. However, they can be calculated theoretically.

The approximate minimum faceplate sensitivity of the camera required can be calculated using the equation below. If the camera specification is already fixed then this equation can be rearranged to determine the most suitable distance from the seafloor or illumination intensity

$$E_{fp} = \frac{It^{2d} \times 0.18}{4f^2d^2}$$

where, E_{fp} is the faceplate sensitivity of the camera (Lux), I is the lamp luminous intensity (Candela), t is the light transmission factor of clear seawater (0.9 m^{-1}), f is the lens aperture (f-stop), and d is the distance from target.

The clarity of the image also depends on the position of the lights in relation to the camera. To reduce glare from reflected particles in the water (backscatter), it is best to reduce the volume of water within the camera field of view that is within the beam of the light(s). This is achieved by mounting the light(s) further away from the camera at an angle. If there is only one light, then the greater the angle, the greater the shadows in the image, and there will be less of a uniformly spread illumination, which will cause a light-to-dark gradient to occur across the image.

Imaging survey design

Survey design is very important in ecological studies, which are concerned with the study of the distribution and abundance of organisms and their interactions with the environment. Many studies of biological populations require estimates of population density or size. To make accurate estimations of these, and other aspects, such as diversity, it is essential to carry out competently designed and conducted quantitative surveys. No analysis or inference theory can make up for fundamental flaws in survey procedure (Buckland *et al*., 2001).

For effective surveys, it is vital that the lines or points surveyed are placed randomly with respect to the distribution of the objects surveyed. It is also vital that a sufficient number of objects are detected and recorded. These ensure that the surveyed lines or points are representative of the whole area.

It is extremely important to consider the sampling unit of surveys. This is not always apparent in underwater imaging surveys. The properties of light in water

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determine the practical size of the photograph. This does not necessarily provide a good reason to use a single photograph as the sampling unit, particularly with changes in sample unit size associated with use of towed camera platforms and in deep-water areas with scarce fauna. Pooling or mosaicking overlapping photographs, or using appropriate intervals of video may be required to generate appropriate sampling units for analysis (Jones *et al*., 2009). Once the sampling unit has been defined, it is important to ensure that sufficient random independent replicates are taken for the sample to be representative of the objects and area under investigation (Sokal & Rohlf, 1995).

Photographic transects

There are four main methods of obtaining photographic transects of the deep-sea floor: (i) towed camera sledges, (ii) towed camera platforms, (iii) ROVs and (iv) AUVs.

The distinction between towed camera sledges and towed camera platforms, in brief, is that sledges are deployed like a trawl and towed in full contact with seafloor behind the ship, taking video and/or still images throughout. Towed camera platforms are deployed from the starboard gantry and lowered to a few metres above the bottom, without making contact. As the ship moves, the camera platform transects the seafloor taking downward-looking video and/or still images.

Towed camera systems have been in use since the 1970s (see Machan & Fedra, 1975; Holmes & Barrett, 1977; Rice *et al*., 1979) and, since the introduction of acoustic mapping of the seafloor, they are often used for ground-truthing sidescan sonar data (e.g. Magorrian *et al*., 1995; Masson, 2001). In biological applications, in particular in the deep sea, the taxonomic identification of many species is difficult, especially if this is done from photographs. In response to this, camera sledges are often used with a small net trailing behind the sledge, which, although non-quantitative (Rice *et al*., 1979), can collect specimens that can be used to identify the organisms in the images with a greater degree of confidence (Ruhl, 2007).

Camera sledges are in full contact with the seafloor and are dragged across the seafloor by the surface vessel via a tow wire. Mounted on the front of the sledge are lights, with either or both still or video cameras. The cameras are often mounted obliquely and forward-facing, vertically downward-looking, or a combination of both. Camera sledges enable descriptions of seafloor attributes, distribution of megabenthic communities and ultimately habitat classification across relatively large areas of seafloor. The images and video stream can either be pre-programmed and logged autonomously (and downloaded after deployment) or streamed to the surface if a conducting cable is available (e.g. fibre optic, though it is rare to risk damage by dragging this across the seafloor). The ship-side operations are very similar to trawling in that they are deployed off the stern and require around 2–3 : 1 wire-out to depth ratio and acoustic monitoring via a pinger or USBL beacon. Camera sledges require a relatively slow towing speed to allow reliable

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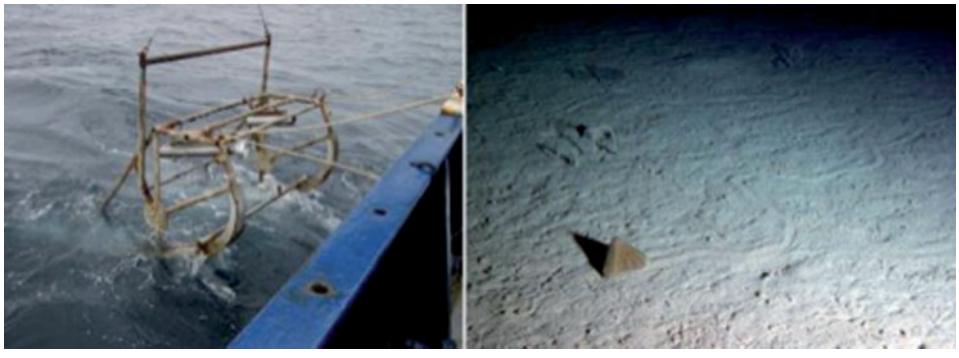

Fig. 7.10 An example of a towed camera sledge; the Scripps camera sledge (left) and an image from the deep-sea floor (right). (Images © Ken L. Smith Jr., MBARI, USA.) (For a colour version of this figure, see Plate 7.6.)

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interpretation of video data: 0.5–2 knots, although 0.75 knots is recommended (Shand & Priestly, 1999). To maintain the correct sledge orientation, a hydrodynamic depressor weight is often attached to the wire a few tens of metres ahead of the sledge (e.g. Barker *et al*., 1999). This depressor keeps the wire close to the seafloor to prevent the main wire from hauling directly on the sledge, particularly in adverse weather conditions. The camera sledge arrangement provides a standardised distance, and, therefore, field of view of the seafloor but special care must be taken when towing to maintain the sledge in the correct position on the seafloor. An example of a deep-sea towed camera sledge and the images it can take is shown in Fig. 7.10.

One disadvantage of camera sledges is their unsuitability on rough terrain or complex topography, which can lead to snagging or damage to the equipment. In unfamiliar areas, it is advised to perform a reliable acoustic sounding of an area to identify a suitable tow path. Some designs have addressed this issue by towing the camera at an altitude of 1 m above bottom (Barker *et al*., 1999). The equipment is held off the seabed at this altitude between positively buoyant flotation and a negatively buoyant drag chain. In the event of snagging, a weak-link is incorporated into the drag chain, which can be snapped if necessary. Alternatively, a towed camera platform is another option for visually mapping the seafloor without making any physical contact.

With the exception of the net facility, towed camera platforms can provide very similar transects of the seafloor to towed camera sledges (Fig. 7.11). Camera platforms typically have downward-facing video and still cameras mounted within a frame, which is lowered from the starboard side of the surface vessel until a few metres above the seafloor. Normally, the data are sent to the ship in real time via a fibre-optic cable but autonomous versions have been used (e.g. WASP; Jones *et al*., 2009). An acoustic pinger/USBL beacon or real-time depth sensor monitors the vehicle's descent. Upon approaching the seafloor, a short-range acoustic altimeter

Fig. 7.11 The Deep Tow Imaging System (DTIS) (left); an example of an image taken of fragile coral mounds taken by DTIS (right). (Images © the National Institute of Water and Atmospheric Research, New Zealand.) (For a colour version of this figure, see Plate 7.7.)

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can indicate the altitude above ground with great accuracy. Alternatively, and more crudely, a drop weight can be suspended below the camera within the field of view at a known distance to indicate to the operator when it is, for example, 2 m above the bottom (though this is not advisable on soft bottoms as a large sediment cloud can be created, which obscures subsequent photographs). Recording commences once the vehicle is within range of the seabed. Some systems have altitude switches that trigger the still camera when a set altitude has been reached. To provide a scale on which to calibrate measurements, parallel lasers are often used (Tusting & Davis, 1992; Pilgrim *et al*., 2000), although the suspended weight technique also provides a known dimension within each shot. The camera platform hovers slowly across the seafloor as the ship moves at about 0.5–2 knots. Any rolling or pitching of the ship can cause the camera to rise and fall, often dramatically. Therefore, regular adjustment by the winch operator is required to maintain the desired altitude above bottom. Even when deployed over the starboard side, to reduce pitch as much as possible, the tow frame can change altitude by several metres resulting in a high percentage of unusable photographs. Another disadvantage to using camera platforms over camera sledges is that the distance covered in a given time period is reduced. However, they do allow photo-transects to be collected in areas of complex topography (such as canyons), areas littered with objects that can damage sledges (boulders, subsea cables, pipelines, etc.) or fragile habitats such as coral mounds. Recent years have seen more sophisticated drop cameras being developed including those with rock-coring capabilities (Fornari, 2003).

Most drop camera systems are designed and constructed by individual institutes, for example WASP and SHRIMP (NOCS), OFOS (IFM-GEOMAR), DTIS (NIWA) and TowCam (WHOI), but there are some commercially available (Table 7.4).

ROV imaging

ROVs can be a very powerful tool for deep-sea imaging (Table 7.5). A large science-class ROV, such as the NOCS *ISIS* vehicle, or work-class vehicle, as is

Table 7.4 Examples of modern towed camera platforms.

Vehicle	Institute, country	Operating depth	Camera type
TowCam	WHOI, USA	6500	Photo
Scampi	iFremer, France	6000	Photo/video
WASP	NOCS, UK	6000	Photo/video
SHRIMP	NOCS, UK	6000	Photo/video
Deep Tow 6KC	JAMSTEC, Japan	6000	Photo/video
Deep Tow 4KC	JAMSTEC, Japan	4000	Photo/video
OFOS	IFM-GEOMAR, Germany	6000	Photo/video
DTIS	NIWA, New Zealand	6000	Photo/video
Seatronics	Commercial, UK	3000	Photo/video

Source: Modified and updated from Humphris (2009).

used in the subsea construction industry, will have space for several camera systems, no practical limits on power for cameras and lights, fast fibre-optic data communication and precision navigation (Fig. 7.12a). The science-class systems optimised for imaging, such as *ISIS* or the MBARI *Ventana* ROV, have multiple High-Definition (HD) video cameras with High-Intensity Discharge (HID) or Hydrargyrum Medium-arc Iodide (HMI) lighting, digital stills cameras and strobe lights, low-light cameras, different lighting set-ups (including red and white lights) and many Standard-Definition (SD) cameras. These enable broadcast quality HD video and high-resolution still images to be obtained from several camera angles. Many of the systems are mounted on hydraulic pan-and-tilt units, allowing continuous adjustment of camera position in response to the subject being imaged. They often have parallel laser systems to provide scale to images. In some vehicles it is possible to obtain simultaneous vertical and oblique HD video and digital stills while carrying out transects with centimetre-scale resolution and relative positional information from a Doppler-velocity log bottom-tracking navigation system.

ROVs have the capacity to carry out highly sophisticated and replicated random sampling of benthic environments. Another benefit is that ROVs can conduct pre- or post-survey collection and detailed imaging of important organisms, which facilitates identification. Although ROVs are at the cutting edge of underwater imaging and very efficient, paradoxically many surveys using this method may

Table 7.5 Examples of modern science ROVs suited to imaging.

Vehicle	Institute, country	Operating depth (m)
ISIS	NOCS, UK	6500
JASON II	WHOI, USA	6000
Victor	Ifremer, France	6000
ROPOS	CSSF, Canada	5000
Hercules	Institute For Exploration, USA	4000
HyperDolphin	JAMSTEC, Japan	3000
Ventana	MBARI, USA	1850
Max Rover	HCMR, Greece	2100
RCV-150	HURL, USA	914

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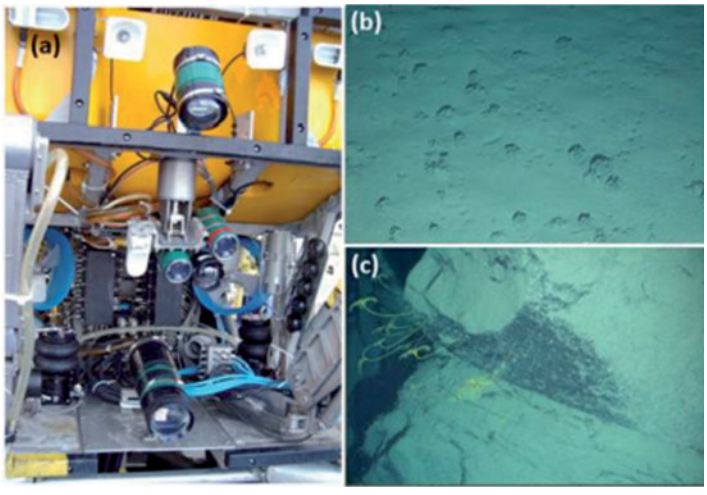

Fig. 7.12 (a) An example of a work-class oceaneering Remotely Operated Vehicle (ROV) set-up for imaging. From top: with lights, strobe (for still camera), pan-and-tilt unit (with low-light video, standard-definition video and high-definition video) and still camera. (b) Examples of horizontal transect showing xenophyophore populations. (c) Examples of vertical transect up a canyon wall showing stalked crinoids. (Images © SERPENT (a) and National Oceanography Centre (b,c).) (For a colour version of this figure, see Plate 7.8.)

be substandard for quantitative seabed assessment. It is extremely important for successful imaging surveys that there should be the following:

(1) A good sampling design (unplanned look-and-see style operations are extremely difficult to analyse quantitatively).
(2) Strict adherence to the survey plan (poor track following makes samples difficult to analyse quantitatively. It can easily occur as a result of pilot boredom or error in long transects, or scientists deviating from sample design to look more closely at or collect a particular organism).
(3) Constant camera set-up to allow for accurate quantification of image area (camera pan/tilt/zoom/altitude should remain constant during the transect).

Before starting a survey, the camera's lighting and recording modes should be adjusted to ensure that the highest quality image be maintained throughout. Testing of the cameras and lighting should ensure that well-lit, high-resolution pictures can be obtained while the ROV is in flight. When using the ROV cameras, they should be positioned at the same orientation throughout the survey in order to ensure a constant view. When mounted on a pan-and-tilt unit the camera pan should be set to straight ahead, while the camera tilt should be set to a known angle. It is usually appropriate to set the camera angle to as near as vertical as possible. It is important that a clear picture of the seabed is obtained, with no obstruction from the ROV frame. The zoom on cameras should be set; in flight it is usually convenient to use the maximum wide-angle setting (minimum zoom), which makes it easy to ensure

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that it is constant for all transects. The lighting should be adjusted to give the best possible picture. These settings should then remain unaltered for the duration of the survey. When flying each of the transects, the ROV should be maintained at a constant speed (e.g. 0.13 m s^{-1} is optimal for ISIS video survey at 2 m altitude) and at a constant height above the seabed. This height should be the minimum practically possible, while still maintaining a good field of view and avoiding sediment clouds that might obscure the image. The ROV should hold a straight course along the entire length of each transect, ignoring any features of interest.

Once images are obtained with a ROV they should be analysed in an identical method to those obtained by towed camera sledges/platforms. Ship-side operation of ROVs is complex and requires large investments in technology and personnel. For this reason, it will not be discussed further here.

AUV imaging

Autonomous underwater vehicles (AUVs) were first used for science in the 1990s and have only recently become viable for imaging. Most of the AUV scientific projects have used acoustics (Stansfield *et al.*, 2001; Millard *et al.*, 2003); direct imaging techniques from AUVs are in their infancy (Jones *et al.*, 2005). At present, several AUVs have still camera systems, for example the Woods Hole Oceanographic Institution Sentry AUV.

At present, AUVs are limited to a certain degree in terms of power, and hence sensors. Although the high power requirements of continuous lighting for video have not as yet been met for deep-sea applications, still photography has been shown to be entirely feasible (Jones *et al.*, 2005). Many AUVs, for example the NOCS Autosub, are limited for photography in some terrains because of their flying mechanism (using lift generated by forward motion) and hence the minimum altitude for safe operations. One of the more scientifically important alternative approaches is the hover-capable vehicle; this approach has been taken by the Woods Hole Oceanographic Institution vehicles ABE and, its successor, Sentry (Yoerger *et al.*, 1992). Hover-capable vehicles are more adapted to precise control over a much shorter range than the lift-generating vehicles (theoretical range 50–100 km for Sentry, 800 km for Autosub). Sentry has four thrusters on pivoting wings and is capable of movement in any direction much like an untethered ROV. These vehicles represent different solutions to AUV imaging for science and as a result will have different scientific uses. While hover-capable systems may be more versatile for imaging over a short range, they will not be able to sample in the more remote environments or at the long ranges that lift-generating vehicles are capable of investigating.

Field operations have demonstrated that AUVs have the capacity to collect high-quality images of the seafloor that are suitable for scientific analysis (e.g. Jones *et al.*, 2007). While the cost of AUVs will be a consideration with regard to their potential use for imaging, their autonomous nature allows them to be used in

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remote environments that cannot be sampled with existing technology. In addition, their autonomy from research vessels and ability to work concurrently with other sampling programmes will make AUVs increasingly important for adding extra data and value to existing research cruises.

Autonomous underwater vehicle photography has a number of novel scientific applications apart from its capability of working in remote environments, predominantly in high-resolution surveys over reasonably large areas. Survey work is the most important area of commercial AUV use (Danson, 2003), with AUV surveys already being explicitly commissioned in deep-water surveys for the oil and gas industry. While this commercial survey work is principally acoustic, it is inevitable that, with imaging technology becoming available, this type of survey will extend to imaging. In many respects these applications could be achieved by other existing technology such as ROVs or towed camera platforms, but in these cases, the use of AUVs will be justified in terms of time or financial savings. AUVs are particularly suited to high-resolution surveys over long distances that can be conducted without the need for human intervention. The AUV can be launched and then left to carry out the scientific task while the mother-ship is conducting other work elsewhere. In addition, the ship requirements of AUVs are potentially low, certainly without the need for the expensive, dynamically positioned support vessels needed for ROV operations.

One particular aspect of AUV operations relevant to photography is the ability of AUVs to follow terrain closely and maintain a constant altitude. Surveys carried out by a large class of imaging platforms, particularly operator-driven ROVs and towed camera platforms, may typically contain large rotational and scale changes between successive images. AUVs, as a stable platform, will avoid these limitations, facilitating photo mosaicking of reasonably wide areas of seafloor (Singh *et al*., 2004, Fig. 7.13).

Time-lapse imaging

There are many deep-sea applications where the information required is temporal rather than spatial. With temporal observations (e.g. one month to one year), using ship-tethered systems is impractical and unrealistic. Similarly, in the study of demersal benthic fauna, such as fishes, the presence of a moving vehicle, particularly with powerful illumination and propulsion can itself create a sampling artefact. A more stealthy, efficient and cost-effective way to extend a vehicle's occupancy in the deep sea is the use of autonomous free-fall landers (also known as 'free-vehicles' in the United States). Landers are deployed from a research vessel and free-fall to the seafloor, unattached to the ship and thereafter perform pre-programmed tasks autonomously for durations of hours to months. From the lander's static position on the seafloor, imaging with either video or stills can be achieved over far longer timescales and more unobtrusively than by alternative methods. At the end of the deployment, the lander is released from the seafloor by jettisoning metal ballast

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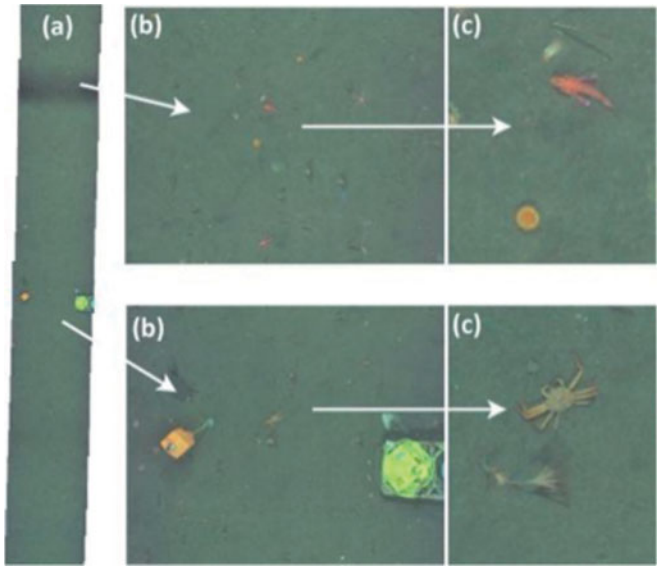

Fig. 7.13 Examples of photo mosaicking from Autonomous Underwater Vehicles (AUVs). Large areas of seafloor can be visualised in a mosaic (a), which are constructed from single still images (b), which can be magnified to identify specific organisms or features (c). (Images © MBARI, USA.) (For a colour version of this figure, see Plate 7.9.)

weights activated either by a timing device or by acoustic command from the ship. The lander then ascends to the surface by means of positively buoyant flotation modules where it is recovered by the surface vessel and downloaded. A more detailed description of lander design and history is reviewed in Tengberg *et al*. (1995) and Bagley *et al*. (2005).

To maximise available power and minimise the effects of the lander's presence, imaging is typically time-lapsed, whereby a short video sequence or still image is taken only every few minutes or hours and not continuously. The time-lapse interval is determined by power requirements, available data storage capacity and the duration of the deployment.

Long-term deployments of landers are typically used to image an area of seafloor to investigate the slow biological and geological phenomena that are otherwise undetectable at short timescales. These include the settling of phytodetrial deposits (Billett *et al*., 1983), benthic resuspension processes (Auffret *et al*., 1994), the growth of deep-sea organisms such as barnacles (Lampitt, 1990) and xenophyophores (Gooday *et al*., 1993), small-scale sediment movement (Wimbush *et al*., 1982), monitoring of manganese nodule environments (Gardner *et al*., 1984) and the detection of changes in feeding activity, density and bioturbation rates of benthic fauna (Bett & Rice, 1993; Vardaro *et al*., 2009).

Examples of this method are the Scripps camera tripod (Smith *et al*., 1993), *Bathysnap* (Lampitt & Burnham, 1983), Module Autonome Pluridisciplinaire

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Fig. 7.14 Examples of images from long-term time-lapse imaging landers: NOCS' Bathysnap (left) and the Scripps camera tripod (right). (Images © DEEPSEAS Group, National Oceanography Centre, Southampton, UK and Ken L. Smith Jr., MBARI, USA.) (For a colour version of this figure, see Plate 7.10.)

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(MAP; Auffret *et al*., 1994) and the Bottom Ocean Monitor (BOM; Gardner *et al*., 1984). These systems, capable of up to 12-month deployments, have been used extensively for over 20 years. They all use still cameras positioned to take oblique images. The Scripps camera tripod camera sits at a height of 2 m at 31° from horizontal with two strobe lights and images a 20 m^{-2} area of seafloor. The *Bathysnap* is smaller with the camera at a height of 0.8 m at 30° from horizontal. It uses only one strobe light and images a 2 m^{-2} area of seafloor. The BOM camera is mounted 1.6 m above bottom at 30° and images an area of 0.86–1.7 m^{-2}, whereas the MAP camera sits at 0.9 m above the seafloor at 40°. These systems are illustrated in Fig. 7.14.

The method of imaging at oblique angles has three advantages: (i) it permits larger areas of visible seafloor relative to the size of the lander; (ii) it images the seafloor outside the lander footprint, thus eliminating any effects of the lander structure and (iii) oblique illumination highlights topographical or biologically induced features such as animal tracks on the sediment surface better than vertical photography. To measure objects and organisms accurately from these photos, the perspective must be calculated following Wakefield and Genin (1987).

Structures deployed in the ocean for extended periods of time are vulnerable to both corrosion and biofouling. Corrosion can be controlled by the correct selection and combination of materials used; however, biofouling is not easily preventable or predictable in the deep sea. While biofouling can cause some problems in the photic zone and can lead to 'artificial reef' effects, the deep-sea environment, though more forgiving, is not entirely free of biofouling. Long-term lander structures are known to foul, and unidentified hydroids have colonised landers as deep as 3000 m over as short a period as six months (pers. obs.), although there is currently no evidence that long-term landers create artificial reefs (Vardaro *et al*., 2007).

At shorter timescales, more mobile animals such as fish or crustaceans are observed by imaging landers (Table 7.6), which typically require the use of bait. Short-term baited landers normally use oily fish such as tuna or mackerel as bait as

Table 7.6 Summary of recent baited and non-baited imaging landers for deep-sea applications.

Lander	Reference	Camera	Bait	Depth	Duration	Other gear
ALBEX	Jeffreys *et al*., 2010	Video	Spinach	6,000	12–66 h	CM
AUDOS	Priede and Bagley, 2000	Stills	Mackerel (×1)	6,000	12 h	CM, FT
Bathysnack	Lampitt *et al*., 1983	Stills	Mackerel (×1)	6,000	1 d	CM
Bathysnap	Lampitt and Burnham, 1983	Stills	Non-baited	6,000	12 mo	CM, ST, C
BOM	Gardner *et al*., 1984	Stills	Non-baited	6,000	12 mo	CM, N
DOBO Mk 1/2	Kemp *et al*., 2006, 2008	Stills	Cetacean/ mackerel (×8)	6,000	6 mo	ADCP
DOVE	Hardy *et al*., 2002	Stills	Unspecified	10,000	4 d	–
EITS	Raymond and Widder, 2007	LL-Video	Fish	6,000	2 d	–
FRESP Mk 2	Bailey *et al*., 2002	Video	Mackerel (×3)	6,000	3 d	RC
FVV	Wilson and Smith, 1984	Video	Mackerel (×3)	6,000	1 d	CM
GEOMAR	Witte, 1999	Stills	Shark	6,000	5 d	–
Hadal-Lander A/B	Jamieson *et al*., 2009a, 2009b	Video/Stills	Mackerel (×1)	11,000	12 h	CTD, AT
ISIT (ICDEEP)	Priede *et al*., 2006	L-L video	Mackerel (×1)	6,000	12 h	CM
LAFF	Jones *et al*., 1998	Stills	Cetacean	6,000	1.5–11 d	AT, FT
MAP	Auffret *et al*., 1994	Stills	Non-baited	6,000	12 mo	CM, N, ST
Photolander	Roberts *et al*., 2005	Stills	Non-baited	6,000	1 mo	CM, TM
ROBIO	Jamieson and Bagley, 2005	Stills	Mackerel (×1)	4,000	12 h	CM, CTD
Scripps Tripod	Smith *et al*., 1993	Stills	Non-baited	6,000	4 mo	ST
SPRINT	Bailey *et al*., 2003	H-S Video	Mackerel (×1)	6,000	12 h	CM, FSI

LL, low light; HS, high speed; CM, current-meter; FT, fish tracking; ST, sediment trap; N, nephelometer; C, compass; CTD, conductivity, temperature and depth; ADCP, acoustic doppler current profiler; RC, respirometry chamber; AT, amphipod trap; TM, transmissometer; FSI, fast start initiator.

they produce the most efficient and readily detected odour plume. As baited cameras simulate a natural food-fall, they can be used to investigate the consumption and dispersal of organic matter by the deep-sea scavenging community. Landers such as AUDOS (Priede & Bagley, 2000), ROBIO (Jamieson & Bagley, 2005) and the FVV (Wilson & Smith, 1984) tether about 2 m above the bottom between the ballast and buoyancy. This allows a relatively small lander to image large areas of seafloor vertically from above. Tethering such landers means that there is very little structure on the seafloor (except for the ballast and bait), which allows a more representative setting around food-falls: i.e. fish are not restricted or distracted by the lander itself (Jamieson *et al*., 2006). These landers are used to study the events at the food-fall, providing information on interception rates, scavenger assemblages, behaviour, physiology, interactions and foraging strategies. Ultimately, the data are then used to calculate species abundance as a non-destructive alternative to trawling. Foraging strategies and abundance estimation from baited landers are discussed in detail in Priede and Merrett (1996; 1998), Bailey and Priede (2002) and Farnsworth *et al*. (2007).

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However, there are some disadvantages to using baited landers that must be considered carefully when selecting the correct equipment to meet scientific requirements. Firstly, baited landers only observe scavengers or predatory fish that will exploit the higher number of small scavenging crustaceans (such as amphipods), collectively known as 'bait-attending' species. Other fish may appear as rarities at baited landers but may otherwise be highly abundant in trawls.

Secondly, although imaging fish vertically from above allows an unobstructed 360° access to the bait and makes body length measurements more accurate, taxonomy is often difficult without horizontal images of fish and physical trawl samples for ground-truthing identifications. Some landers such as ROBIO (Jamieson & Bagley, 2005) can be set up with different camera angles (vertical, horizontal and oblique) that can be changed between deployments at the same site to give a variety of different information (Fig. 7.15). Other systems such as the LAFF lander (Jones *et al*., 1998) have vertical and horizontal cameras operating simultaneously on the same frame.

The issue of ground-truthing from trawls is very important. There are some ubiquitous scavenging fish such as the macrourid family that are notoriously difficult to distinguish to species level, with some impossible to distinguish from photographs

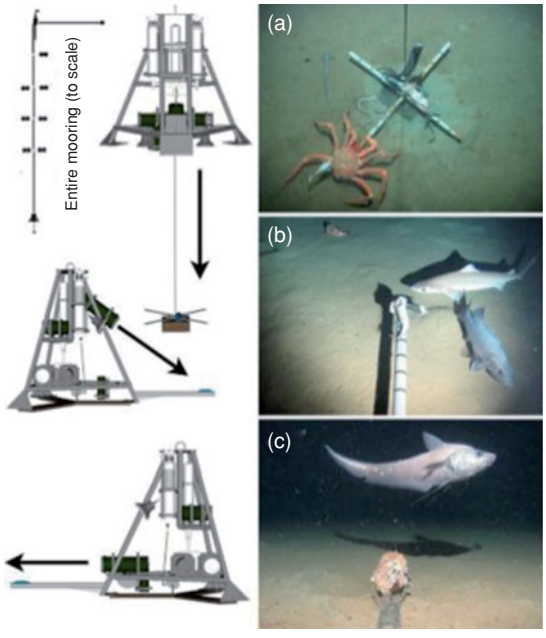

Fig. 7.15 Examples of different baited camera configurations of the ROBIO lander. (a) Vertical imaging from 2 m above the seafloor (field of view = 2.1 m × 1.6 m). (b) Oblique 30° imaging at 1 m above bottom (field of view = 1.4 m across centre). (c) Horizontal imaging at 0.3 m above bottom (field of view = 0.55 m along bottom). (Images © the University of Aberdeen, UK.) (For a colour version of this figure, see Plate 7.11.)

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(Wilson & Waples, 1983). Relating the images to known trawl data and comparing geographic and bathymetric distributions is often sufficient. However, one of the main advantages of using baited landers is that it permits deep-sea fish surveys to operate in areas where trawling is not possible (e.g. MPAs, in the vicinity of subsea oil and gas infrastructure or in areas too deep for trawling activities); therefore taxonomic identification from photographs and video must be treated with caution.

Baited landers are also used to investigate the scavenging community at depths where trawling is no longer possible from most vessels (>6000 m). Hadal-Lander A and B (Jamieson *et al*., 2009a, 2009b) have been used extensively in recent years at depths between 6000 and 10,000 m in the deep trenches of the Pacific Ocean. This method is much the same as shallower baited landers except that descent and ascent times are much longer. Other systems rated to 6000 m + include the DOVE camera (Hardy *et al*., 2002), which is an extremely compact system. Miniaturising baited cameras in this way could in theory address the spatial coverage and relatively low number of deployments per day by deploying an array of cameras across an area or along a depth contour.

Apart from taxonomic cataloguing and abundance estimation, baited landers have been used in a variety of other applications such as behavioural and physiological observations (Priede & Bagley, 2000; Bailey *et al*., 2002, 2003). Other systems such as the ISIT (now ICDEEP) lander (Priede *et al*., 2006) of the HOV/ROV delivered Eye-In-The-Sea (EITS; Widder *et al.*, 2005) are used with ultra-low-light video cameras that observe the benthos under extremely low-light conditions (thus eliminating potential illumination artefacts) or the bioluminescence activity in the vicinity of food-falls (Heger *et al*., 2007; Raymond & Widder, 2007).

There are also methods used to extend the occupancy of the baited lander from short- to long-term (days to months). Larger bait such as dolphin, porpoise or shark carcasses have been used to look at the fate of these larger food-falls over longer periods of time (Jones *et al*., 1998; Witte, 1999; Kemp *et al*., 2006). Similarly, the DOBO lander, which originally used porpoise carcasses, has been refitted with a multiple-bait release that can introduce new single mackerel baits at two-week intervals (Kemp *et al*., 2008). This multi-bait system exploits the high-pressure and low-temperature environment of the deep sea to keep the bait fresh for longer. The multi-bait system achieves the equivalent of repeated deployments over longer timescales without the presence of a surface vessel.

Most landers are fitted with other sensors on board to place the images in larger environmental and hydrographic context. Landers used for survey work tend to have current-meters on board that aid in odour plume modelling, while others carry CTD and ADCP sensors to investigate the hydrographic regimes in the vicinity.

When using baited systems it is important to be aware that the camera field of view is not the only sampled area. The bait creates an odour plume that disperses laterally in the near-bottom currents and, therefore, emanates across the seafloor in an elliptical shape within the currents over time. Although some species may detect the presence of the lander irrespective of the odour plume (e.g. due to noise

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or bioluminescent activity), it is the odour plume that is effectively the catchment area. The effective area of the plume depends on the current velocities and bait characteristics, both of which can vary a great deal over time and can complicate interpretation. Potential foraging strategies and interactions with theoretical odour plumes are described in Bailey and Priede (2002). Based on this study and others (Stanley *et al*., 1985; Sainte-Marie and Hargrave, 1987), the area of a simple odour plume can be calculated theoretically in two-dimensions. This theoretical model predicts a plume area of 0.01 km^{-2} after one hour, 0.6 km^{-2} after six hours and 2.4 km^{-2} after 12 hours (assuming an average current speed of 0.05 m s^{-1}); however, as in reality the concentration of the plume will decrease with time, at some point it will inevitably fall below the detection threshold of a given species.

There are also other more complex methods of calculating odour plumes in three-dimensions (Rowe *et al*., 1986). However, in the actual situation, those who intercept the bait first often remove and consume large pieces, which temporarily increases the odour concentration before the concentrations is depleted over time. As a result of this, and changing current velocity and direction, the concentration at a given distance from the lander at any given time is largely unknown, as are the detection thresholds for most if not all deep-sea fish. Consequently, odour plume models are typically used theoretically for the first half hour of the deployment. Interpretation of data obtained from baited cameras is reviewed in Bailey *et al*. (2007).

Interpretation of images

To geo-reference data accurately from transects, the video and still images should be date and time-stamped and, where possible, the GPS position should be included, as it is often the vessel position that is logged rather than the position of the sledge or platform. Alternatively, albeit perhaps less accurately, the position of sledges relative to the ship can be calculated using Pythagorean theorem from the sounding depth, tow wire angle and length of wire-out. ROVs and AUVs will be equipped with extremely accurate positioning data that can be related to each video sequence or image.

With field of views up to $\sim$5 m^{-2}, photographic transects may cover an area of hundreds or even thousands of square metres across survey lines of a few kilometres. For example, towing a camera at 2 knots for one hour will cover $\sim$3600 m of seafloor. Accurately determining the distance travelled and the resultant area imaged (derived from the field of view) are paramount in the interpretation of the images.

There are three main options for scaling seabed images and hence for calculating the area covered: (i) a trigonometric approach using altitude and optical camera angles; (ii) a photogrammetric approach using stereo-images and (iii) direct scaling using parallel lasers to project a pattern of known dimensions onto the seabed. In some imaging applications scaling can be achieved by placing an object of known

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size in the field of view, although this is not usually possible if moving camera systems are used.

Vertical photography can be interpreted as point-source quadrats and can provide more easily measurable images than oblique images. Given that towed camera platforms are not imaging from a fixed altitude nor, when they are suspended in the water column, a fixed angle, the area of visible seafloor must be calculated for each image. Vertical images can be stitched together in a mosaic format to digitally reconstruct large areas of the seafloor; this can provide a basis on which to identify large-scale patterns not visible from single images (Pizarro & Singh, 2003; Singh *et al*., 2004). This requires each image to be scaled up or down to standardise the scale in all the images. In fact, each image will not neatly line up to the next, and there will undoubtedly be some overlapping of areas and others where gaps occur. Once the area of each image is known and any gaps of overlaps have been found, the area of the whole transect can be calculated.

For individual vertical photographs, altitude can be converted to areal coverage using the following equation, where a is the camera altitude (e.g. metres), θ is the horizontal camera acceptance angle (degrees) and ω is the vertical camera acceptance angle (degrees):

$$\text{Area of photograph} = 4a^2 \tan\left(\frac{\theta}{2}\right)\left(\frac{\omega}{2}\right)$$

If there is no overlap between photographs then the areal coverage of the survey is just the sum of the areas of the photographs. If there is constant overlap between regular photographs, mean altitude can be used to calculate an approximate area of the whole transect, where v is the towed camera platform speed (m s^{-1}), t is the time between frames (seconds) and n is the number of frames:

$$\text{Area of transect} = 4a^2 \tan\left(\frac{\omega}{2}\right) \times \tan\left(\frac{\theta}{2}\right) + 2anvt \times \tan\left(\frac{\theta}{2}\right) - 2avt \times \tan\left(\frac{\theta}{2}\right)$$

As accurately identifying organisms and other objects from vertical photography can often be difficult, many systems often use oblique photography, which provides a trapezoidal field of view. Mosaic reconstructions are not possible from oblique photography; however, implementing a digital perspective grid (or 'Canadian grid') over the raw image allows accurate and precise measurements of objects, organisms or distances between them. Any pitching or rolling of the sledge over uneven terrain can skew the accuracy of such measurements so the grid must be corrected to suit each image. Applying a digital perceptive grid to images is a lengthy, complicated procedure and is described in full in Wakefield and Genin (1987). With advantages

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and disadvantages of both vertical and oblique photography, the solution is to operate both techniques simultaneously on the same vehicle.

Interpretation of video footage can be achieved using one of three methods: (i) extraction of still frame grabs, (ii) complete counts or (iii) video mosaicking (Jones *et al.*, 2009). Frame-grabbing entails the deconstruction of video sequences into individual still images at set intervals. These images are then processed in the same manner as raw still photography, as detailed above. However, this does result in large amounts of data being lost. The resolution of video frame grabs is often far lower than still images, although, with the introduction of HD cameras, frame grabs are now more comparable to digital still images. Complete count analysis makes full use of the video footage. Objects or organisms can be counted as they pass a line perpendicular to the direction of travel (Jones *et al.*, 2006). The total area of seafloor imaged using this method can be estimated by the line width multiplied by the distance travelled. The line width can be calculated by trigonometry. For example, to calculate the line width at the bottom of the displayed video image (the shortest horizontal distance in an oblique image and hence the best resolution part of the image available), the following equation can be used; where a is the camera altitude (e.g. metres), θ is the horizontal camera acceptance angle (degrees), ω is the vertical camera acceptance angle (degrees) and δ is the angle of the camera from vertical (degrees):

$$\text{Line width at base of screen} = 2\sin(0.5\theta)\sqrt{\alpha\sin(90-\delta-0.5\omega)^2+\alpha^2}$$

By using sophisticated algorithms, similar to the still image mosaicking, video footage can also be mosaicked, which utilises all available data to reconstruct large areas of seafloor (see Pizarro & Singh, 2003). This approach is becoming more and more successful as algorithms develop.

Interpreting images from landers whether baited or non-baited is done much in the same way, except that the images represent temporal quadrats, mapping changes over time. The oblique photography of the long-term non-baited landers requires the use of perspective grids (Wakefield & Genin, 1987). Vertical photography from baited landers requires a scale in the field of view. Rather than using expensive laser scales, a metal strip of a known length with a scale marked on it is typically placed in the field of view close to the seafloor. This acts as a calibration scale on which to make measurements of organisms or objects using commercially available proprietary software. Horizontal images do not lend themselves to accurate measurements. Perspective grids no longer work for horizontal images as most of the image is above the horizon. Indeed the percentage error of measurements derived from perspective grids using images taken at less than 30° can be considerable. The horizontal images are generally used for taxonomic purposes and depending on the orientation of, for example, a fish, relative measurements such as percentage head length of body length can still be measured.

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Most video data from baited landers is analysed manually and has the advantage, particularly if using high-speed video, of being slowed down to allow the interpretation of fast-moving events. Likewise, video data can be deconstructed into lower-resolution frame grabs to give a higher interval time lapse, albeit at lower resolution.

7.6 Biogeochemistry of the deep-sea floor

The ability to sample the marine benthos and recover organisms to the surface is fundamental to deep-sea research. However, there are other biogeochemical processes that have crucial implications for the biology and ecology of benthic communities that are not readily detected or accurately measurable from laboratory analysis (Glud, 2008). Removing sediment and water from the natural environment and recovering it to the surface can cause alteration of chemical, physical and biological processes (Hall *et al*., 2007). Discrepancies between laboratory (*ex situ*) and *in situ* measurements have been shown to exist in deep-sea sediment analyses, for example, total carbonate (or dissolved inorganic carbon, DIC) and alkalinity in pore waters (Murray *et al*., 1980; Sayles, 1981). In addition, oxygen penetration depths and pore-water gradients have been found to differ between *ex situ* cores and *in situ* cores (Glud *et al*., 1994, 1999a; Epping *et al*., 2002). Oxygen fluxes measured *in situ* with incubating chambers are lower than equivalent *ex situ* incubations (Smith & Hinga, 1983; Reimers *et al*., 1986; Glud *et al*., 1994). Other discrepancies have been observed in ammonium (Berelson *et al*., 1990; Glud *et al*., 1994; Aller *et al*., 1998), nitrate (Hammond *et al*., 1996; Martin & Sayles, 1996; Aller *et al*., 1998), silicate (Fanning & Pilson, 1971; Jahnke *et al*., 1989; Aller *et al*., 1998) and urea (Epping *et al*., 2002).

As a result, *in situ* measurements of relatively undisturbed systems have become commonplace in understanding sediment biogeochemistry and exchange fluxes in the deep sea. These *in situ* measurements are obtained using benthic chambers and microprofilers (Tengberg *et al*., 1995; 2005).

Benthic incubation chambers

Benthic chambers (also referred to as bell jars) are systems that are placed on the sediment, enclosing an area of the sediment surface and overlying water (Tengberg *et al*., 1995). Measurements of solute concentrations in the overlying water are measured from discrete water samples and *in situ* oxygen and sometimes pH levels are monitored throughout (Glud *et al*., 1994, 1999b; Stueben *et al*., 1998).

Benthic incubation chambers are typically delivered to the seafloor using the free-fall lander method (Fig. 7.16a), where, after touchdown, the chamber is very slowly lowered into the seafloor by mechanical activators. An extremely slow penetration minimises disturbance to the sediment/water interface. Once the chamber is in place and watertight, a small stirrer motor is activated to maintain ambient

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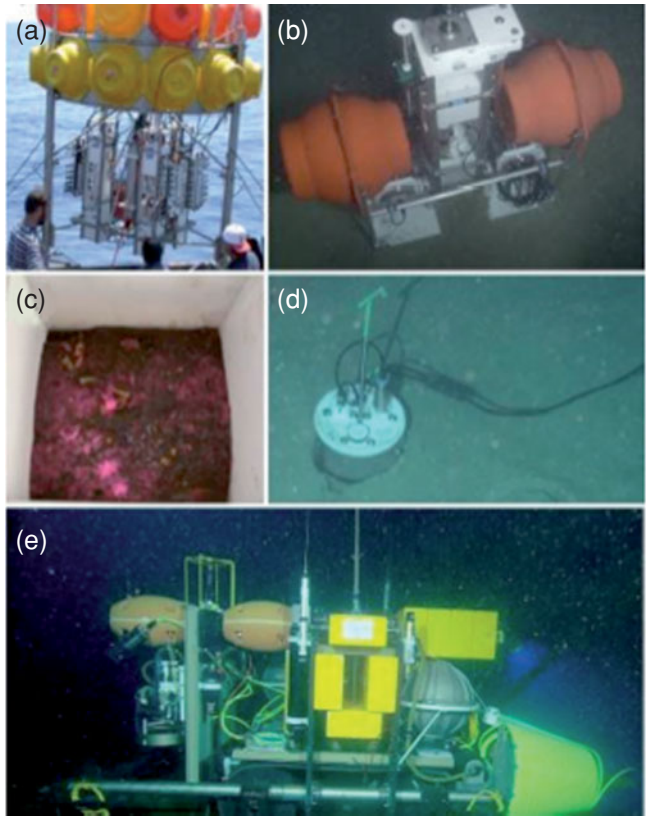

Fig. 7.16 Examples of deep-sea chamber incubating systems. (a) Modular chamber lander carrying three chambers. (b) A single autonomous chamber delivered by Remotely Operated Vehicle (ROV). (c) Sediment recovered from the chamber with ^{13}C-labelled algae and luminophore deposits visible of the sediment surface. (d) Simple ROV-operated chamber system. (e) Two chambers mounted on the front of a deep-sea crawler vehicle. (Images © the University of Aberdeen, UK and Ken L. Smith Jr, MBARI, USA.) (For a colour version of this figure, see Plate 7.12.)

hydrodynamic conditions (and prevent stratification artefacts) within the chamber (Tengberg *et al*., 2005). Measurements of oxygen and other parameters commence within the chamber and are continued throughout. A syringe sampler can be programmed to withdraw small water samples at regular intervals during the incubation. At the end of the incubation the underside of the chamber can be closed and retracted to recover the incubated sediment to the surface. There are many closing mechanisms in use, such as Ekman grab style scoops (Smith *et al*., 1979b), hydraulically-activated spades (Black *et al*., 2001) or motor-driven shutters (Pfannkuche & Linke, 2003). Once recovered, the sediment can be processed in the same manner as grab and core samples but with the additional *in situ* biogeochemical measurements.

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To increase the quantity and replication of data, benthic chambers landers are often modular, enabling the simultaneous delivery of several chambers to the seafloor (Pfannkuche & Linke, 2003). Furthermore, to extend the incubation period or for operations in areas where oxygen is extremely low (e.g. oxygen minimum zones; Levin *et al*., 2000) or consumption is rapid (e.g. cold seeps; Sommer *et al*., 2006), chamber landers have been fitted with OxyStat 'gill' systems (Woulds *et al*., 2007; Sommer *et al*., 2008). Gills circulate the incubated water through silicone tubing, which is exposed to and thus absorbs oxygen from either ambient water or highly oxygenated water in a closed reservoir, and pumps back into the chamber. By monitoring the O_2 concentrations inside and outside the chambers, the total O_2 consumption can be calculated by how much is needed to maintain the ambient O_2 rather than how much it has been depleted.

Benthic chambers are also used with the additional capability of injecting material into the chamber. Simple methods include the injection of a quantity of phytodetritus to observe the changes in community oxygen consumption in response to food supply. Alternatively, the injection of labelled isotope (^{13}C) material can simulate a settling food pulse allowing the pathway of ^{13}C through the benthic community to be followed (known as pulse chase experiments (Aberle & Witte, 2003; Witte *et al*., 2003a, 2003b; Bühring *et al*., 2006). Alternative injection experiments include the deposition of dyed sediment particles (luminophore tracers; Fig. 7.16c) into the chamber. After the incubation, the sediment is recovered and subcored, which provides an indication of bioturbation rates.

Although most benthic chambers are delivered to the deep by free-falling landers (Fig. 7.16a), the impact of the lander can often cause disturbance to the surficial layer. To account for this, chamber landers can either insert the chamber moments before the lander reaches the seafloor or insert the chamber some time after touchdown, the latter being the preferred method (Tengberg *et al*., 1995). Alternatively, benthic chambers can be designed for use with ROVs (Fig. 7.16b). The chamber comprises a self-contained autonomous system that is temporarily secured to an elevator or to the ROV itself. Once on the seafloor, the ROV decouples the chamber and places it very gently on the seafloor and activates a start switch, after which the remaining chamber operations are performed autonomously. Alternatively, simpler (and, therefore, cheaper) systems can be inserted and retracted into the sediment by the ROV itself (Fig. 7.16d). Although they do not recover the sediment or inject any tracer material, they do provide a simple way of achieving multiple chamber incubations.

For the same disturbance-reducing reasons combined with the need for repeated measurements over longer timescales, benthic chambers have been integrated into crawler vehicles (Smith *et al*., 1997; Sherman & Smith, 2009; Fig. 7.16e). The crawler can operate for six months at a time and traverse the seafloor, travelling up-current to new and undisturbed sites, to stop, insert and incubate a chamber of sediment.

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Sediment profiling systems

An alternative approach to quantifying benthic exchange processes *in situ* are profiling systems that insert mini- or microelectrodes into sediment to make precision measurements of chemical profiles (O_2, pH, pCO_2) and then interpret the results with mathematical models (Reimers, 1987; Archer *et al*., 1989; Gundersen & Jørgensen, 1990; Cai & Reimers, 1993; Glud *et al*., 1994). Profilers are typically deployed using free-fall landers (Tengberg *et al*., 1995). After landing, an array of microelectrodes is lowered towards the sediment surface. Most systems have a device to locate the sediment surface accurately (resistivity probes; Andrews & Bennett, 1981). Once the sediment has been penetrated, the probes take vertical stepped measurements at sub-millimetre intervals. The depth resolution depends partly on the size of these steps, but is mainly limited by the outer tip diameter of the electrode, which can vary from 0.025 to 1 mm for different profiling instruments (Tengberg *et al*., 1995). There are some commercially available micro- and mini-electrodes, although most research institutes tend to make their own. Oxygen electrodes have recently been somewhat superseded by optical sensors or optodes (Klimant *et al*., 1995). Micro-optodes are now often used in profiling as they are simpler to manufacture, have superior long-term stability, are more robust (1 mmØ) and, therefore, can achieve deeper penetration (Wenzhöfer *et al.*, 2001).

The development and success of micro-optodes in the marine environment inspired the development of planar optodes, where the O_2 quenchable luminescent chemistry is immobilised on transparent support foils. Excitation light is supplied from the outside and by using a digital camera the O_2-sensitive luminescence is imaged and converted into two-dimensional O_2 images (Glud *et al*., 1996, 2001, 2005; Fig. 7.17c). The planar optodes' principle has also been developed to image pH profiles (Ståhl *et al*., 2007). Planar optodes share the same basic principles as the Sediment Profile Imaging system (SPI, also known as REMOTS; Rhoads & Cande, 1971; Fig. 7.17a) whereby the optical path of a downward-looking camera is redirected by 90° by a 45° mirror and onto a faceplate. The faceplate is positioned on one edge of a wedge and inserted into the sediment either by gravity if lowered from a wire or motor-driven from a lander or ROV package. This system, essentially an upside-down periscope, images a cross-section of the sediment/water interface, thus imaging either a photograph of the sediment profile (SPI) or 2D image of O_2 concentration (planar optode).

Other solute concentration gradients across the sediment/water interface such as iron and manganese can be profiled using gel probes (also known as *gel peepers*). Gel probes can achieve a greater resolution than traditional core slicing and dialysis procedures (Carrignan *et al*., 1985; Shaw *et al*., 1990). Gel probes use a 'Diffusive Gradient in Thin films' principle (DGT; Davison *et al*., 2000) whereby a gel impregnated with chelating resin is inserted into the sediment. Metals continuously diffuse across the outer gel layer and accumulate on the resin. After the deployment, the resin is then sliced and analysed in the laboratory where the measured flux can

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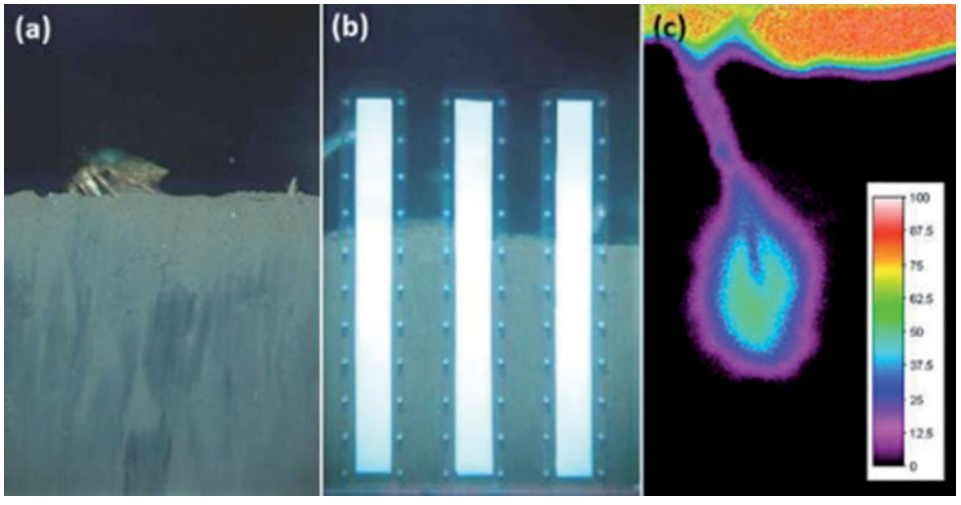

Fig. 7.17 Examples of imaging the sediment profile. (a) A typical Sediment Profile Imaging (SPI) image showing a crab on the sediment surface, (b) a SPI image with three integrated gel strips and (c) a 2D oxygen image of a brittlestar burrow from a Planar Optode. (Images © the University of Aberdeen, UK (a,b) and the Scottish Association for Marine Science, UK (c).) (For a colour version of this figure, see Plate 7.13.)

be quantitatively interpreted as concentration (Zhang *et al*., 1995; Harper *et al*., 1998).

Gel probes can also be integrated into the faceplates of SPI cameras (Teal *et al*., 2009; Fig. 7.17b) to visualise *in situ* faunal activity and correlate the relationship between pore-water metal gradients and sediment colour, which is often used as a proxy for biogeochemical state (Rhoads & Cande, 1971; Grizzle & Penniman, 1991).

7.7 *In situ* manipulative experiments

With the ever-increasing access to real-time underwater vehicles, particularly ROVs, there has been a great increase in the number of *in situ* manipulative experiments, which had previously been restricted to the laboratory and inshore environments. The diversity and capabilities of such experiments are vast and as a result there are too many to mention in full here. Many of these experiments are based on enclosed manipulation with the capability of inclusion or exclusion of an organism, species or foreign substance. For example, Gallucci *et al*. (2008) enclosed six small areas of seafloor (0.24 m^{-2}), which prohibited epifauna and their associated disturbance. Using imaging and coring techniques, they revisited the site years later to investigate the role of epifaunal disturbance (or lack of) on the community structure. In contrast, Hudson *et al*. (2004) deployed a similar cage

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and using a collection scoop deposited one holothurian into the cage. Fluorescent luminophore tracers were added to the cage and the experiment was left for approximately a day. The holothurian was later collected and dissected whereupon the throughput time of the gut was measured. Further tracer experiments comprise submersible deployed enclosures in which luminophores and/or ^{13}C-labelled algae (to simulate natural carbon deposition) are delivered to investigate sediment mixing processes and uptake rate by the benthic community (Blair *et al*., 1996; Fornes *et al*., 1999). The area of enclosed sediment is again left for a period of time before being revisited and cored by the submersible. Larger-scale experiments using a submersible have been able to introduce slowly-dispersing CO_2 reservoirs to study the potential effects of CO_2 sequestration on the benthic community. Thistle *et al*. (2005) introduced 60 litres of CO_2 at 3250 m and measured its effect on the benthos by coring at 2 and 40 m away from the source 30 days later. This experiment showed that CO_2-rich water had an adverse effect on infaunal communities. This experiment was developed further to include the setting of invertebrate traps 75 m away from the CO_2 source to examine the effects on more mobile fauna, in this case copepods (Thistle *et al*., 2007). In a further development the CO_2 sequestration reservoirs were placed in the vicinity of caged megafauna (octopus and fish), the results of which showed that some species can survive month-long episodic exposure to acidic, CO_2-rich water (Barry & Drazen, 2007).

Other experiments involved the collection of an organism by the submersible and placing it into an experimental apparatus on the seafloor. Using submersibles has the advantage of being able not only to select a particular species but also the number of specimens and/or the size preferred for the experiment. Whereas some experiments use suction samplers to isolate an organism in an experimental chamber for direct monitoring *in situ* (Smith & Hessler, 1974) or *ex situ* (Shillito *et al*., 2008), epifauna such as echinoids and holothurians can be sampled using scoops and placed inside watertight respirometry chambers and left for a period of time. Using Benthic Incubation Chamber Systems (BICS; NOCS, unpublished data), measurements of O_2 uptake and pH levels can be made with the flexibility of flushing the chamber at will to provide replicates, or of adding food to the chamber to measure the organism's response. Other experiments include depositing holothurians into small cages with gridded floors overlying sample bottles in order to examine gut and faecal matter content (NOCS, unpublished data). The specimens are left for a period of time, and the faecal matter is collected in the tubes for trophic analysis.

In all the described applications, the experimental equipment used was relatively simple, flexible in experimental design and relatively low-cost to produce. As the use of ROVs for manipulative experiments is fairly new to science, researchers must allow for modifications to experimental design, equipment and expected timescales in their planning. Thought must be given as to how the equipment should be mounted and dismounted from the ROV or elevator and ROV operators should be involved in all the design stages. The necessity to revisit an extremely small area of seafloor over long timescales (like those of Blair *et al*., 1996; Fornes *et al*.,

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1999; Hudson *et al*., 2004; Gallucci *et al*., 2008) and the requirement to sample specific areas over large distances (like that of Thistle *et al*., 2005, 2007; Barry & Drazen, 2007) simply cannot be undertaken without access to real-time vehicles. The ever-increasing access to such vehicles and the flexibility and potential for experimental science has opened up an area of deep-sea research not previously available.

7.8 Future developments

Techniques for sampling the deep benthos are developing in many directions, namely, sensory capabilities, increased replication, operational capability, access to deep-submergence vehicles and permanent underwater infrastructure.

The capabilities of sensors in areas such as sampling resolution, accuracy, precision, power management and storage continue to expand and are likely to enhance the value of measurements made in the future. The miniaturisation of instruments may provide the facility to improve sampling replication, the lack of which deep-sea research has always suffered from. Mimicking the approach in undersea seismics and physical oceanography, which have been revolutionised with the onset of Ocean Bottom Seismometer (OBS) arrays and lagrangian drifters, could and should be applied to benthic investigations. For example, miniaturising free-fall landers for short-term baited deployments, long-term non-baited deployments, chamber incubation and profiling applications may provide the financial and operational opportunity to increase replication through the deployment of multiple systems within a locality.

Access to deep-submergence vehicles is of paramount importance for future research in the deep sea. Though the number of scientific ROVs, AUVs and Crawlers continues to increase, it still lags behind the numbers in operation in the commercial sector. To address this issue, there are initiatives in place to exploit the down-time of commercially-operated ROVs, which are continuing to push into deeper waters, for scientific use (e.g. SERPENT project; Hudson *et al*., 2005; Jones, 2007). AUVs with increasing capabilities are also becoming more common. There are at present about a dozen in use for scientific exploration worldwide (Humphris, 2009). Currently in development are plans for long-range AUVs designed to survey greater areas of seafloor than previously possible (Barrouil & Lemaire, 1998). The autonomous nature of AUVs results in maximum usage of valuable ship-time and with their photographic capabilities they may even supersede other more labour-intensive imaging methods such as camera sleds.

Access to deep-submergence technology via permanent deep-sea cabled observatory networks is another promising area for deep-sea research. Cabled infrastructures such as ARENA, MARS and NEPTUNE (Massion *et al*., 2004) are already in place in the Pacific, with plans to develop similar networks in Europe (Favali & Beranzoli, 2009). The opportunity to develop scientific instrumentation into

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existing underwater Neutrino telescope observatories and network large mooring arrays is also highly likely (Howe *et al*., 2006). There is currently extensive research and development into the design of cabled docking systems, for recharging and data download, which will allow deep-submergence vehicles to operate for long periods without surfacing (Galletti di Cadilhac & Brighenti, 2003). This will greatly expand scientific research by allowing temporally replicated wide-area, high-resolution imaging without human intervention. Docking technology may allow high temporal resolution through increased access to deep-water environments and substantial savings in ship-time. While it is perhaps inevitable that more sophisticated systems such as AUVs will replace towed camera platforms in the future, at present they are not yet capable of producing images of comparable quality and at a comparable cost. However, the prospect of providing real-time, semi-permanent and/or permanent access to the benthos without the need for surface support vessels will revolutionise deep-sea research.

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Abbreviations

ADCP	Acoustic Doppler Current Profiler (oceanographic sensor)
ALBEX	Autonomous Lander for Biological Experiments (lander)
AUDOS	Aberdeen University Deep Ocean Submersible (baited camera lander)
AUV	Autonomous Underwater Vehicle
BRIL	Biogenic Reef Ichthyofauna Lander (baited camera lander)
CTD	Conductivity, Temperature and Depth (sensor)
DIC	Dissolved Inorganic Carbon
DOBO	Deep Ocean Benthic Observer (long-term baited lander)
DOVE	Deep-Ocean Visualisation Experimenter (baited lander)
DP	Dynamic Positioning (vessel positioning)
DTIS	Deep Tow Imaging System (camera platform)
FRESP	Fish Respirometer (baited camera/trap lander)
FVGR	Free Vehicle Grab Respirometer (chamber incubating lander)

FVV	Free-fall Video Vehicle (baited lander)
HD	High-Definition (video resolution)
HID	High-Intensity Discharge (lighting)
HMI	Hydrargyrum medium-arc iodide (lighting)
HOV	Human Occupied Vehicle (aka manned submersible)
ICDEEP	Image Intensified Charge Coupled Device for Deep-sea Research (low-light video)
ISIT	Intensified Silicone Intensified Target (low-light video)
LAFF	Large Abyssal Food Fall (baited camera lander)
LBL	Long Baseline (acoustic positioning system)
MBARI	Monterey Bay Aquarium Research Institute (USA)
NIOZ	Netherlands Institute for Sea Research
NIWA	National Institute of Water and Atmospheric research (New Zealand)
NOCS	National Oceanographic Institute, Southampton (UK)
OBS	Ocean Bottom Seismometer
OTSB	Otter Trawl Semi Balloon (trawl)
PAL	Photographic and Acoustic Lander (baited camera lander)
ROBIO	Robust Biodiversity lander (baited camera lander)
ROV	Remotely Operated Vehicle
SD	Standard Definition (video resolution)
SPI	Sediment Profile Imaging
USBL	Ultra-Short BaseLine (Acoustic Positioning System)
USNEL	United States Naval Electronics Laboratory (USA)

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References

Aberle, N. & Witte, U. (2003) Deep-sea macrofauna exposed to a simulated sedimentation event in the abyssal NE Atlantic: *in situ* pulse chase experiments using ^{13}C labeled phytodetritus. *Marine Ecology Progress Series*, **251**, 37–47.

Aldred, R.G., Thurston, M.H., Rice, A.L. & Morley, D.R. (1976) An acoustically monitored opening and closing epibenthic sledge. *Deep-Sea Research*, **23**, 167–174.

Aller, R.C., Hall, P.O.J., Rude, P.D. & Aller, J.Y. (1998) Biogeochemical heterogeneity and suboxic diagenesis in hemipelagic sediments of the Panama Basin. *Deep-Sea Research I*, **45**, 133–165.

Andrews, D. & Bennett, A. (1981) Measurements of diffusivity near the sediment-water interface with a finescale resistivity probe. *Geochimica et Cosmochimica Acta*, **45**, 2169–2175.

Archer, D., Emerson, S. & Smith, C.R. (1989) Direct measurements of the diffusive sublayer at the deep sea floor using microelectrodes. *Nature*, **340**, 623–626.

Auffret, G., Khripunoff, A. & Vangriesheim, A. (1994) Rapid post-bloom resuspension in the northeastern Atlantic. *Deep-Sea Research I*, **41**, 935–939.

Bailey, D.M. & Priede, I.G. (2002) Predicting fish behaviour in response to abyssal food-falls. *Marine Biology*, **141** (5), 831–840.

Bailey, D.M., Jamieson, A.J., Bagley, P.M., Collins, M.A. & Priede, I.G. (2002) Measurement of *in situ* oxygen consumption of deep-sea fish using an autonomous lander vehicle. *Deep-Sea Research*, **49**, 1519–1529.

Bailey, D.M., Bagley, P.M., Jamieson, A.J., Collins, M.A. & Priede, I.G. (2003) *In situ* investigation of burst swimming and muscle performance in the deep-sea fish *Antimora rostrata (Günther, 1878)*. *Journal of Experimental Marine Biology and Ecology*, **285-286**, 295–311.

Bailey, D.M., King, N.J. & Priede, I.G. (2007) Camera and carcasses: historical and current methods for using artificial food falls to study deep-water animals. *Marine Ecology Progress Series*, **350**, 179–191.

Bagley, P.M., Priede, I.G., Jamieson, A.J., Bailey, D.M., Battle, E.G., Henriques, C. & Kemp, K.M. (2005) Lander techniques for deep ocean biological research. *Underwater Technology*, **26** (1), 3–11.

Barker, B.A.J., Helmond, I., Bax, NJ., Williams, A., Davenport, S. & Wadley, VA. (1999) A vessel-towed camera platform for surveying seafloor habitats of the continental shelf. *Continental Shelf Research*, **19**, 1161–1170.

Barnett, P.R.O., Watson, J. & Connelly, D. (1984) The multiple corer for taking virtually undisturbed samples from shelf, bathyal and abyssal sediment. *Oceanologica Acta*, **7**, 399–408.

Barrouil, C. & Lemaire, J. (1998) An integrated navigation ystem for a long range AUV. In: *Engineering for Sustainable Use of the Oceans. Proceedings of IEEE OCEAN' 98*, 28 September to 1 October. IEEE Oceanic Engineering Society, Nice.

Barry, J.P. & Drazen, J.C. (2007) Response of deep-sea scavengers to ocean acidification and odor from a dead grenadier. *Marine Ecology Progress Series*, **350**, 193–207.

Bellan-Santini, D. & Thurston, M.H. (1996) Amphipoda of the hydrothermal vents along the mid-Atlantic Ridge. *Journal of Natural History*, **30**, 685–702.

Bett, B.J. & Rice, A.L. (1993) The feeding behaviour of an abyssal echurian revealed by in situ time-lapse photography. *Deep-Sea Research I*, **40** (9), 1767–1779.

Bett, B.J., Vanreusal, A., Vincx, M., Soltwedel, T., Pfannkuche, O., Lambshead, P.J.D., Gooday, A.J., Ferrero, T. & Dinet, A. (1994) Sampler bias in the qualitative study of deep-sea meiobenthos. *Marine Ecology Progress Series*, **104**, 197–203.

Bett, B.J., Malzone, M.G., Narayanaswamy, B.E. & Wigham, B.J. (2001) Temporal variability in phytodetritus and megabenthic activity at the seabed in the deep Northeast Atlantic. *Progress in Oceanography*, **50**, 349–368.

Berelson, W.M., Hammond, D.E., O'Neil, D., Xu, X.-M., Chin, C. & Zukin, J. (1990) Benthic fluxes and pore water studies in sediments from the central equatorial north Pacific: nutrient diagenesis. *Geochimica Et Cosmochimica Acta*, **50**, 3001–3013.

Billett, D.S.M., Lampitt, R.S., Rice, A.L. & Mantoura, R.F.C. (1983) Seasonal sedimentation of phytoplankton to the deep-sea benthos. *Nature*, **302**, 520–522.

Billett, D.S.M., Bett, B.J., Rice, A.L., Thurston, M.L., Galéron, J., Sibuet, M. & Wolff, G.A. (2001) Long-term change in the megabenthos of the Porcupine Abyssal Plain (NE Atlantic). *Progress in Oceanography*, **50**, 325–348.

Black, K.S., Fones, G.R., Peppe, O.C., Kennedy, H.A. & Bentaleb, I. (2001) An autonomous benthic lander: preliminary observations from the UK BENBO thematic programme. *Continental Shelf Research*, **21**, 859–877.

https://telegram.me/Geologybooks

Blair, N.E., Levin, L.A., DeMaster, D.J. & Plaia, G. (1996) The short-term fate of fresh algal carbon in continental slope sediments. *Limnology and Oceanography*, **41** (6), 1208–1219.

Blankenship, L.E., Yayanos, A.A., Cadien, D.B. & Levin, L.A. (2006) Vertical zonation patterns of scavenging amphipods from the hadal zone of the Tonga and Kermadec Trenches, *Deep-Sea Research I*, **53**, 48–61.

Boland, G.S. & Rowe, G.T. (1991) Deep-sea benthos sampling with the Gomex box corer. *Limnology and Oceanography*, **36**, 1015–1020.

Bouma, A.H. & Marshall, N.F. (1964) A method for obtaining and analyzing undisturbed oceanic sediment samples. *Marine Geology*, **6**, 231–241.

Buckland, S.T., Anderson, D.R., Burnham, K.P., Laake, J.L., Borchers, D.L. & Thomas, L. (2001) *Introduction to Distance Sampling: Estimating Abundance of Biological Populations*, Oxford University Press, Oxford, 432 pp.

Bühring, S.I., Lampadariou, N., Moodley, L., Tselepides, T. & Witte, U. (2006) Benthic microbial and whole-community responses to different amounts of ^{13}C-enriched algae: in situ experiments in the deep Cretan Sea (Eastern Mediterranean). *Limnology and Oceanography*, **51**, 157–165.

Cai, W.-J. & Reimers, CE. (1993) The development of pH and pCO2 microelectrodes for studying the carbonate chemistry of pore waters near the sediment-water interface. *Limnology and Oceanography*, **38**, 1776–1778.

Carrignan, R., Rapin, F. & Tessier, A. (1985) Sediment pore water sampling for metal analysis: a comparison of techniques. *Geochimica Et Cosmochimica Acta*, **49**, 2493–2497.

Charmasson, S.S. & Calmett, D.P. (1987) Distribution of scavenging Lysianassidae amphipods *Eurythenes gyllus* in the northeast Atlantic: comparison with studies held in the Pacific. *Deep-Sea Research*, **34** (9), 1509–1523.

Danson, E.F.S. (2003) AUV tasks in the offshore industry. In: *Technology and Applications of Autonomous Underwater Vehicles* (ed. G. Griffiths), Taylor and Francis, London, pp 127–138.

Davison, W., Fones, G.R., Harper, M., Teasdale, P. & Zhang, H. (2000) Dialysis, DET, and DGT: in situ diffusional techniques for studying water, sediments, and soils. In: *In Situ Monitoring of Aquatic Systems: Chemical Analysis and Speciation* (eds J. Buffle & G. Horvai), John Wiley & Sons, Chichester, pp 496–569.

Drazen, J.C., Buckley, T.W. & Hoff, G.R. (2001) The feeding habits of slope dwelling macrourid fishes in the eastern North Pacific. *Deep-Sea Research I*, **48**, 909–995.

Drazen, J.C., Bird, L.E. & Barry, J.P. (2005) Development of a hyperbaric trap-respirometer for the capture and maintenance of live deep-sea organisms. *Limnology and Oceanography: Methods*, **3**, 488–498.

Epping, E., Van Der Zee, C., Soetaert, K. & Helder, W. (2002) On the oxidation and burial of organic carbon in sediments of the Iberian margin and Nazare Canyon (NE Atlantic). *Progress in Oceanography*, **52**, 399–431.

Fanning, K.A. & Pilson, M.E.Q. (1971) Interstitial silica and pH in marine sediments: some effects of sampling procedures. *Science*, **173**, 1228–1231.

Farnsworth, K.D., Thygesen, U.H., Ditlevsen, S. & King, N.J. (2007) How to estimate scavenger fish abundance using baited camera data. *Marine Ecology Progress Series*, **350**, 223–234.

https://telegram.me/Geologybooks

Favali, P. & Beranzoli, L. (2009) EMSO: European multidisciplinary seafloor observatory. *Nuclear Instruments and Methods in Physics Research, A*. doi: 10.1016/j.nima.2008.12.214.

Fornari, D.J. (2003) A new deep-sea towed digital camera and multi-rock coring system. *EOS, Transactions, American Geophysical Union*, **84** (8), 69–76.

Fornes, W.L., DeMaster, D.J., Levin, L.A. & Blair, N.E. (1999) Bioturbation and particle transport in Carolina slope sediments: a radiochemical approach. *Journal of Marine Research*, **57**, 335–355.

Gage, J.D. (1975) A comparison of the deep-sea epibenthic sledge and anchor-box dredge samplers with van Veen grab and hand coring by divers. *Deep-Sea Research*, **22**, 693–702.

Gage, J.D. & Bett, B.J. (2005) Deep-sea benthic sampling. In: *Methods for the Study of the Marine Benthos* (eds A. Eleftheriou & A.D. McIntyre), 3rd edn, Blackwell Publishing Ltd., Oxford, pp 273–325.

Gage, J.D., Lightfoot, R.H., Pearson, M. & Tyler, P.A. (1980) An introduction to a sample time-series of abyssal macrobenthos: methods and principle sources of variability. *Oceanologica Acta*, **3**, 169–176.

Galletti di Cadilhac, R. & Brighenti, A. (2003) Docking systems. In: *Technology and Applications of Autonomous Underwater Vehicles* (ed. G. Griffiths), Taylor and Francis, London, pp 93–108.

Gallucci, F., Sauter, E., Sachs, O., Klages, M. & Soltwedel, T. (2008) Caging experiment in the deep sea: efficiency and artefacts from a case study in the Arctic long-term observatory Hausgarten. *Journal of Experimental Marine Biology and Ecology*, **354**, 39–55.

Gardner, W.D., Sullivan, L.G. & Thorndike, E.M. (1984) Long-term photographic, current, and nephelometer observations of manganese nodule environments in the Pacific. *Earth and Planetary Science Letters*, **70**, 95–109.

Gerdes, D. (1990) Antarctic trials of the multi-box corer, a new device for benthos sampling. *Polar Record*, **26** (156), 35–38.

Glud, R.N. (2008) Oxygen dynamics of marine sediments. *Marine Biology Research*, **4**, 243–289.

Glud, R.N., Gunderson, J.K., Joergensen, B.B., Revsbech, N.P. & Schulz, H.D. (1994) Diffusive and total oxygen uptake of deep sea sediments in the eastern South Atlantic Ocean: *in situ* and laboratory measurements. *Deep-Sea Research I*, **41**, 1767–1788.

Glud, R.N., Ramsing, N.B., Gundersen, J.K. & Klimant, I. (1996) Planar optodes, a new tool for fine scale measurements of two dimensional O_2 distribution in benthic communities. *Marine Ecology Progress Series*, **140**, 217–226.

Glud, R.N., Klimant, I., Holst, G., Kohls, O., Meyer, V., Kühl, M. & Gundersen, J.K. (1999a). Adaptation, test and in situ measurements with O_2 microopt(r)odes on benthic landers. *Deep-Sea Research I*, **46**, 171– 183.

Glud, R.N., Gunderson, J.K. & Holby, O. (1999b). Benthic in situ respiration in the upwelling area off central Chile. *Marine Ecology Progress Series*, **186**, 9–18.

Glud, R.N., Tengberg, A., Kuhl, M., Hall, P.O.J., Klimant, I. & Holst, G. (2001) An in situ instrument for planar O_2 optode measurements at benthic interfaces. *Limnology and Oceanography*, **46**, 2073–2080.

Glud, R.N., Wenzhöfer, F., Tengberg, A., Middelboe, M., Oguri, K. & Kitazato, H. (2005) Distribution of oxygen in surface sediments from central Sagami Bay, Japan: in situ

https://telegram.me/Geologybooks

measurements by microelectrodes and planar optodes. *Deep-Sea Research I*, **52**, 1974–1987.

Goffredi, S.K., Paull, C.K., Fulton-Bennett, K., Hurtado, L.A. & Vrijenhoek, R.C. (2004) Unusual benthic fauna associated with a whale fall in Monterey canyon, California. *Deep-Sea Research I*, **51**, 1295–1306.

Gooday, A.J., Bett, B.J. & Pratt, D.N. (1993) Direct observation of episodic growth in an abyssal xenophyophore (Protista). *Deep-Sea Research I*, **40** (11/12), 2131–2143.

Gordon, J.D.M. (1986) The fish populations of the Rockall Trough. *Proceedings of the Royal Society of Edinburgh*, **88B**, 191–204.

Gordon, J.D.M. & Duncan, J.A.R. (1987) Deep sea bottom living fishes at two repeat stations as 2200 and 2900 m in the Rockall Trough, north-eastern Atlantic Ocean. *Marine Biology*, **96**, 309–325.

Gordon, J.D.M., Merrett, N.R., Bergstad, O.A. & Swan, S.C. (1996) A comparison of the deep water fish assemblages of the Rockall Trough and Porcupine Seabight, eastern Atlantic: continental slope to rise. *Journal of Fish Biology*, **49A**, 217–238.

Greinert, J., Bohrmann, G. & Suess, E. (2001) Gas hydrate-associated carbonates and methane-venting at hydrate ridge: classification, distribution, and origin of authigenic lithologies. In: *Natural Gas Hydrates: Occurrence, Distribution, and Detection (Geophysical Monograph)*, Vol. 124 (eds. C.K. Paull & P.W. Dillon), American Geophysical Union, Washington, DC, pp 99–113.

Grizzle, R.E. & Penniman, C.A. (1991) Effects of organic enrichment on estuarine macrofaunal benthos—a comparison of sediment profile imaging and traditional methods. *Marine Ecology Progress Series*, **74**, 249–262.

Gundersen, J.K. & Jørgensen, B.B. (1990) Microstructure of di!usive boundary layers and the oxygen uptake of the seafloor. *Nature*, **345**, 604–607.

Hall, P.O.J, Brunnegård, J., Hulthe, G., Martin, W.R., Stahl, H. & Tengberg, A. (2007) Dissolved organic matter in abyssal sediments; core recovery artifacts. *Limnology and Oceanography*, **52**, 19–31.

Hammond, D.E., Mcmanus, J., Berelson, W.M., Kilgore, T.E. & Pope, R.H. (1996) Early diagenesis of organic material in equatorial Pacific sediments: stoichiometry and kinetics. *Deep-Sea Research II*, **43**, 1365–1412.

Hardy, K., Olsson, M., Yayanos, A.A., Prsha, J. & Hagey, W. (2002) Deep ocean visualisation experimenter (DOVE): low-cost 10 km camera and instrument platform. In: *Oceans 2002 Conference Proceedings, MTS/IEEE-OES*, IEEE Oceanic Engineering Society, Washington, DC, pp 2390–2394.

Hargrave, B.T., Phillips, G.A., Prouse, N.J. & Cranford, P.J. (1995) Rapid digestion and assimilation of bait by the deep-sea amphipod *Eurythenes gryllus*. *Deep-Sea Research I*, **42** (11/12), 1905–1921.

Harper, M.P., Davison, W., Zhang, H. & Tych, W. (1998) Kinetics of metal exchange between solids and solutions in sediments and soils interpreated from DGT measured fluxes. *Geochimica Et Cosmochimica Acta*, **62**, 2757–2770.

Harrison, K. (1988) Seasonal reproduction in deep-sea Crustacea (Isopoda: Asellota). *Journal of Natural History*, **22**, 175–197.

Heger, A., King, N.J., Wigham, B.D., Jamieson, A.J., Bagley, P.M., Priede, I.G. & Pfannkuche, O. (2007) Bioluminescent aggregations of *Vargula norvegica* (Ostracoda)

https://telegram.me/Geologybooks

at artificial food falls at 1000 m depth in carbonate mound provinces of the NE Atlantic. *Marine Biology*, **151** (4), 1471–1478.

Hessler, R.R. & Jumars, P.A. (1974) Abyssal community analysis from replicate box cores in the central North Pacific. *Deep-Sea Research*, **21**, 185–209.

Hessler, R.R. & Sanders, H.L. (1967) Faunal diversity in the deep sea. *Deep-Sea Research*, **14**, 65–78.

Hessler, R.R., Ingram, C.L., Yayanos, A.A. & Burnett, B.R. (1978) Scavenging amphipods from the floor of the Philippine Trench. *Deep-Sea Research*, **25**, 1029–1047.

Holmes, N.A. & Barrett, R.L. (1977) A sledge with television and photographic cameras for quantitative investigation of the epifauna on the continental shelf. *Journal of the Marine Biological Association of the United Kingdom*, **57**, 391–403.

Howe, B.M., McGinnis, T. & Gobat, J. (2006) Moorings for ocean observatories: continuous and adaptive sampling. In: *Proceedings of the IEEE Workshop on Scientific Submarine Cable 2006 Conference*, 7–10 February 2006, Marine Institute, Dublin, pp 172–181.

Hudson, I.R., Wigham, B.D. & Tyler, P.A. (2004) The feeding behaviour of a deep-sea holothurians *Stichopus tremulus* (Gunnerus) based on in situ observations and experiments using a remotely operated vehicle. *Journal of Experimental Marine Biology and Ecology*, **301**, 75–91.

Hudson, I.R., Jones, D.O.B. & Wigham, B.D. (2005) A review of the uses of work-class ROVs for the benefits of science: lessons learned from the SERPENT project. *Underwater Technology*, **26** (3), 83–88.

Humes, A.G. (1988) Copepoda from deep-sea hydrothermal vents and cold seeps. *Hydrobiologia*, **167–168** (1), 549–554.

Humphris, S.E. (2009) Vehicles for deep sea exploration. In: *Encyclopedia of Ocean Science* (eds J.H. Steele, K.K. Turekian & S.A. Thorpe), 2nd edn, Academic Press, San Diego, CA, pp 4647–4659.

Ingram, C.L. & Hessler, R.R. (1983) Distribution and behavior of scavenging amphipods from the central North Pacific. *Deep-Sea Research*, **30** (7A), 683–706.

Isaacs, J.D. & Schick, G.B. (1960) Deep-sea free instrument vehicle. *Deep-Sea Research*, **7** (1), 61–67.

Isaacs, J.D. & Schwartzlose, R.A. (1975). Active animals of the deep sea floor. *Scientific American*, **233** (4), 84–91.

Jahnke, R.A., Emerson, S.R., Reimers, C.E., Schuffert, J., Ruttenberg, K. & Archer, D. (1989) Benthic recycling of biogenic debris in the eastern tropical Atlantic Ocean. *Geochimica Et Cosmochimica Acta*, **53**, 2947–2960.

Jamieson, A.J. & Bagley, P.M. (2005) The ROBIO and DOBO landers: deep-sea biodiversity surveys in areas of anthropogenic activity. *Sea Technology*, **46** (1), 54–57.

Jamieson, A.J., Solan, M. & Fujii, T. (2009a) Imaging deep-sea life beyond the abyssal zone. *Sea Technology*, **50** (3), 41–46.

Jamieson, A.J., Fujii, T., Solan, M. & Priede, I.G. (2009b) HADEEP: free-falling landers to the deepest places on Earth. *Marine Technology Society Journal*, **43** (5), 151–159.

Jamieson, A.J., Bailey, D.M., Wagner, H.-J., Bagley, P.M. & Priede, I.G. (2006) Behavioural responses to structures on the seafloor by the deep-sea fish *Coryphaenoides armatus*: implications for the use of baited landers. *Deep-Sea Research I*, **53**, 1157–1166.

https://telegram.me/Geologybooks

Jeffreys, R., Lavaleye, M., Bergman, M., Duineveld, G., Witbaard, R. & Linley, TD. (2010) Deep-sea macrourid fishes scavenge on plant material: evidence from in situ observations. *Deep-Sea Research I*, **57** (4), 621–627.

Jones, D.O.B. (2007) Using existing industrial remotely operated vehicles for deep-sea science. *Zoologica Scripta*, **38** (Suppl. 1), 41–47.

Jones, D.O.B., Hudson, I.R. & Bett, B.J. (2006) Effects of physical disturbance on the cold-water megafaunal communities of the Faroe-Shetland Channel. *Marine Ecology Progress Series*, **319**, 43–54.

Jones, D.O.B., Bett, B.J. & Tyler, P.A. (2007) Depth-related changes in the arctic epibenthic megafaunal assemblages of Kangerdlugssuaq, East Greenland. *Marine Biology Research*, **3**, 191–204.

Jones, D.O.B., Bett, B.J., Wynn, R.B. & Masson, D.G. (2009) The use of towed camera platforms in deep-water science. *Underwater Technology*, **28** (2), 41–50.

Jones, D.O.B, McPhail, S.D., Bett, B.J., Flewellen, C. & Conquer, M. (2005) Seabed photography from an autonomous underwater vehicle. *Journal of Marine Science and Environment*, **C3**, 29–36.

Jones, E.J., Collins, M.A., Bagley, P.M., Addison, S. & Priede, I.G. (1998) The fate of cetacean carcasses in the deep sea: observations on consumption rates and succession of scavenging species in the abyssal north-east Atlantic Ocean. *Proceedings of the Royal Society B,* **265**, 1119–1127.

Jones, E.G., Tselepides, A., Bagley, P.M., Collins, M.A. & Priede, I.G. (2003) Bathymetric distribution of some benthic and benthopleagic species attracted to baited cameras and traps in the deep eastern Mediterranean. *Marine Ecology Progress Series*, **251**, 75–86.

Jumars, P.A. (1975) Methods for measurement of community structure in deep-sea macrobenthos. *Marine Biology*, **30**, 245–252.

Kato, C., Li, L., Tamaoka, J. & Horikoshi, K. (1997) Molecular analyses of the sediment of the 11000-m deep Mariana Trench. *Extremophiles*, **1**, 117–123.

Kemp, K.M., Jamieson, A.J., Bagley, P.M., McGrath, H., Bailey, D.M., Collins, M.A. & Priede, I.G. (2006) Consumption of a large bathyal food fall, a six month study in the north-east Atlantic. *Marine Ecology Progress Series*, **310**, 65–76.

Kemp, K.M., Jamieson, A.J., Bagley, P.M., Collins, M.A. & Priede, I.G. (2008) A new technique for sequential periodic bait-release at a camera platform in the deep sea; trial at 3664 m depth in the Charlie Gibbs Fracture Zone, Mid-Atlantic Ridge, *Deep-Sea Research I*, **55** (1–2), 218–228.

Klimant, I., Meyer, V. & Kühl, M. (1995) Fiber-optic oxygen microsensors, a new tool in aquatic biology. *Limnology and Oceanography*, **40**, 1159–1165.

Klinkhammer, G.P., Chin, C.S., Keller, R.A., Dahlmass, A., Sahling, H., Sarthou, G., Peterson, S., Smith, F. & Wilson, C. (2001) Discovery of new hydrothermal vent sites in Bransfield Strait, Antarctica. *Earth and Planetary Science Letters*, **193**, 395–407.

Koyama, S., Miwa, T., Horii, M., Ishikawa, Y., Horikoshi, K. & Aizawa, M. (2002) Pressurestat aquarium system designed for capturing and maintaining deep-sea organisms. *Deep-Sea Research I*, **49**, 2095–2102.

Kullenberg, B. (1956) The technique of trawling. In: *The Galathea Deep Sea Expedition* (eds A.F. Bruun, S.V. Greve, H. Mielche & R. Spärck), George Allen and Unwin Ltd., London, pp 112–118.

https://telegram.me/Geologybooks

Lampitt, R.S. (1990) Directly measured growth of a deep-sea barnacle. *Nature*, **345**, 805–807.

Lampitt, R.S. & Burnham, M.P. (1983) A free fall time-lapse camera and current meter system "Bathysnap" with notes on the foraging behaviour of a bathyal decapod shrimp. *Deep-Sea Research*, **30**, 1009–1017.

Lampitt, R.S., Merrett, N.R. & Thurston, M.H. (1983) Inter-relations of necrophagous amphipods, a fish predator, and tidal currents in the deep-sea. *Marine Biology*, **74**, 73–78.

Lawson, G.S., Tyler, P.A. & Young, C.M. (1993) Attraction of deep-sea amphipods to macrophyte food falls. *Journal of Experimental Marine Biology and Ecology*, **169**, 33–39.

Levin, L.A., Gage, J.D., Martin, C. & Lamont, P.A. (2000) Macrobenthic community structure within and beneath the oxygen minimum zone, NW Arabian Sea. *Deep-Sea Research II*, **47**, 189–226.

MacDonald, A.G. & Gilchrist, I. (1978) Further studies on the pressure tolerance of deep-sea crustacean, with observations using a new high-pressure trap. *Marine Biology*, **45**, 9–21.

MacDonald, A.G. & Gilchrist, I. (1982) The pressure tolerance of deep-sea amphipods collected at their ambient high pressure. *Comparative Biochemistry and Physiology*, **71A**, 349–352.

Machan, R. & Fedra, K. (1975) A new towed underwater camera system for wide-range benthic surveys. *Marine Biology*, **33**, 75–84.

Magorrian, B.H., Service, M. & Clarke, W. (1995) An acoustic bottom classification survey of Strangford Lough, Northern Ireland. *Journal of the Marine Biological Association of the United Kingdom*, **75**, 987–992.

Martin, W.R. & Sayles, F.L. (1996) $CaCO_3$ dissolution in sediments of the Ceara Rise, western equatorial Atlantic. *Geochimica Et Cosmochimica Acta*, **60**, 243–263.

Massion, G., Asakawa, K., Chave, AD., Howe, B., McGinnis, T., Phibbs, P., Rodgers, D., Shirasaki, Y., Mikada, H. & Kawaguchi, K. (2004) New scientific cabled observing systems: NEPTUNE and ARENA. *Suboptic 2004*, 29 March to 1 April, pp. 197–199, SubOptic, Monaco.

Masson, D. (2001) Sedimentary processes shaping the eastern slope of the Faroe-Shetland Channel. *Continental Shelf Research*, **21** (8–10), 825–857.

McIntyre, A.D. (1971) Meiofauna and microfauna sampling. In: *Methods for the Study of the Marine Benthos* (eds N.A. Holme & A.D. McIntyre), 1st edn, Blackwell Scientific Publications, Oxford, pp 131–139.

Merrett, N.R. & Marshall, N.B. (1981) Observations on the ecology of deep-sea bottom living fishes collected off northwest Africa (08°–27° N). *Progress in Oceanography*, **9**, 185–244.

Millard, N.W., McPhail, S.D., Stevenson, P., Pebody, M., Perrett, J.R., Webb, A.T., Squires, M., Griffiths, G., Thorpe, S.A., Collins, M.B., Statham, P., German, C., Burkill, P.H., Stansfield, K. & Smeed, D.A. (2003) Multidisciplinary ocean science applications of an AUV: the Autosub science missions programme. In: *The Technology and Applications of Autonomous Underwater Vehicles* (ed. G. Griffiths), Taylor and Francis, London, pp 139–159.

https://telegram.me/Geologybooks

Murray, J.W., Emerson, S. & Jahnke, R. (1980) Carbonate saturation and the effect of pressure on the alkalinity of interstitial waters from the Guatemala Basin. *Geochimica Et Cosmochimica Acta*, **44**, 963–972.

Paul, A.Z. (1973) Trapping and recovery of living deep-sea amphipods from the Arctic Ocean floor. *Deep-Sea Research*, **20**, 289–290.

Pfannkuche, O. & Linke, P. (2003) GEOMAR landers as long-term deep-sea observatories. *Sea Technology*, **44** (9), 50–55.

Phleger, C.F., McConnaughey, R.R. & Crill, P. (1979) Hyperbaric fish trap operation and deployment in the deep sea. *Deep-Sea Research*, **26A**, 1405–1409.

Pilgrim, D.A., Parry, D.M., Jones, M.B. & Kendall, M.A. (2000) ROV image scaling with laser spot patterns. *Underwater Technology*, **24** (3), 93–103.

Pizarro, O. & Singh, H. (2003) Toward large-area mosaicing for underwater scientific applications. *IEEE Journal of Oceanic Engineering*, **28** (4), 651–672.

Priede, I.G. & Bagley, P.M. (2000) In situ studies on deep-sea demersal fishes using autonomous unmanned lander platforms. *Oceanography and Marine Biology: An Annual Review*, **38**, 357–392.

Priede, I.G., Bagley, P.M., Way, S., Herring, P.J. & Partridge, J.C. (2006) Bioluminescence in the deep sea: free-fall lander observations in the Atlantic Ocean off Cape Verde. *Deep-Sea Research I*, **53**, 1272–1283.

Priede, I.G. & Merrett, N.R. (1996) Estimation of abundance of abyssal demersal fishes; a comparison of data from trawls and baited cameras. *Journal of Fish Biology*, **49** (Suppl. A), 207–216.

Priede, I.G. & Merrett, N.R. (1998) The relationship between numbers of fish attracted to baited cameras and population abundance: studies on (demersal grenadiers *Coryphaenoides (Nematonurus) armatus* in the abyssal NE Atlantic Ocean. *Fisheries Research*, **36**, 133–137.

Raymond, E.H. & Widder, E.A. (2007) Behavioral responses of two deep-sea fish species to red, far-red, and white light. *Marine Ecology Progress Series*, **350**, 291–298.

Reimers, C.E. (1987) An *in-situ* microprofiling instrument for measuring interfacial pore water gradients: methods and oxygen profiles from the North Pacific Ocean. *Deep-Sea Research*, **34**, 2019–2035.

Reimers, C.E., Fischer, K.M., Merewether, R., Smith, K.L., Jr & Jahnke, R.A. (1986) Oxygen microprofiles measured *in situ* in deep ocean sediments. *Nature*, **320**, 741–744.

Rhoads, D.C. & Cande, S. (1971) Sediment profile imaging camera for in-situ study of organism–sediment interactions. *Limnology and Oceanography*, **16**, 110–114.

Rice, A.L., Aldred, R.G., Billett, D.S.M. & Thurston, M.H. (1979) The combined use of an epibenthic sledge and a deep-sea camera to give quantitative relevance to macrobenthos samples. The Deep Sea: Ecology and Exploitation; Ambio Special Report, No. 6, Royal Swedish Academy of Science, pp 59–63, 65–72.

Rice, A.L., Aldred, R.G., Darlington, E. & Wild, R.A. (1982) The quantitative estimation of the deep-sea megabenthos: a new approach to an old problem. *Oceanologica Acta*, **5**, 63–72.

Roberts, J.M., Peppe, O.C., Dodds, L.A., Mercer, D.J., Thomson, W.T., Gage, J.D. & Meldrum, D.T. (2005) Monitoring environmental variability around cold-water coral reefs: the use of a benthic photolander and the potential of seafloor observatories.

https://telegram.me/Geologybooks

In: *Cold-Water Corals and Ecosystems* (eds A. Freiwald & J.M. Roberts), Springer-Verlag, Berlin, Heidelberg, pp 483–502.

Rowe, G.T. & Clifford, C.H. (1973) Modifications of the Birge-Ekman box corer for use with SCUBA or deep submergence research vessels. *Limnology and Oceanography*, **18**, 172–175.

Rowe, G.T., Suibuet, M. & Vangriesheim, A. (1986) Domains of occupation of abyssal scavengers inferred from baited cameras and traps on the Demerara Abyssal Plain. *Deep-Sea Research I*, **33** (4), 501–522.

Ruhl, H.A. (2007) Abundance and size distribution dynamics of abyssal epibenthic megafauna in the northeast Pacific. *Ecology*, **88** (5), 1250–1262.

Sainte-Marie, B. & Hargrave, B.T. (1987) Estimation of scavenger abundance and distance of attraction to bait. *Marine Biology*, **94**, 431–443.

Sayles, F.L. (1981) The composition and diagenesis of interstitial solutions–II. Fluxes and diagenesis at the water-sediment interface in the high latitude North and South Atlantic. *Geochimica Et Cosmochimica Acta*, **45**, 1061–1086.

Shand, C.W. & Priestly, R. (1999) A towed sledge for benthic studies. Scottish Fisheries Information. Pamphlet Number 22/1999, Fisheries Research Services.

Shaw, T.J., Giekes, J.M. & Jahnke, R.A. (1990) Early diagenesis in differing depositional environments; the response of transition metals in pore water. *Geochimica Et Cosmochimica Acta*, **54**, 1233–1246.

Sherman, A.D. & K.L. Smith, Jr. (2009) Deep-sea benthic boundary layer communities and food supply: a long term monitoring strategy. *Deep-Sea Research II*, **56**, 1754–1762.

Shillito, B., Hamel, G., Duchi, C., Cottin., D., Sarrazin, J., Sarradin, P-M., Ravaux, J. & Gaill, F. (2008) Live capture of megafauna from 2300 m depth, using a newly designed pressurized recovery device. *Deep-Sea Research I*, **55**, 881–889.

Shirayama, Y. & Fukushima, T. (1995) Comparisons of deep-sea sediments and overlying water collected using multiple corer and box corer. *Journal of Oceanography,* **51**, 75–82.

Shulenberger, E. & Hessler, R.R. (1974) Scavenging abyssal benthic amphipods trapped under oligotrophic central North Pacific Gyre waters. *Marine Biology*, **28**, 185–187.

Singh, H., Howland, J. & Pizarro, O. (2004) Advances in large-area photomosaicking underwater. *IEEE Journal of Oceanic Engineering*, **29**, 872–886.

Smith, K.L., Jr (1982) Zooplankton of a bathyal benthic boundary layer: In situ rates of oxygen consumption and ammonium excretion. *Limnology and Oceanography*, **27** (3), 461–471.

Smith, K.L., Jr & Baldwin, R.J. (1982) Scavenging deep-sea amphipods: effects of food odor on oxygen consumption and a proposed metabolic strategy. *Marine Biology*, **68**, 287–298.

Smith, K.L., Jr & Baldwin, R.J. (1984) Vertical distribution of the necrophagous amphipods, *Eurythenes gryllus,* in the North Pacific: spatial and temporal variation. *Deep-Sea Research*, **31** (10), 1179–1196.

Smith, K.L., Jr & Hessler, R.R. (1974) Respiration of benthopelagic fishes: In situ measurements at 1230 metres. *Science*, **184**, 72–73.

Smith, K.L., Jr & Hinga, K.R. (1983) Sediment community respiration in the deep sea. In: *The Sea* (ed. G. T. Rowe), John Wiley & Sons, New York, pp 331–370.

Smith, K.L. & Howard, J.D. (1972) Comparison of a grab sampler and large volume corer. *Limnology and Oceanography*, **28**, 882–898.

https://telegram.me/Geologybooks

Smith, K.L., Jr & Laver, M.B. (1981) Respiration of the bathypelagic fish *Cyclothone accliniens*. *Marine Biology*, **61** (4), 1432–1793.

Smith, K.L., Jr, Kaufmann, R.S. & Wakefield, W.W. (1993) Mobile megafaunal activity monitored with a time-lapse camera in the abyssal North Pacific. *Deep-Sea Research I*, **40** (11/12), 2307–2324.

Smith, KL., Jr, White, G.A. & Laver, M.B. (1979a) Oxygen uptake and nutrient exchange of sediments measured *in-situ* using a free vehicle grab respirometer. *Deep-Sea Research*, **26**, 337–346.

Smith, K.L., Glatts, R.R.C., Baldwin, R.J., Beaulieu, S.E., Uhlman, A.H., Horn, R.C. & Reimers, C.E. (1997) An autonomous, bottom-transecting vehicle for making long time-series measurements of sediment community oxygen consumption to abyssal depth. *Limnology and Oceanography*, **42**, 1601–1612.

Smith, K.L., Jr, White, G.A., Laver, M.B., McConnaughey, R.R. & Meador, J.P. (1979b) Free vehicle capture of abyssopelagic animals. *Deep-Sea Research*, **26A**, 57–64.

Smith, W. & McIntyre, A.D. (1954) A spring loaded bottom sampler. *Journal of the Marine Biology Association of the United Kingdom*, **33**, 257–264.

Sokal, R.R. & Rohlf, F.J. (1995) *Biometry: The Principles and Practice of Statistics in Biological Research*. W.H. Freeman and Company, New York.

Sommer, S., Pfannkuche, O., Linke, P., Luff, R., Greinert, J., Drews, M., Gubsch, S., Pieper, M., Poser, M. & Viergutz, T. (2006) Efficiency of the benthic filter: biological control of the emission of dissolved methane from sediments containing shallow gas hydrates at Hydrate Ridge. *Global Biogeochemical Cycles*, **20**, GB2019.

Sommer, S., Turk, M., Kriwanek, S. & Pfannkuche, O. (2008) Gas exchange system for extended in situ benthic chamber flux measurements under controlled oxygen conditions: first application - sea bed methane emission measurements as Captain Arutyunov mud volcano. *Limnology and Oceanography: Methods*, **6**, 23–33.

Ståhl, H., Glud, A., Schröder, C.R., Klimant, I., Tengberg, A. & Glud, R.N. (2007) Time-resolved pH imaging in marine sediments with a luminescent planar optode. *Limnology and Oceanography Methods*, **4**, 336–345.

Stanley, B.H., Hummel., H.E. & Ruesink, W.G. (1985) Estimating maximum horizontal area of pheromone plumes. *Journal of Chemical Ecology*, **11**, 1129–1146.

Stansfield, K., Smeed, D., Gasparini, G., McPhail, S.D., Millard, N., Stevenson, P., Webb, A., Vetrano, A. & Rabe, B. (2001) Deep Sea, high resolution, hydrography and current measurements using an AUV: the overflow from the Strait of Sicily. *Geophysical Research Letters*, **28**, 2645–2648.

Stein, D.L. (1985) Towing large nets by single warp at abyssal depths: methods and biological results. *Deep-Sea Research*, **32**, 183–200.

Stockton, W.L. (1982) Scavenging amphipods from under the Ross Ice Shelf, Antarctica. *Deep-Sea Research*, **29**, 819–835.

Stueben, D., Koelbl, R., Haushahn, P. & Schaupp, P. (1998) Measuring and sampling diffuse submarine hydrothermal vents. *International Ocean Systems Design*, **2**, 6–12.

Teal, L.R., Parker, E.R., Fones, G. & Solan, M. (2009) Simultaneous determination of in situ vertical transitions of colour, porewater metals and visualisation of infaunal activity in marine sediments. *Limnology and Oceanography*, **54** (5), 1801–1810.

Tengberg, A., De Bovee, F., Hall, P., Berelson, W., Chadwick, D., Crassous, P., Devol, A., Emerson, S., Gage, J., Glud, R., Graziottini, F., Gundersen, J., Hammond, D., Helder, W.,

https://telegram.me/Geologybooks

Hinga, K., Holby, O., Jahnke, R., Khripounoff, A., Liebermann, S., Nuppenau, V., Pfannkuche, O., Reimers, C., Rowe, G., Sahami, A., Sayles, F., Schurter, M., Smallman, D., Wehrli, B. & De Wilde, P. (1995) Benthic chamber and profiling landers in oceanography – a review of design, technical solutions and functioning. *Progress in Oceanography,* **35**, 253–294.

Tengberg, A., Andersson, U., Hall, P., Linden, B., Styrenius, O., Boland, G., De Bovee, F., Carlsson, B., Ceradini, S., Devol, A., Duineveld, G., Friemann, JU., Glud, R., Khripounoff, A., Leather, J., Linke, P., Lund-Hansen, L., Rowe, G., Santschi, P., De Wilde, P. & Witte, U. (2005) Intercalibration of benthic flux chambers II: hydrodynamic characterization and flux comparisons of 14 different designs. *Marine Chemistry*, **94**, 147–173.

Thistle, D., Carman, K.R., Sedlacek, L., Brewer, P.G., Fleeger, J.W. & Barry, J.P. (2005) Deep-ocean, sediment-dwelling animals are sensitive to sequestered carbon dioxide. *Marine Ecology Progress Series*, **289**, 1–4.

Thistle, D., Sedlacek, L., Carman, K.R., Fleeger, J.W., Brewer, P.G. & Barry, J.P. (2007) Exposure to carbon dioxide-rich seawater is stressful for some deep-sea species: an *in situ*, behavioral study. *Marine Ecology Progress Series*, **340**, 9–16.

Thorson, G. (1957) Sampling the benthos. In: *Treatise on Marine Ecology and Paleoecology* (ed. J. Hedgepeth), Geological Society of America, New York, pp 61–86.

Thurston, M.H. (1979) Scavenging abyssal amphipods from the northeast Atlantic Ocean. *Marine Biology*, **51**, 55–68.

Treude, T., Janßen, F., Queisser, W. & Witte, U. (2002) Metabolism and decompression tolerance of scavenging lysianasoid deep-sea amphipods. *Deep-Sea Research I*, **49**, 1281–1289.

Tusting, R.F. & Davis, D.L. (1992) Laser systems and structured illumination for quantitative undersea imaging. *Marine Technology Society Journal*, **26** (4), 5–12.

van Dover, C.L. & Fry, B. (1994) Microorganisms as food resources at deep-sea hydrothermal vents. *Limnology and Oceanography*, **39** (1), 51–57.

Vardaro, M.F., Parmley, D. & Smith, K.L., Jr (2007) A study of the possible "reef effects" caused by long-term time lapse camera in the deep-sea. *Deep-Sea Research I*, **54**, 1231–1240.

Vardaro, M.F., Ruhl, H.A. & Smith, K.L., Jr (2009) Climate variation, carbon flux and bioturbation in the abyssal North Pacific. *Limnology and Oceanography*, **54**, 2081–2088.

Vetter, E.W. & Dayton, P.K. (1998) Macrofaunal communities within and adjacent to a detritus-rich submarine canyon system. *Deep-Sea Research II*, **45**, 25–54.

Wakefield, W.W. & Genin, A. (1987) The use of Canadian (perspective) grid in deep-sea photography. *Deep-Sea Research*, **34** (3), 469–478.

Wenzhöfer, F., Holby, O. & Kohls, O. (2001) Deep penetrating benthic oxygen profiles measured in situ by oxygen optodes. *Deep-Sea Research I*, **48**, 1741–1755.

Wickins, J.F. (1983) Catches of large lysianassid amphipods in baited traps at the Nuclear Energy Authority dump site during June 1979. *Deep-Sea Research*, **30** (1), 83–86.

Widder, E.A., Robison, B.H., Reisenbichler, K.R. & Haddock, S.H.D. (2005) Using red light for in situ observations of deep-sea fishes. *Deep-Sea Research I*, **52**, 2077–2085.

Wigley, R.L. (1967) Comparative efficiencies of Van Veen and Smith-McIntyre grab samplers as revealed by motion pictures. *Ecology*, **48**, 168–169.

https://telegram.me/Geologybooks

Wilson, R.R. & Smith, K.L., Jr (1984) Effect of near-bottom currents on detection of bait by the abyssal grenadier fishes, *Coryphaenoides* spp. recorded *in situ* with a video camera on a free vehicle, *Marine Biology*, **84**, 83–91.

Wilson, R.R. & Waples, R.S. (1983) Distribution, morphology, and biochemical genetics of *Coryphaenoides armatus* and *C. yaquinae* (Pisces: Macrouridae) in the central and eastern North Pacific. *Deep-Sea Research*, **30**, 1127–1145.

Wimbush, M., Nemeth, L. & Birdsall, B. (1982) Current-induced sediment movment in the deep Florida straits: observations. In: *The Dynamic Environment of the Ocean Floor* (eds A.K. Fanning & F.T. Manheim), Heath, Lexington, MA, pp 77–94.

Witte, U. (1999) Consumption of large carcasses by scavenger assemblages in the deep Arabian Sea: observations by baited camera. *Marine Ecology Progress Series*, **183**, 139–147.

Witte, U., Wenzhöfer, F., Sommer, S., Boetius, A., Heinz, P., Aberle, N., Sand, M., Cremer, A., Abraham, W-R., Jørgensen, B.B. & Pfannkuche, O. (2003a) *In situ* experimental evidence of the fate of a phytodetritus pulse at the abyssal sea floor. *Nature*, **424**, 763–766.

Witte, U., Aberle, N., Sand, M. & Wenzhöfer, F. (2003b) Rapid response of a deep-sea benthic community to POM enrichment: an *in situ* experimental study. *Marine Ecology Progress Series*, **251**, 27–36.

Wolff, T. (1976) Utilization of seagrass in the deep sea. *Aquatic Botany*, **2**, 161–174.

Woulds, C., Cowie, G.L., Levin, LA., Andersson, J.H., Middelburg, J.J., Vandewiele, S., Lamont, P.A., Larkin, K.E., Gooday, A.J., Schumacher, S., Whitcraft, C., Jeffreys, R. & Schwartz, M. (2007) Oxygen as a control on sea floor biological communities and their role in sedimentary carbon cycling. *Limnology and Oceanography*, **52**, 1698–1709.

Yayanos, A.A. (1981) Reversible inactivation of deep-sea amphipods (*Paralicella caperesca*) by a decompression from 601 bars to atmospheric pressure. *Comparative Biochemistry and Physiology*, **69A**, 563–565.

Yeh, H.-M. & Ohta, S. (2002) Influence of velocity and types of beam trawl towing in deep-sea demersal fish and decapods crustacean samples. *Journal of Oceanography*, **58**, 505–517.

Yoerger, D.R., Bradley, A.M. & Walden, B.B. (1992) Autonomous benthic explorer. *Sea Technology*, **33**, 50–54.

Zhang, H., Davison, W., Miller, S. & Tych, W. (1995) In situ high-resolution measurements of fluxes of Ni, Cu, Fe, and Mn and concentrations of Zn and Cd in porewaters by DGT. *Geochimica Et Cosmochimica Acta*, **59**, 4181–4192.

https://telegram.me/Geologybooks

Chapter 8

Measuring the Flow of Energy and Matter in Marine Benthic Animal Populations

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Abstract

Traditionally, the rationale for energy flow studies was found in the elucidation of energy transfers within ecosystems or within the practical context of the rational management of resources, but it is now widely recognised that its scope embodies almost all biology, including the field of population dynamics and evolutionary studies. Here, we first describe conceptual models of energy and mass budgets at the level of the individual, the population and the community. However, the emphasis is on the next part in which the practicalities of measuring the various components of these budgets in the marine zoobenthic community are described in detail. The measurement of, among other things, ingestion, absorption, defaecation, excretion, growth, reproduction and respiration is discussed. Finally, attention is paid to the estimation of secondary production of benthic populations and to community-level modelling methods.

Keywords macrozoobenthos, meiobenthos, energy budget, dynamic energy budget, scope for growth, secondary production, demography, linear inverse modelling

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8.1 Introduction

The flow of energy and matter through an ecosystem starts with the primary producers, who store solar energy into a collection of organic compounds. By using water, carbon dioxide and minerals as nitrogen and phosphor, the primary producers (autotrophs) basically produce all organic compounds (such as polysaccharides, lipids and proteins) they need themselves. In contrast, herbivores, carnivores and decomposers (heterotrophs) feed on these energy-rich compounds, which they need both for extracting energy (in order to be able to do 'work') and for obtaining the required 'building blocks' for tissue growth and reproductive material.

Because energy is stored into chemical compounds, the fluxes of energy and mass are closely linked. Yet there is one important difference between these fluxes: ecosystems require the input of (solar) energy from outside, which finally dissipates as heat into outer space. Ecosystems are thus open systems in terms of energy. Material cycles on the other hand, can be closed, since autotrophs use simple minerals to synthesise complex compounds, which are then decomposed again into these simple minerals by heterotrophs. If only heterotrophic organisms or populations are considered, the intake of energy and material is nevertheless closely coupled. Hence, the flow of energy may be used as a description of the productivity of herbivores and carnivores. This production is often called secondary production, and in the past much effort has been put into the measurement of secondary production.

The study of energy flows is based on the principle that biological systems obey the laws of thermodynamics. These laws hold at all levels of organisation in biology and thus enable a link between the various levels. The first law, for example, states that energy can neither be created nor destroyed. Hence, energy flows at the population level are directly linked to energy flows at the level of the individual. Indeed, modern work on population dynamics deals with structured populations, in which individuals are no longer treated as identical, but are characterised by their size, their energy stores and (possibly) other state variables. State-dependent energy fluxes (i.e. energy intake and allocation to maintenance, growth and reproduction) eventually determine birth and death processes. A similar link exists between the level of the individual and cellular and molecular processes. The study of life-history strategies also profits from an energetics point of view, because the evolutionary demands put on survival and reproduction work only within the constraints on energy fluxes set by the laws of thermodynamics. Hence, while traditionally the rationale for energy flow studies was found in the elucidation of energy transfers within ecosystems or within the practical context of the rational management of resources, its scope embodies almost all biology, including the field of population dynamics and evolutionary studies.

The benthic habitat may contain both primary producers, the so-called phytobenthos, and herbivores and carnivores, the zoobenthos. In this chapter, we merely focus on the measurement of the flow of energy and matter in the zoobenthos.

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Measurements of primary production of the phytobenthos and microbial ecology are not discussed here (Underwood & Kromkamp, 1999).

State variables and units of measurement

Energy flow studies by different authors are often difficult to compare, for two main reasons. First, there has been a lack of a general theoretical framework and, second, different units have been used in which the energy flow or other processes are expressed. With respect to the latter, we strongly recommend the use of the SI system (Taylor, 1995). One should use the metre as the unit of length, the kilogram as the unit of mass, newton as the unit of force, joule (not kcal) as the unit of energy and watt (not kJ per day) as the unit of power (or energy expenditure rate). Time is, as far as possible, expressed in seconds, but the use of day and year (notation *a*) are (if really appropriate) allowed.

The state of a single animal is usually given by its body mass (unit kg, and often incorrectly termed weight), whereas biomass denotes the mass of the sum of animals per unit area (e.g. kg m^{-2}). This mass consists of living tissue and dead structures (shells, teeth, etc.) that have been built by the animal. Mass is expressed either directly or it is represented in terms of the mass (or the number of molecules) of a specific chemical component (element), e.g. carbon, or by its energy content (see Section 8.3 for details).

The lack of a general theoretical framework is, of course, a greater source of concern. However, over the last decade many studies have applied the so-called Dynamic Energy Budget (DEB) model developed by Kooijman (2010) for analysing flows of energy and matter in marine benthic animals. Such a common framework makes comparisons among different studies and different species much easier.

8.2 Energy and mass budgets of individual organisms

The flow of energy and matter into, within and out of an individual organism can be divided into a number of separate processes. In the International Biological Programme (IBP) various fluxes were distinguished, the most important ones being:

- *Consumption or ingestion*: total uptake of energy or mass.
- *Absorption*: part of the ingestion that crosses the gut wall.
- *Defaecation*: part of the ingestion that is not absorbed, but leaves the gut as faeces.
- *Growth*: part of the absorption that is incorporated in the body tissue of the organism.
- *Reproduction*: part of the absorption that is released as reproductive bodies.
- *Excretion*: part of the absorption that is released out of the body in the form of urine, or other exudates (with the exception of reproductive bodies).

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- *Respiration*: Part of the absorption that is released in association with the oxidation of organic compounds, and thus causes a net loss of CO_2.
- *Assimilation*: The IBP definition of assimilation as 'physiologically useful energy' has revealed some confusion. Sometimes the term has been regarded as a synonym to absorption, but it also has been circumscribed as absorption minus excretion. Penry (1998) defines assimilation as an anabolic process, i.e. the incorporation of absorbed products into the tissue of the organism. Absorbed products that are not assimilated are used for catabolic processes. Penry's definition of assimilation is thus equivalent to the IBP definition of growth. We follow that definition in this chapter, but we realise that it is ambiguous, for example with respect to reproductive products that are temporarily stored in a buffer. Only dynamic models can solve the associated timescale problems.

Ratios or 'efficiencies'

Traditionally, several ratios between the fundamental variables defined above are being reported. Sometimes they are easier to observe than the basic variables and are studied for that reason. They are called 'efficiencies'.

Following Penry (1998), three efficiencies related to food uptake can be defined. Digestion efficiency is defined as the fraction of ingested food that is broken down to digestive products during gut passage, whether or not these products are absorbed by the organism. Digestion efficiency is calculated from measurements of the amount of a substance in ingested food S_i, and the undegraded amount of substance in faeces S_f, and equals $1 - S_f/S_i$. Absorption efficiency is sometimes defined as the fraction of the digestive products absorbed, but this is not a practical definition because it is very difficult to measure the rate of digestion. Similarly, assimilation efficiency can be defined as the fraction of absorbed products that is assimilated, but again the practical definition uses food intake as the denominator. Whenever confusion could arise, it is important to state which definition has been used. The practical definitions are thus as follows: absorption efficiency is defined as the fraction of the food taken in that is absorbed S_a/S_i, where S_a is the amount of the substance that is taken up across the gut wall. Assimilation efficiency is defined as the fraction of the food assimilated, i.e. built into tissues S_s/S_i, where S_s is the amount of the substance assimilated. Absorption and digestion efficiencies are equal for compounds that are not changed by digestive processes, e.g. easily absorbable components like glucose, or radioactive or stable isotopes (Penry, 1998). For ill-defined 'food', as is often the case in sediment organic material, the difference between digestion and absorption efficiencies may be caused by egestion of digested molecules, or by bacterial uptake of digestion products in the gut. This difference is often not easy to determine.

Absorption efficiency is usually measured based on the formula of Conover (1966). It makes use of the fact that inorganic matter ingested into the digestive system is egested in unchanged form, whereas part of the organic matter ingested is absorbed and, therefore, not egested. If the organic fraction f_i in the ingested

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material is determined, as well as the organic fraction f_f in the egested material, then absorption efficiency is given by $(f_i - f_f)/(1 - f_f)$.

Energy flux modelling

To establish a link between these energy fluxes, a strategic modelling approach is required. Kooijman (2010) has written the most encompassing textbook on energy budget models. The DEB model of Kooijman (2010), here restricted to the case of isomorphs (animals that do not alter in shape as they grow in size) and ectotherms (animals that do not heat their body to a constant body temperature), first assumes that organisms basically consist of two parts: a structural body and a reserve pool. Hence the model uses two state variables: structural body volume and energy density. Structural body volume is related to any practical measure of body length by means of a cubic relationship. Energy density is defined as the amount of energy reserves per unit of structural volume.

The model assumes that absorbed food enters the reserve pool and that all the energy that subsequently leaves the pool is allocated to either maintenance and growth (where maintenance has priority) or maturity and reproduction. It is further assumed that (i) ingestion is proportional to the surface area of the organism and is related to food density through a Holling type II curve; (ii) a constant fraction of the ingested energy is absorbed and enters the reserve pool; (iii) the utilisation of the reserve density follows a first-order process; (iv) a fixed proportion of the utilised energy (catabolic rate) is spent on growth plus maintenance, the rest being spent on maturity (in case of juveniles) and reproduction (in case of adults) and (v) maintenance costs are proportional to body volume. The model predicts that under constant food conditions growth will follow the Von Bertalanffy growth equation. We refer the reader to the original work by Kooijman (2010) not only but also to a more accessible introduction by van der Meer (2006). Over the last decade an increasing number of DEB models have been constructed for quite a few marine benthic invertebrate species, among others many bivalve and crustacean species (Ren & Ross, 2001; van der Veer *et al*., 2003; Bos *et al*., 2006; Cardoso *et al*., 2006; Kooijman, 2006; Ren & Schiel, 2008; Bourles *et al*., 2009; Campos *et al*., 2009; Freitas *et al*., 2009; Maar *et al*., 2009; Ren, 2009; Freitas *et al*., 2010; Saraiva *et al*., 2011; Zaldivar *et al*., 2011).

8.3 Methods for estimating the energy budget of an individual organism

The following section focuses on the practical aspects (the main objective of this handbook) of measuring the energy budget of an individual organism. However, one should realise that this is only the first step. Modelling the DEB of the individual, which includes both the definition of the model structure and the estimation of

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the model parameters, is the important second step, but will not be discussed further here.

Mass, size, chemical composition and energy content

In studies of energy and mass flows, the state of an individual animal is usually expressed in terms of its body mass or its energy content or, alternatively, in terms of some specific chemical component, e.g. total organic carbon content, total organic nitrogen content, DNA, Adenosine TriPhosphate (ATP), etc. Overviews are provided in Tables 8.1 and 8.2. Brey *et al*. (1988), for example, argue for the use of organic carbon content as a biomass indicator. Biomass can be determined either directly on a balance (or with a device based on a string, which actually measures weight) or indirectly through the measurement of body size, i.e. length or volume. A calibration relationship is subsequently used to transform size in mass.

Table 8.1 Direct determination of biomass and chemical composition of individuals.

Variable	Problems	Recommended procedures
Wet mass	Water content Gut content Hard parts (shells, spines, jaws, bones, etc.)	
Dry mass	As with wet mass, in addition temperature and duration of drying Static electricity for small animals	60°C during 24 h 80°C during 48 h Degaussing of weighing pan
Ash-free dry mass	Temperature and duration of burning in muffle furnace	550°C during 4 h 570–580°C during 2 h
Total carbon and nitrogen		Determined directly in an Element Analyser
Carbohydrates		Total carbohydrates according to Gerchacov and Hatcher (1972). Acid soluble carbohydrates extracted in 0.1 M HCl (2 h at 50°C)
Lipids		Extraction from dried sediments after sonication by direct elution with chloroform and methanol. Analysis according to Bligh and Dyer (1959) and Marsh and Weinstein (1966)
Proteins		Extraction with 0.5 M NaOH during 4 h. Determination according to Hartree (1972) and Rice (1982)
Energy content	Determination of calorific content requires in the order of 5–10 mg dry mass and, therefore, is very difficult for small animals	Determination of calorific content of dried tissue with a micro-bomb calorimeter (Phillipson, 1964). Calorific values can be calculated from CHN analysis (Gnaiger & Bitterlich, 1984), which requires less material

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Table 8.2 Various general conversion factors that have been used to compare carbon content, mass and energy content data of benthic animals. For specific factors see Brey *et al*. (2010) and references therein.

Protozoa		
C/WM	0.165	Jensen, 1984
C/DM	0.580	Jensen, 1984
C/AFDM	0.540	Finlay & Uhlig, 1981
Nematodes		
C/WM	0.124	Jensen, 1984
C/DM	0.400	Feller & Warwick, 1988
Meiofauna		
C/WM	0.116	Debovee & Labat, 1993
C/DM	0.463	Sikora *et al.*, 1977
Macrofauna		
DM/WM	0.234	Brey *et al.*, 2010
AFDM/DM	0.677	
C/DM	0.363	
C/AFDM	0.504	
kJ/gC	42.68	

Particularly when a large number of specimens have to be treated, it is much easier to determine individual biomass indirectly.

If the aim is to characterise the animal by a structural part and a pool of reserves, no simple methods are available and many caveats are to be expected (van der Meer & Piersma, 1994).

Body mass

When dealing with energy budgets, definition and determination of body mass are crucial. An animal's body consists of organic matter (proteins, lipids, carbohydrates), inorganic body parts (carbonates, salts, etc.) and water in organs and cells. Additionally, there is water inside the body (e.g. in the bivalve mantle cavity) or on outer surfaces of the body, and there is organic and inorganic material in the gut. One might argue that body mass should be representative of all energetically relevant body constituents and exclude all others. This is difficult to achieve owing to technical limits and problematic definitions, e.g. a bivalve invests energy to grow its carbonate shell, but this carbonate is of no energetic value for the consumer of this bivalve. The procedures to determine mass seem straightforward, but there are many caveats in the methodologies that should be described in detail in any study. Unfortunately, despite many efforts there are as yet no standardised procedures. See Brey *et al*. (1988, 2010) for an overview of different methodologies used.

Three measures of mass are regularly used in benthic studies, called (i) Wet Mass (WM), (ii) Dry Mass (DM) and (iii) Ash-Free Dry Mass (AFDM). For animals with shells the Shell Mass (SM) is often determined separately from shell-free body mass that usually is denoted as Shell-Free Wet Mass (SFWM) or

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Shell-Free Dry Mass (SFDM). WM is the mass of a specimen, either living or dead, not subjected to drying procedures. Thus WM includes all bodily constituents mentioned above, whereas SFWM is WM after mechanical or chemical removal of the shell. DM is obtained by drying the specimens (usually at 60–80°C) until constant mass (after 24–48 hours). Most of the water will be removed then. In order to determine the AFDM, specimens are ashed in a muffle furnace. The ash mass is then subtracted from the DM in order to obtain the AFDM. Specimens that do not contain carbonate are ashed at temperatures around 550–580°C until constant ash mass, usually for two hours. Specimens that contain carbonate should be cleaned either mechanically, e.g. by separating the shell from the tissue, or chemically, e.g. by boiling the whole animal in 10% by mass of aqueous caustic alkali (see Crisp (1984) for a more detailed description). When the shell can be removed from the soft tissues, AFDM of the shell can be found by decalcifying it in dilute hypochloric acid or by using calcium-chelating agents in mildly acid media. When the separation of carbonate from tissue is deemed impractical (too many samples) or impossible (echinoderms, brachiopods, etc.), specimens must be ashed at a temperature lower than 520°C, as higher temperatures cause oxidation of $CaCO_3$ (Crisp, 1984). Exposure time must be extended accordingly, usually to 24 hours. AFDM still includes organic material present in the gut, but, in general, AFDM is less variable within and between species than WM or DM. However, the major disadvantage of using AFDM is the destruction of the specimens. It is our belief that this should be avoided in most ecological work and that specimens should be conserved whenever possible to allow for later analysis, including molecular analysis (which also has consequences for fixation and preservation, see below). Thus, the use of conversion factors from WM to the desired measure is highly recommended. Such factors can be produced from a subset of the available sample, taken from the literature or extracted from a suitable data bank, e.g. the one provided by Brey *et al*. (2010).

Determination of biomass is most often done on specimens fixed in some preservative. For this purpose, mostly formalin (in seawater buffered with 40 g/litre borax) and ethyl alcohol are used. Formalin (4% solution of formaldehyde in water) is most frequently used but is harmful to human health. When using formalin, good ventilation of the working place is essential. Some substitutes for formalin have been tested, but are rarely used (Brey, 1986). Formalin can result in loss of DM and AFDM, which is due to leaching of organic compounds. Brey (1986) found that during 100 days' preservation of two mollusc species DM remained unchanged but AFDM stabilised at 22–23% of the initial value. Few parallel data are available on meiofauna. Widbom (1984) found that DM of formalin-preserved animals was 44% greater than that of unpreserved animals, and for AFDM the figure was 29%. Jensen (1984) found carbon losses of 8–24% in nematodes preserved in 4% formaldehyde at room temperature, compared to unpreserved animals or specimens kept frozen in formaldehyde. Effects on specific body components, such as low molecular weight compounds, lipids, proteins or carbohydrates, may be much more pronounced (Danovaro *et al.*, 1999; Moens *et al.*, 1999a).

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Body size and size–mass relationships

Determination of length and width are important basic requirements in many methods used in energy budget studies. In hard-bodied species (fish, crustaceans) these linear dimension are easy to measure but in soft-bodied species they have to be determined from drawings or photographs or calculated using Image Analysis software. This requires calibration. In specific cases, derived measures of width can be used when this is more convenient or when this is the tradition for a certain taxonomic group. Examples are measurement of width of a certain segment in polychaetes, the measurement of length or width of jaws, of a certain distance on the carapax of crustaceans, etc. When using such proxies, again, calibration is essential. In meiofauna ecology it has long been a tradition to determine volumes. Volumes are calculated from scale drawings made under the camera lucida or from microphotographs but are now increasingly calculated using Image Analysis software.

For isomorphic animals, which do not change in shape with increasing size, such as nematodes in the meiobenthos, a two-step procedure can be used to predict mass from length and width measurements. First, volume V is predicted from length l and width w by the relationship

$$V = alw^2$$

where a is a dimensionless body shape factor (Warwick & Price, 1979). Feller and Warwick (1988) made clay models of irregularly shaped meiofauna and measured shape factors for 12 groups of meiofauna (Table 8.3). For copepods, the value of the shape factor varied between 0.23 for depressed and 0.75 for more cylindrical species (Fig. 8.1). Note that a perfect cylinder has a shape coefficient equal to $\pi/6$. Knowledge on the specific density can subsequently be used to transform volume into biomass.

Table 8.3 Estimated values of the dimensionless shape factor a from the equation $V = alw^2$, where l is the length, w is the maximum width and V is the volume, for different meiofauna groups (Feller & Warwick, 1988).

Nematodes	0.530
Ostracods	0.450
Halacarids	0.399
Kinorhynchs	0.295
Turbellarians	0.550
Gastrotrichs	0.550
Tardigrades	0.614
Hydroids	0.385
Polychaetes	0.530
Oligochaetes	0.530
Tanaids	0.400
Isopods	0.230

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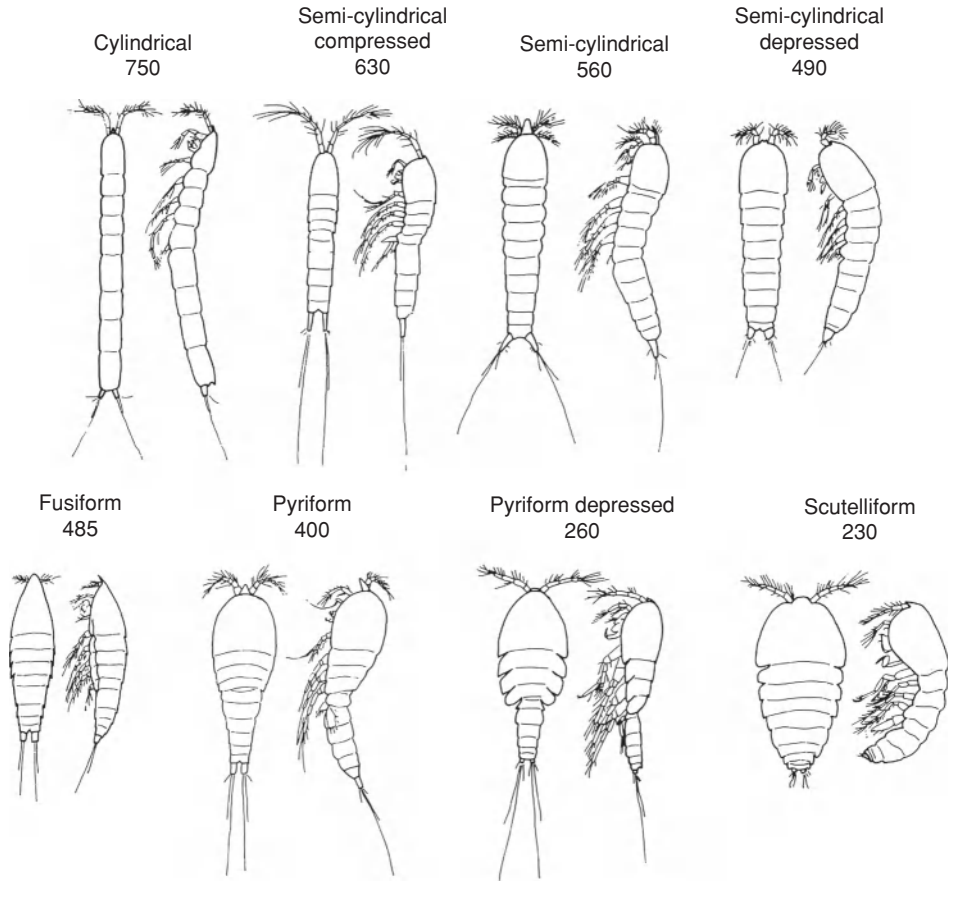

Fig. 8.1 Harpacticoid copepod body forms with the dimensionless conversion factors *a* ($\times$ 1000) from the equation $V = alw^2$, where *V* is body volume, *l* is length and *w* is width. (Reproduced from Warwick and Gee (1984) with permission.)

More often, however, the assumption that animals within a species are of the same shape regardless of size, is not made. Mass is predicted from size *S* (length, width or some other measure) using an empirically derived allometric relationship of the form

$$M = aS^b$$

thus, without the requirement that *b* should be equal to 3 when length is the measure for size. Brey (1999) provided an overview of parameter values that have been used. Usually, the parameters *a* and *b* are estimated from a linear regression of log *M* versus log *S*. However, this procedure reveals biased parameter estimates and we recommend a non-linear weighted least squares procedure using untransformed data (Wetherill, 1986).

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Mass can be directly calculated from volume when the specific density is known. Density has been determined by flotation in different mixtures of kerosene and bromobenzene (Wieser, 1960) or in a gradient column of bromobenzene and xylene (Low & Richards, 1952). For nematodes, for example, the only datum used is 1.13 (Wieser, 1960).

Specific chemical components: organic carbon, organic nitrogen and ATP

The standard method to determine tissue contents of organic carbon *C* and of nitrogen *N* is the elemental analyser. Inorganic carbon is removed by acid leaching before the sample is combusted at temperatures equal or above 1000°C using an oxidation catalyst containing Cr_2O_3 and $AgCo_3O_4$. The combustion products subsequently pass a reduction reactor containing elemental copper at 650°C to transform NO_x compounds to N_2 and to remove excess oxygen. After removal of water, N_2 and CO_2 are separated by gas chromatography and detected by thermal conductivity (Nieuwenhuize *et al*., 1994). The minimum amount of material required for carbon is about 30 μg dry mass. Previously commonly used methods include wet oxidation (*C*) and the Kjeldahl method (*N*). According to Brey *et al*. (2010), mean ± standard deviation *C* and *N* content in the dry mass of benthic animals amount to 0.363 ± 0.114 *C*/*DM* and to 0.078 ± 0.024 *N*/*DM*.

Adenosine triphosphate (ATP) content is a measure of instantly available energy. It can be measured on the basis of the principle that when luciferin reacts with oxygen – a reaction that is driven by ATP – in the presence of the enzyme luciferase, light is emitted. This light emission can be monitored luminometrically. Samples need to be stored frozen at −80°C (−20°C can only be considered for short-term storage). Upon analysis, they should immediately be transferred from the freezer to a boiling water bath for 15 minutes in order to destroy ATPase activity, at the same time effectively releasing ATP. Particulate matter is precipitated by centrifugation, and the supernatant containing the ATP is (sub)sampled. Unless detailed information on expected ATP concentrations is already available, it is usually necessary to analyse an ATP dilution series (Braeckman *et al.*, 2001) as well as several dilutions prepared from the sample supernatant. Therefore, the use of a luminometer equipped with a microplate reader greatly facilitates analysis. To avoid bias from slight short-term variability in reaction kinetics, it is advisable to integrate light emission over a time span rather than to perform point measurements (Braeckman *et al*., 2001). ADP can be completely converted to ATP – and is then measurable as excess ATP – in a coupled reaction catalysed by pyruvate kinase, where phosphoenolpyruvate is converted into pyruvate.

Energy content

The energy content can be determined directly in a bomb calorimeter (Phillipson, 1964; Fraschetti *et al*., 1994), where the heat production of small tissue samples (1 g or less; 5–10 mg in micro-bomb calorimeters) is determined. The literature on

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energy content of macrobenthos was summarised, e.g. by Brey *et al*. (1988) and Beukema (1997). Brey *et al*. (2010) provide a database on conversion factors in aquatic organisms that includes values of energy conversion for several hundred benthic species. Mean conversion factors from mass to energy content in benthic animals are 3.48 kJ g^{-1} WM, 15.80 kJ g^{-1} SFDM and 22.22 kJ g^{-1} AFDM. The average energy content depends on the amount of carbohydrates, proteins and lipids. The energy values of these compounds are 17.55 kJ g^{-1} DM for carbohydrates, 19.17 kJ g^{-1} DM for proteins and 37.54 kJ g^{-1} DM for lipids (Craig *et al*., 1978; Gnaiger & Bitterlich, 1984; Esminger & Esminger, 1995; Schraer & Stoltze, 1999; Sadava & Orians, 2000).

Ingestion, absorption and defaecation

Ingestion, absorption and defaecation will be separately discussed for deposit-feeders, predators and filter-feeders, as each group requires its specific approach. Several of the approaches described for deposit-feeders and predators also apply to meiofaunal-sized animals, yet some specific methods exist and caveats need to be considered when dealing with these small organisms; these are briefly discussed in a separate paragraph.

Ingestion and absorption by deposit-feeders

Deposit-feeders either ingest whole sediment or selectively choose a particular fraction of the sediment (e.g. based on particle size or density), and digest part of the organic matter incorporated into that sediment. The estimation of their ingestion and absorption rates is fraught with a number of difficulties, related to the nature of the substrate. In a sediment matrix, a number of potential food sources are present: benthic microalgae, bacteria, detritus particles, sorbed organic matter, dissolved organic matter, chemoautotrophs that may be associated with the animals, and possibly others. The first difficulty is that animals may selectively digest only part of these food sources, but are forced to ingest them together with the inedible part of the sediment. The second is that any of the potential food sources are poorly specified. Most bacteria in sediments are unknown and not yet cultured and their specific rates of growth, growth efficiency, etc. are unknown; the largest part of the sediment organic matter is unspecified, and methods are lacking to chemically characterise all of these; high-quality dissolved organic matter, for example metabolic intermediates, may have low concentration but high turnover in sediments. When performing feeding experiments on deposit-feeders, we are actually offering a black box of potential food to the animals, without the possibility of controlling exactly what is offered. The final difficulty is that deposit-feeders typically have low absorption efficiency. As a consequence, it is not uncommon that a higher organic concentration is measured in the faeces compared with the ambient sediment. Therefore, direct comparisons of organic content in faeces and sediment are not informative (see Lopez and Levinton (1987) for discussion).

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Several methods have been developed to circumvent these problems. Depending on the aims of the study, they have tried to selectively label and follow the fate of a specific food class (e.g. microphytobenthos) or to label all organic matter as unselectively as possible. In some experiments, artificial 'sediments' have been prepared, with a uniform and well-specified composition, so as to avoid problems associated with selectivity of the animals.

In our discussion, we make a distinction between 'bulk sediment ingestion rate' and 'organic ingestion rate'. The first measure quantifies the intake rate of the organic and inorganic fraction, whereas the second considers only the amount of organic matter taken per unit time. With two different definitions of ingestion rate, the definition of efficiencies is ambiguous. Following most authors active in the field, we define efficiencies relative to the organic ingestion rate, thus neglecting the inorganic material ingested. As it can be assumed that inorganic matter leaves the gut relatively unaltered, variability in the ingestion of inorganic matter would introduce undue variability in the estimated values of all efficiencies where total (bulk) ingestion would be used as a yardstick for efficiencies.

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Bulk sediment ingestion rate

Since, in general, organic matter is only a small fraction of the total sediment volume (order 0.1–10% by dry mass (DM); see Berner, 1982), and absorption efficiencies of animals for this organic matter are low, the mass of the ingested bulk sediment changes little during gut passage. Consequently, the bulk sediment egestion rate (rate of faeces production) can be taken as a measure for bulk sediment ingestion rate (Taghon, 1988). Bulk sediment ingestion rate is then estimated as the total mass of faeces produced per unit of time per animal.

Several methods have been described for the measurement of faeces egestion rate. For species defecating at the sediment surface, simple collection of faeces by pipette is possible (Taghon, 1988; Lopez & Elmgren, 1989; Forbes & Lopez, 1990). Alternatives are direct visual inspection of faeces production rate in short experiments (Mayer *et al.*, 1993), or videotaping of worms over longer time intervals (Taghon & Greene, 1998). For animals defecating below the sediment surface, sieving out faecal pellets may be possible. Wheatcroft *et al*. (1998) used a 180 μm sieve to remove worms and tubes of *Mediomastus ambiseta*, and a 45 μm sieve for the pellets. Faecal pellets of many species are relatively strongly bound, but it is advisable to reintroduce counted pellets in the sediment, and repeat the procedure to check for losses, as was done by these authors. Sieve mesh sizes may have to be adapted for other species. Collection of faeces not only allows the estimation of bulk sediment ingestion rates but also offers the opportunity to study the composition of these faeces.

Cammen (1980) used fluorescent particles experimentally deposited onto the sediment surface to estimate the bulk sediment ingestion rate of a surface deposit-feeder. After a short incubation time, during which the animal ingested sediment

coloured by the particles, the animal was killed and its gut contents dissected. In the gut, a distinction could be made between coloured contents in the anterior part of the gut, and uncoloured contents in the posterior part. This allows a quantification of the ingestion during the period when colour was present on the sediment. This type of experiment can be used only if (i) animals feed at the surface where the coloured particles can easily be applied; (ii) animals do not select entirely against the coloured particles (note that mild negative selection is not a problem); (iii) gut contents are not vigorously mixed, but rather pass the gut on a first in, first out basis. A modification of this method has later been used by Lopez and Elmgren (1989) to determine feeding depth of amphipods.

Organic ingestion rate

Because the major problem for estimating organic ingestion rate from bulk ingestion rate is selectivity of the animals on the particles they take up, a solution may be to offer artificial homogeneous sediment to the animals. As an example, Taghon (1988) used silica sand enriched with baby food (see Tenore (1977) and many later publications – the food value of baby food for worms is better known than that of natural organic matter).

Using radiolabelled food, organic ingestion rate can be estimated in very short incubation experiments. Unlabelled animals are allowed to feed for a short time on labelled food and their gut contents are dissected out or, in meiofauna, whole animals are chemically digested and analysed. Assuming no substantial digestion has taken place during the short incubation, the radioactivity of the gut contents represents a measure for the ingestion rate of the labelled food type. This approach was followed by Forbes and Lopez (1989) to estimate feeding of *Hydrobia* on radiolabelled benthic algae. Organic ingestion rate is calculated as the amount of ^{14}C ingested per unit of time multiplied by the chlorophyll *a* to ^{14}C ratio in the sediment multiplied by the C to chlorophyll *a* ratio in the algae. More elaborated models may be used to model ingestion, absorption and defaecation of radiolabelled food items in animals (Herman & Vranken, 1988; Kofoed *et al.*, 1989). Discrimination of ingestion from absorption is more difficult in meiofauna, firstly, because it is not possible to dissect the gut out of the body, so whole animals have to be analysed; and, secondly, because gut passage times in some meiofauna may be extremely short, at least at high food availability (Moens *et al.*, 1999a).

Food labelling in experiments is increasingly done with stable isotopes. We refer to Section 8.5 for details on the approach, which apart from field studies can also be used in laboratory experiments.

Absorption efficiency

The 'dual labelling technique' with ^{51}Cr and ^{14}C deserves special mention as a methodology to measure absorption efficiency. The method was developed by

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Calow and Fletcher (1972) and adapted for use with deposit-feeders by Lopez and Cheng (1983). Lopez *et al*. (1989) and Charles *et al*. (1995) give critical accounts of the method. The idea is to apply two tracers to the sediment, one of which is not absorbed by the animal and leaves the gut unaltered, while the other can be absorbed with the food. ^{51}Cr is used as the unabsorbed tracer, as biological membranes are nearly impermeable to the trivalent form of chromium and sediment can easily be labelled with chromium, which readily adsorbs to particles. One or more organic fractions of the sediment mix are ^{14}C labelled (e.g. heterotrophic bacteria by offering a labelled substrate, algae by offering light and inorganic ^{14}C, all organic matter by adsorbing ^{14}C formaldehyde to it). When the sediment is taken up, the chromium passes the gut almost unaltered. However, part of the ^{14}C is taken up, and as a consequence, the ratio of ^{14}C to ^{51}Cr is different in ingested and egested sediment. Organic absorption efficiency is estimated in dual labelling experiments by the Conover ratio (see the section entitled 'Ratios or "efficiencies"'), which in this case equals:

$$1 - \left[\left(^{14}\mathrm{C}/^{51}\mathrm{Cr}\right)_{\text{faeces}} / \left(^{14}\mathrm{C}/^{51}\mathrm{Cr}\right)_{\text{food}}\right]$$

Lopez and Crenshaw (1982) and Lopez and Cheng (1983) pioneered the use of ^{14}C-formaldehyde to non-selectively label all organic matter in the sediment. As an alternative to dual labelling, a mass balance approach with a single labelled food source used in pulse-chase experiments may be used. Animals are allowed to feed on labelled food for a period shorter than the gut residence time (but see the section entitled 'Organic ingestion rate' for meiofauna), and then allowed to defecate while being on unlabelled sediment. Faeces are collected quantitatively, and the label activity in faeces and animals at the end of the experiment are determined. This method estimates assimilation, rather than absorption efficiency. Assimilation efficiency is given as $L_a/(L_a + L_f)$, where L_a and L_f are label activity in the animal and faeces, respectively. See Ahrens *et al*. (2001), for an example, and Penry (1998) for a discussion of advantages and disadvantages of the method. If respiration of the absorbed material in the course of the experiment can be neglected, the assimilation efficiency thus determined will approximate absorption efficiency.

Ingestion and absorption by predators

Important aspects for the qualification of a predator–prey relationship are the functional response and the selectivity of the predator. The functional response expresses how the rate of predation (usually expressed as number of prey taken per unit time) varies with the density of prey. Selectivity can be between species, but often is studied between size or age classes of a single species of prey. This aspect is extremely important for the dynamics of the prey species, because many predators select for small sizes and the prey can consequently outgrow predation pressure. However, it is also an important consideration for the study of the predator. Theories

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of optimal foraging (Stephens & Krebs, 1986) try to predict predator behaviour based on optimisation of energy intake, minimisation of risks, optimisation of time use, etc. Many studies of (epi)benthic predators have focused on this aspect (see, e.g. Cote *et al*., 2001; Mascaró & Seed, 2001; Hiddink *et al*., 2002).

Estimation of ingestion rate

Most predators are relatively large, and can be inspected visually. In the laboratory, rates of prey intake may be directly observable in relatively short experiments, for instance, experiments on predation by starfish on mussels by Sommer *et al*. (1999), or experiments in which predators are encaged in the lab or the field together with a known number of prey (Moens *et al*., 2000; Dahlhoff *et al*., 2001). Note that there may be a difference between the biomass of prey killed by the predator and biomass actually ingested, e.g. due to kleptoparasites (Morissette & Himmelman, 2000). In some cases – for instance predation of benthic metazoans on ciliates – prey can actively reproduce during the time frame of even short experimental incubations. Hence, proper controls are required to allow assessment of prey population growth in the absence of predators, and accurate calculation of predation rate implies that a prey population increase model is incorporated (Frost, 1972). Vice versa, significant decreases of prey abundance during experimental incubation may result in biased estimates of predation rates at the initial prey abundance (Moens *et al*., 2000).

Ejdung *et al*. (2000) labelled prey species radioactively, and compared the label activity in the predator with the numbers of prey that had disappeared from the experiments, to show that the predator not only killed, but also actually ate the prey. Also potentially relevant for benthic predators is the use of natural radiotracers (in particular ^{137}Cs) to estimate ingestion rates (Forseth *et al*., 1992; Rowan & Rasmussen, 1994; Gingras & Boisclair, 2000).

Gut content analysis can be used both in controlled experiments and as a way to estimate the intake rate of predators caught in the field. Animals are quickly killed, but taking care that emptying of the gut just before death is avoided. In such cases, the use of chemical preservatives such as formalin at ambient temperature should be avoided, because it induces rapid regurgitation and defaecation (Moens *et al.*, 1999a). For experiments with meiofauna, the sudden cooling of the animals often largely inactivates the predators and prevents them from emptying their guts (Moens *et al.*, 1999a). Alternatively, chemical preservation at high temperatures ($\geq$70°C) can also instantaneously kill the fauna. Macrofaunal predators are subsequently dissected, and hard remains of prey in the digestive tract are enumerated. Estimates of the predator's gut turnover time are needed in order to calculate the effect of the predator on the prey species of interest. Hiddink *et al.* (2002) used this approach for infaunal predators. If emphasis is placed on the intake rate of the predator itself, a condition for the use of this methodology is that most prey leave measurable and identifiable remains in the predator's digestive tract, which may be difficult to prove. Gut content analysis is an important methodology in fisheries

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research. Cortés (1996) reviewed the method, providing many references to the basic methods. Many of these may also find application in studies of (epi)benthic predators (Cartes & Maynou, 1998). Gut content analysis on meiobenthos may be difficult and tedious, and is rarely used for quantification of food ingestion (but see further). When applied, it is generally performed on intact animals using light microscopy.

Clearance and pumping rates of filter-feeders

Pumping rate of filter-feeders is defined as the volume of water pumped over the filter per unit of time. Sometimes it is used as a synonym of filtration rate, but the latter term is also used to indicate the mass of solids filtered per unit of time, and we will use it in that sense throughout this text. Operationally, it is usually easier to measure clearance rate, i.e. the volume of water cleared of suspended particles per unit of time. Clearance rate *Cl* is defined as the product of pumping rate and filtering efficiency. It is equal to pumping rate when the filtering efficiency (the fraction of suspended particles in the water retained by the filter) is 1 (100%). It is lower than pumping rate when filtering efficiency is lower than one.

After a period of confusion and discussion in the literature, Riisgård (2001) made a critical review of laboratory methods for the measurement of 'filtration rates' (which, according to our use of the term, is actually pumping rates) in bivalves, and this account follows his description of methods. Measurement of pumping rates in other invertebrates may require special technical adaptations, but fall within the same general categories as discussed here (Riisgård & Larsen, 1995; Riisgård & Larsen, 2000). The critical evaluation of methods and results by Riisgård (2001) gave rise to some discussion itself, which is understandable in the light of the severe controversy in the literature on fundamental aspects of filter-feeder physiology (and, as a corollary, on the validity of experimental methods), expressed by the papers of Jørgensen (1996) and Bayne (1998). The intercalibration exercise by Petersen (2004) has cleared up many misunderstandings, and led to agreement on fundamentals, if not on details (Bayne, 2004; Petersen, 2004; Riisgård, 2004). It constitutes a turning point in the research methodology. However, readers should be aware of the discussions if they want to interpret correctly the older literature values on these important parameters.

Riisgård (2001) attempted to define minimum requirements for the quality of measurements. In particular, he proposed that any method employed to study physiological regulation of pumping rate should always be employed under optimal conditions (where food is a silt-free culture of suitable algal species, with a concentration between lower and upper thresholds where valve opening is reduced, and animals are well acclimated to the laboratory conditions) and that these measurements should be used as a methodological check: they should yield pumping rates in the range of published studies summarised in Riisgård (2001). The latter check seems overly restrictive, as it would automatically reject measurements on

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populations that for one reason or another would have lower pumping rates. However, it remains a useful suggestion to perform and report a measurement under optimal conditions (if possible on a well-studied species such as *Mytilus edulis*) as part of all measurement programmes. The following classification of methods was proposed by Riisgård (2001).

Direct measurement of pumping rate

In these methods, the exhaled water is physically separated from the surrounding water. Separation is possible, using, for example, a rubber apron. The method dates back to the beginning of the twentieth century; older literature is reviewed by Jørgensen (1966). A more recent implementation by Famme *et al*. (1986) has demonstrated its extreme sensitivity to the build-up of backpressure on the pumping system. Reliable estimates of 'natural' pumping rates can be obtained only when this backpressure is carefully avoided. Direct measurements seem most useful to study pumping under experimentally manipulated backpressures, allowing the physical characteristics of the animal pump to be defined.

Interestingly, a variation on this approach has been applied to estimate ingestion rate in rhabditid nematodes. These animals have a double valve in the pharyngeal metacorpus, and the pulsations of this valve apparatus can be readily observed at low magnification when nematodes are foraging. The volume of 'medium' ingested per pulsation (estimated from the volume dilation of the pharyngeal metacorpus) multiplied by the frequency of pulsation and by the number of food particles per volume of 'medium' yields a reasonable approximation of the amount of food particles ingested per unit time (Woombs & Laybournparry, 1984; Moens *et al*., 1996).

Flow-through chamber method

Animals are placed in a chamber, a suspension of constant and pre-defined composition is pumped through this chamber, and concentrations of suspended matter are measured at the inflow and outflow of the chamber. The main advantage of the method is that the composition of the feeding suspension can be kept constant during the experiment. The basic form of the experimental set-up assumes that refiltration by the animal of previously filtered water is excluded. This requires a high flow throughput through the experimental chamber, which moreover should have a proper geometry (Riisgård, 1977). Under these conditions the clearance rate is given by

$$Cl = Q\left(1 - C_2/C_1\right)$$

where Q is the flow rate through the chamber and C_1 and C_2 are the concentrations of inflow and outflow, respectively. The derivation of this equation is as follows: the mass flux flowing into the grazing chamber per unit time is given as QC_1, the mass

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flux flowing out is QC_2. The animals take out the difference, i.e. $Q(C_1 - C_2)$. As the animals filter water with a concentration C_1, the volume swept clear is the division of the mass flux by the concentration. It is obvious from the equation that clearance rate calculated according to this formula cannot be larger than the flow rate Q through the chamber. In fact, the validity of the equation above, and in particular the assumption of no refiltration, can only be guaranteed if $Q \gg C_1$. A compromise must be sought, since the precision of the measurement of C_2/C_1 decreases with Q (smaller concentration differences are more difficult to measure). As a methodological check, it may be useful to plot apparent clearance rate calculated according to the equation versus Q (Riisgård, 2001). Hildreth and Crisp (1976) used a modified version of the equation that can, under certain conditions, overcome this difficulty. If (and only if) the water in the grazing chamber is perfectly mixed, clearance rate may be estimated from

$$Cl = Q\left(C_1/C_2 - 1\right)$$

which is easily derived by assuming perfect mixing of the water in the grazing chamber, so that the animals filter water with a concentration equal to the outflowing concentration C_2.

Filgueira *et al*. (2006) give an account of the different methodological checks for flow-through methods, needed to validate the methodology for routine measurements. Flow-through methods have also been used on groups of filter-feeders in raceway tracks. This does not necessarily give the same results as measurements on individuals, because a stronger depleted benthic boundary layer can develop around the groups of animals. It may better approximate clearance in field situations. An example using flow cytometry to measure incoming and outgoing seston is given by Li *et al*. (2009).

Suction method

In this method, described by Møhlenberg and Riisgård (1979) and used by Kiørboe and Møhlenberg (1981), Famme *et al*. (1986) and Kryger and Riisgård (1988), samples of inhaled and exhaled water are sucked through glass pipes placed a few mm above inhalant and exhalant openings of the filtering animal (Fig. 8.2). The flow rate through the tubes is adjustable, and the clearance rate is calculated with the second equation of the previous paragraph, where C_2 is the concentration in the water collected from the exhalant current, whereas C_1 is the concentration in the inhalant flow. Just as in the flow chamber method using this equation, suction flow must be larger than clearance rate for the method to work, and this should be checked by plotting apparent clearance rate versus suction flow rate. An advantage of the suction method is that it can be applied to animals in a natural position (e.g. buried in sediment) and even, with proper adjustment of the methods, in the field. A recent implementation of the method is the 'InEx' method of Yahel *et al*. (2005).

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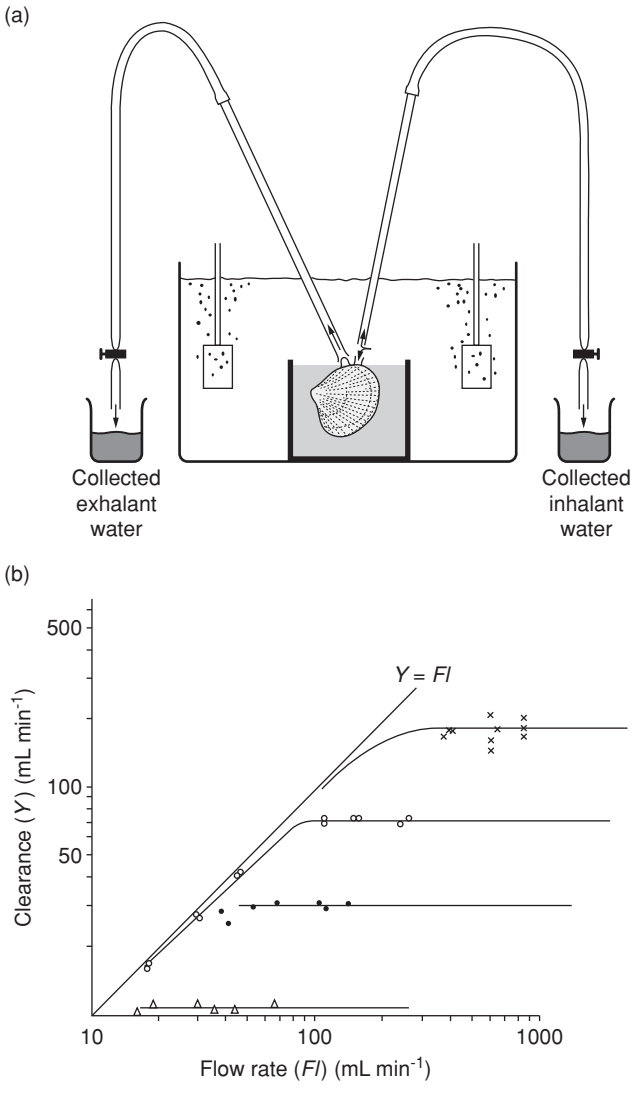

Fig. 8.2 Illustration of the application of the suction method for the measurement of clearance rate. (a) Set-up used for measurement of filtration rates in suspension-feeding bivalves. The glass tubes collect water from the inhalant and exhalant siphons by means of gravity. (b) Clearance rate, estimated as a function of suction flow rate. Real clearance rates are reliably estimated at the plateau where they become independent of suction flow rate. The example shows measurements on *Modiolus modiolus* (four individuals of differing size). (Reproduced from Riisgård (2001).)

Clearance method

This is a basic method, where animals are placed in a container with a food suspension, and the decrease of food concentration is monitored over time. Thorough mixing of the water in the container is needed to avoid the build-up of concentration gradients around the animals and, thus partial refiltration. A disadvantage of the method

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is that food concentration decreases during the experiment, but this can be overcome by periodically adding new food supply to the container. The food concentration in the container, at constant clearance rate, follows an exponential decline

$$C_t = C_0 \exp\left(-\frac{Cl}{V}t\right)$$

where V is the volume of the container and C_0 is the concentration at time 0. It is customary to take several measurements of concentration over time, and estimate clearance as V times the slope of the regression of log(concentration) versus time.

The use of flow cytometry to determine both quantity and quality of the particles in suspension (Atkinson *et al*., 2011) extends this method with the possibility of studying particle selectivity. It determines a clearance rate for different types of particles during one experimental run. Another application of flow cytometry emphasised the measurement of individual condition of mussels (Duchemin *et al.*, 2008).

Controlled addition methods

In these methods, controlled additions of food suspension to a (well-mixed) grazing container maintain a constant concentration of food around the animals. Additions of food are measured and clearance rates calculated from these. Winter (1973) and Riisgård and Mohlenberg (1979) used light-based systems to maintain constant concentration of food in an aquarium. When the concentration fell below a set minimum, food was added from a stock solution. The number of food additions was recorded, and clearance calculated from this number. In a slightly different design, Riisgård and Randløv (1981) and Poulsen *et al*. (1982) used a chemostat algal culture to provide a continuous addition of food in a long-term (45 days) study of mussel filtration. Clearance rate was calculated from the steady-state concentration of algae in the mussel aquarium and the concentration in the water flowing from the algal chemostat.

Measurement of exhalant current velocity

Current velocity in the exhalant stream may be measured with thermistor probes (Vogel (1994), and references therein). If properly calibrated in a flow from an aperture with the correct geometry, continuous measurements at a single spot within the exhalant current can be sufficient to calculate the volume flux from the exhalant opening. As an alternative, Jones *et al*. (1992) used a small impeller to measure the exhalant current velocity. Troost *et al*. (2009) used particle image velocimetry to estimate inhalant and exhalant currents very precisely in oysters, mussels and cockles. This method is suited for qualitative considerations, for example on the filtration of larger particles or larviphagy, but is not suited to routinely estimate clearance rates.

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Observations of valve opening or exhalant siphon area

Video-based methods recording the opening state of the valves or the surface area of the exhalant siphon have been used in field and laboratory set-ups as an approximation of clearance rate (Macdonald & Nodwell, 2003; Saurel *et al*., 2007). Maire *et al*. (2007) describe an automated image analysis system for the approach, and argue that it gives reliable estimates. Macdonald *et al*. (2009) evaluate their method against classic clearance rate measurements, showing that the method gives an approximation at best.

Biodeposit method

In this method, faeces and pseudofaeces of the filter-feeders are collected, and the proportion of inorganic and organic material in the food suspension and the biodeposits is determined (see, e.g. Hawkins *et al*., 1996; Cranford *et al*., 1998). Clearance rate is estimated as

$$Cl = \frac{f_b P_b}{f_i C_{\mathrm{TPM}}}$$

where f_b and f_i are the fractions of inorganic matter in the biodeposits and the food suspension, respectively, P_b is the production rate (mass time^{-1}) of biodeposits and C_{TPM} is the concentration (mass volume^{-1}) of total particulate matter in the food suspension. Riisgård (2001) suggests that a slightly modified version of this equation, as used by Cranford and Hargrave (1994), does not take into account pseudofaeces production, but this assertion is in error. He correctly points out that, as for the other methods, the clearance rate determined by the biodeposit method is equal to the pumping rate only if all the particulate matter is filtered with 100% efficiency. Since the food suspension used in this method is usually naturally occurring suspended matter, some particles may be smaller than the critical size for efficient particle retention. This can lead to estimates of clearance rates lower than pumping rates based on retention of larger and efficiently retained algal suspensions. In the intercalibration exercise by Petersen *et al.* (2004), this method was shown to be very sensitive and easily biased, although this conclusion was doubted by Bayne (2004).

Video observation method

Several authors have made use of video imaging of particles approaching the gills. From the approach velocity and the surface of the gills, an estimate of pumping may be obtained. As with all aspects of filter-feeding physiology, this methodology has also been a matter for debate (Beninger, 2000; Riisgård & Larsen, 2000; Silverman *et al.*, 2000; Ward *et al*., 2000). The utility of video observation is primarily in detecting particle selection mechanisms as well as basic characteristics of the filter, and its use in estimating pumping rates as part of a study of the bioenergetics of the

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Table 8.4 Rates and ratios relevant for the study of filter-feeder physiology.

Feeding parameter	Symbol	Units	Calculation
Clearance rate	Cl	Volume time^{-1}	$Cl = \frac{f_p P_p + f_f P_f}{f_i C_{TPM}}$
Filtration rate	Fi	Mass time^{-1}	$Fi = Cl\ C_{TPM} = \frac{f_p P_p + f_f P_f}{f_i}$
Ingestion rate	I	Mass time^{-1}	$I = Fi - P_p$
Net organic ingestion rate	I_{no}	Mass time^{-1}	$I_{no} = Fi\,(1 - f_i) - P_p\,(1 - f_p)$
Net organic absorption rate	A_{no}	Mass time^{-1}	$A_{no} = I_{no} - P_f\,(1 - f_f)$
Net organic selection efficiency		–	$1 - (1 - f_p)/(1 - f_i)$
Net absorption efficiency from ingested organics		–	A_{no}/I_{no}
Organic content of ingested matter		–	$I_{no}/(Fi - P_p)$

Note that the net organic ingestion rate and the net organic selection efficiency are influenced by loss of mucus in pseudofaeces; the net organic absorption rate and the net absorption efficiency from ingested organics are influenced by loss of organics in pseudofaeces and by metabolic faecal losses (Hawkins *et al.*, 1996).

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animal is limited. The reader is referred to the references cited above as a useful introduction to the uses, advantages and disadvantages of this type of studies.

Absorption efficiencies

Collection of pseudofaeces and faeces, and determination of the organic and inorganic content both in these and in the food suspension, allows calculation of a number of rates and ratios relevant for the study of filter-feeder physiology (Table 8.4). The table is slightly modified from Hawkins *et al*. (1998) where references can be found to many studies using this approach. Basic methods are complicated due to pseudofaeces production. The fractions of organic and inorganic matter in the total DM of food suspension, faeces and pseudofaeces, which are relatively easy to determine, are used as the basis for the calculations. The equations are generally applicable; in the absence of pseudofaeces production, its rate can be set equal to zero.

The following basic observations can be made:

P_p	Rejection rate is the production rate of pseudofaeces (mass time^{-1})
P_f	Faeces production rate (mass time^{-1})
f_i	Fraction of inorganic material in the food suspension (–)
f_p	Fraction of inorganic material in pseudofaeces (–)
f_f	Fraction of inorganic material in true faeces (–).

The rejection and faeces production rates may be estimated by collecting (separately) the pseudofaeces and faeces of the filter-feeding animal. Organic and inorganic fractions may be determined from CHN analyses or from mass loss on ignition.

Ingestion and absorption in meiofauna

Feeding of meiobenthic predators, such as certain nematode and many turbellarian species, may be quantified in much the same ways as described for macrobenthos, albeit special attention needs to be paid to incubation time and substratum. However, most meiobenthic species are probably bacterivores and/or herbivores, feeding more selectively than deposit-feeders on bacteria and unicellular protists such as diatoms. Many approaches use radioactive or, increasingly, stable isotope tracers to measure food ingestion in ways comparable to those described under 'organic matter ingestion', however, usually with a specific labelling of certain food items (bacteria, diatoms) rather than of bulk sediment organic matter. Food particles may be pre-labelled and then added to the experimental containers, or labels may be added directly to the containers, thus becoming incorporated by the target food organisms during incubation. The latter approach in particular often requires an extensive set of controls to account for label uptake by meiofauna through ways other than grazing on the target food (Montagna, 1983; Montagna & Bauer, 1988).

In addition, gut content analysis is sometimes used to qualify, rather than quantify, food sources of meiobenthic animals. Caution is necessary when interpreting such observations, because, for instance, some gut contents of predacious meiobenthos may at least in part be derived from the gut contents of their prey (Moens *et al*., 1999b). One specific approach on gut content analysis does quantify food uptake in meiofauna: High-Performance Liquid Chromatography (HPLC) to quantify grazing on microalgae by harpacticoid copepods (Santos *et al*., 1995; Souza-Santos *et al*., 1995; Buffan-Dubau *et al*., 1996; Buffan-Dubau & Carman, 2000) and nematodes (Majdi *et al.*, submitted). The sample size needed for reproducible measurements mainly depends on the targeted pigment(s) and the biomass of the grazers. As few as 10 harpacticoid copepods may be sufficient for an analysis of chlorophyll *a* (Souza-Santos *et al*., 1995), but many hundreds may be required to detect other pigments in meiofaunal guts (Buffan-Dubau & Carman, 2000) or when working with small (for instance deep-sea) species.

Any attempt at quantifying food ingestion and absorption in meiofauna *ex situ* requires careful consideration of substratum choice and incubation conditions. The patchy distribution of food items and their association with sand grains can be highly relevant in determining feeding rates (Moens & Vincx, 1997), but are very hard to mimic adequately in the laboratory. Even subtle changes in sediment grain size, water content, temperature, light, etc. may profoundly impact feeding rates of meiofauna (Buffan-Dubau & Carman, 2000; Moens *et al*., 2000; Gallucci *et al.*, 2005; De Troch *et al.*, 2006).

Excretion

Urine products

In the deamination step in the catabolisation of proteins, the highly toxic compound ammonium is released into the cells of an animal. Some is transformed into

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other compounds that are less toxic (such as urea) before it is released into the environment. The release of ammonium and its derivatives requires an effective nitrogen drain. The excretory products have only marginal energy content (Elliot & Davison, 1975). Although of minor importance in terms of energy, energy budgets are traditionally approached using mass balances, in which the products can be significant. Moreover, excretion is studied because the nitrogen compounds can stimulate primary production of N-limited phytoplankton in adjacent waters.

Experimental designs

Excretion processes are measured by monitoring the increase in excretory products in the overlying water of different experimental settings. Closed incubations are the most frequently used setting to measure excretion processes. Animals are placed in a closed system (flask, core, chamber or aquarium) with filtered seawater; rotors circulate the water inside the system. Sterilised sand or glass beads or glass tubes might be used to mimic a more natural environment for polychaetes. During the incubation the increase of excretory products is monitored in the overlying water. The production rate can be estimated by the slope of the linear increase in concentration of the excretory product. The production rate is multiplied by the volume of the core (see Fig. 2A in Glud (2008) for application of oxygen production) and divided by the desired unit, such as animal mass. Descriptions of experimental settings are given in Migne and Davoult (1997), Hatcher (1994) and Smaal and Vonck (1997). This method is also valid for estimating N production of the whole sediment as benthic chambers or using intact sediment cores (Chapelle *et al*., 2000).

In a flow-through system, the overlying water is continuously refreshed and excretion rates are calculated based on the difference in concentration of excretory products between the outflow and inflow, and are corrected for flow rate and sediment surface (Chapelle *et al*., 2000; Lavrentyev *et al*., 2000). Prior to laboratory incubations, it is useful to allow gut clearance to prevent any leakage of products from ingested food or faeces to prevent over-estimation of true excretion rates (Hatcher, 1994).

It is a common phenomenon that temperature and nutritional condition influence the excretion of ammonium (see Clarke & Prothero-Thomas (1997) and references therein). For example, ammonium excretion in two benthic cnidarians was found to decrease by 51% after seven days of starvation and temperature accounted for 44% of the observed variation in the seasonal trend (Migne & Davoult, 1997). Peak excretion rates of mussels (Smaal & Vonck, 1997) and *Monoporeia*/*Pontoporeia* spp. (Lehtonen, 1995) coincided with blooms of phytoplankton. Brockington and Clarke (2001) estimated that 15–20% of the summer increase in metabolism of polar sea urchins was due to temperature increase, and 80–85% was caused by increased physiological activity. Estimating 'field' excretion rates of collected organisms in laboratory or mesocosm environments thus necessitates short handling and acclimatisation times and maintenance of temperature at ambient level.

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Methods to measure excretion products

Possible excretion products comprise ammonium, nitrate/nitrite, amino acids and urea. Most benthic invertebrates are ammonotelic, which means that ammonium dominates the excretory products. Therefore, in the majority of studies only ammonium excretion is monitored, and only some studies additionally measure other end products. What follows is a short overview of the analytical procedures to determine concentration of the excretion products with relevant references.

Ammonium is generally measured with colorimetric method based on Solórzano (1969) and is explained in most handbooks on seawater analysis (Strickland & Parsons, 1972; Grasshoff *et al.*, 1983; Crompton, 1989). The method is based on the reaction of ammonia with hypochlorite that, with some other additives, gives a blue colour and the intensity of the colour is measured at 625 nm. The precision at which ammonium can be measured is about 0.1 μg $N\text{-}NH_4^+$ dm^{-3} (Strickland & Parsons, 1972). It is most easily applied in an autoanalyser, or flow-segmented analyser. This set-up greatly increases the reproducibility.

Nitrate and nitrite are measured by colorimetric analysis, and the analytical procedure can again be found in all standard handbooks (Strickland & Parsons, 1972; Grasshoff *et al*., 1983; Crompton, 1989). The method can be handled manually, but an autoanalyser greatly improves reproducibility of the method. The detection of nitrite and nitrate is below 1 μmol N dm^{-3} (Crompton, 1989).

Basically three different amino acid fractions can be distinguished: (i) Free Amino Acids (FAA), (ii) Dissolved Combined Amino Acids (DCAA) and (iii) Particulate Amino Acids (PAA). In fact, only FAA can be measured directly and the latter two need hydrolysation prior to determination of the concentration (Cowie & Hedges, 1992). Cowie and Hedges (1992) also provide an accurate description of measuring amino acids using HPLC, as first described by Lindroth and Mopper (1979). The HPLC method has been successfully applied to measure the uptake of dissolved amino acids from natural seawater by the mussel *M. edulis* (Manahan *et al*., 1982, 1983).

Urea can be measured by (i) enzymatic (with urease as enzyme) hydrolysation to ammonium at elevated temperatures or (ii) by chemically complexing the urea. In the first method, the ureum is hydrolysed and the concentration of ammonium is continuously monitored during the incubation. Strickland and Parsons (1972) describe the method and note that the detection limit is about 0.05 μg N dm^{-3}. The latter method is described in Grasshoff *et al.* (1983) and they report that a detection limit is at 1.4 μg N dm^{-3} with a relative standard deviation of approximately 15% just above the detection limit and 4.5% at higher levels.

Mucus

The production of organic mucus is related to multiple physiological processes ranging from feeding and production of pseudofaeces in bivalves, locomotion and reproduction of molluscs to drifting capability of larvae (Davies & Hawkins, 1998).

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The comprehensive review by Davies and Hawkins (1998) provides references on different aspects of mucus production in molluscs. Here we will describe only the main methods to measure mucus production in the context of energy budgets.

Gastropods produce mucus on the pedal foot for multiple reasons such as locomotion, attachment, navigation and possibly as food trap (Davies & Hawkins, 1998; Davies & Beckwith, 1999). Some papers report it as being a dominant component of the energy budget. Two methods are described in the literature: either mucus is collected from the pedal foot or from an artificial substrate on which the gastropod has roamed around. Of course, a combination of the two is possible. Peck *et al.* (1993) estimate mucus production by scraping deposited mucus from a glass plate with a razor blade. Subsequent analysis included C, H, N, protein, lipid, carbohydrate and energy content of mucus. Mucus is collected from the foot by using the rounded end of a pair of forceps (Davies & Williams, 1995) or glass rod (Horn, 1986). Based on the difference between a 30-minute and 24-hour 'walk' of limpets on a glass plate, Peck *et al*. (1993) concluded that reattachment (i.e. the short walk) to the glass plate constituted ~80% of the daily mucus production. This implies that experiments where mucus was scraped from the pedal feet possibly over-estimate field rates of mucus excretion.

Bivalve mucus production is primarily related to pre-ingestive selection processes and the production of pseudofaeces (Davies & Hawkins, 1998). For example, Beninger and St-Jean (1997) use sophisticated techniques to determine the role of mucus in feeding processes and the production location. Quantification of mucus production has proved to be difficult and no single satisfactory method has emerged (see examples in Davies & Hawkins, 1998). Yet, Urrutia *et al*. (2001) propose a quantitative method to estimate mucus that is voided with pseudofaeces.

We found no quantitative assessments of mucus excretion by polychaetes in the marine literature. A paper in the field of soil biology describes the quantification of mucus deposition by earthworms (Scheu, 1991). ^{14}C-labelled earthworms were allowed to burrow in an unlabelled environment; the ^{14}C signal in the burrow wall was taken as a measure for mucus excretion by the body surface and the ^{14}C faeces signal as mucus excretion by the intestines. Another paper (Schmidt *et al.*, 1999) describes a method to qualitatively collect mucus from earthworms for stable isotope analyses by placing them briefly in a slightly acidified bath. These methods might be starting points for mucus production measurements on polychaetes. Some suspension-feeding polychaetes spin a mucus net to capture food particles, and subsequently swallow the enriched mucus net (Harley, 1950; Riisgård, 1991; Riisgård & Larsen, 1995). In the literature, the focus is only on costs of pumping and on the structure of the mucus net (see Riisgård and Larsen (1995) for references).

Copious mucus production can also be observed in nematodes, and may be implemented in locomotion, feeding and different forms of sediment aggregation (Riemann & Schrage, 1978; Nehring *et al*., 1990; Moens *et al*., 2005). To our

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knowledge, no attempts have been made to quantify and include mucus production in meiofaunal energy budgets.

Respiration

Adenosine triphosphate (ATP) is the energy currency of the cell that supports the cell with energy for all sorts of processes and its production is a continuous process.

Generation and hydrolysis of ATP result in loss of energy in the form of heat. Heat production measurements seem a logical step in respiration measurements, as they simultaneously measure aerobic and anaerobic processes. Microcalorimetry is based on heat production, but has not found many successful applications in research on benthic animals due to methodological difficulties (but see Pamatmat, 1978, 1983; Shick *et al*., 1983). Other methods for measuring respiration focus on the consumption of oxygen or the formation of carbon dioxide as an end product of aerobic respiration. Thus, in a majority of studies, respiration is considered from the mass balance viewpoint, rather than from that of the energy budget. Because hypoxic, dysoxic or anoxic conditions are common, if not prevalent, in many marine benthic environments, respiration estimates based on measurements of oxygen consumption under fully aerobic conditions may not provide an adequate assessment of *in situ* activity.

Oxygen consumption

Measuring decreases in oxygen concentration or oxygen partial pressure in a closed or (semi-)open system inhabited by organisms is a widespread approach to assess aerobic respiration under both laboratory and field conditions; various methods are currently available: the Winkler method, Cartesian divers, electrodes and optodes.

Winkler method

The classical Winkler titration, dating back to 1888, is still one of the most accurate techniques for measuring oxygen concentration in water. It is based on the precipitation of oxygen with manganese as manganese oxide and the subsequent oxidation of iodine to iodide ions in a strongly acidic environment. Based on the titration of the iodide ions formed and on stoichiometry, one can calculate the original oxygen concentration (see, e.g. Carpenter (1965) or Strickland & Parsons (1972) for a full methodological description). There is extensive literature available on the automation of the Winkler method (Anderson *et al*. (1992); Pomeroy *et al*. (1994), and references therein). Despite this, it remains rather a labour-intensive technique, however, providing very accurate data in the micromolar range. At concentrations below 3 μM, the analytical precision may be somewhat less (Strickland & Parsons, 1972).

Cartesian diver

The principle of the Cartesian diver was developed into a very sensitive method for use in cell biology and the physiology of small organisms by the Danes

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Linderstrøm-Lang, Holter and Zeuthen (Lasserre, 1976). The basic set-up consists of a sealed vessel partially filled with liquid and a small free-floating diver. The diver consists of a glass capillary (0.16–0.5 mm diameter) with a reservoir at one end, into which a sample of living material can be introduced, and a head space above. When the diver's compound density equals that of the surrounding medium, it floats. Oxygen consumption inside the diver reduces its floatation capacity because the respired CO_2 is being absorbed by NaOH in the diver seal. Consequently, the pressure above the medium must be lowered for the diver to keep floating, and the required pressure change is directly proportional to the amount of oxygen consumed. Alternatively, gradient diver approaches (Hamburger, 1981) do not keep the diver in a fixed position, but introduce it into an aqueous density gradient and measure its migration in that gradient. Good reviews of diver methodology are available (Holter & Zeuthen, 1966; Klekowski, 1971; Lasserre, 1976). The main merit of the diver methodology is its unsurpassed sensitivity, oxygen consumption values down to 0.01 nM h^{-1} still being accurately measurable. However, it should be stressed that the Cartesian diver is a very labour-intensive, tedious and delicate methodology, requiring substantial experience before reliable measurements can be obtained. In the past three decades, the method has been used only exceptionally in studies on marine benthos.

Electrodes

Polarographic electrodes as originally described by Clark (1956) have a lower sensitivity than divers (approximately 3–6 nM h^{-1}; Holter & Zeuthen, 1966), yet are more suitable for routine use when sufficient biomass (ten to thousands of meiobenthos specimens and one to a few macrobenthos specimens, depending on size and metabolic activity) is available. Polarographic oxygen electrodes allow continuous monitoring of oxygen tension in a solution, enabling the integration of respiration over different intervals of time or oxygen pressure. One should be aware that electrodes have their own, background, oxygen consumption. Miniaturised Clark-type oxygen electrodes have been used in many different designs, including closed (Riisgård, 1989) as well as flow-through systems (Riisgård & Ivarsson, 1990). The performance and reliability of these systems, especially at low oxygen consumption rates, thus depend on a number of factors, including sensor characteristics, materials constituting the respiration chamber and diffusion in and out of the system via the titration cannula. An in-depth discussion of these factors is given in Haller *et al*. (1994).

Several commercially available devices allow parallel, simultaneous measurement of oxygen consumption of many samples, and easy, computer-assisted data processing. Some systems combine polarographic oxygen sensors with pCO_2 and/or pH electrodes, thus enabling direct assessment of the respiratory quotient. A further advantage of polarographic electrode measurements is that, provided oxygen consumption rates are sufficiently high, short incubation times (less than 30 minutes) yield good results.

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Optodes

The use of optodes or optrodes as a tool to measure oxygen concentration was introduced in aquatic ecology by Klimant *et al*. (1995). The measuring principle of the O_2 optode is based on the ability of oxygen to act as a dynamic fluorescence quencher that decreases the fluorescence quantum yield of an immobilised fluorophore, often a metalloporphyrin complex (Kautsky, 1939; Kohls & Scheper, 2000), which is most often coated onto a fibre optic device. Contrary to microelectrodes, they are easy to manufacture, insensitive to stirring, do not consume oxygen and show fairly long-term stability (Klimant *et al.*, 1995). Optodes can be introduced into a variety of closed and flow-through (Sanchez-Pedreno *et al*., 2000) incubation chambers or even in microtitre plate-format (Kim *et al.*, 1998). Most applications imply the introduction of a fibre into a closed chamber. Recently, however, non-invasive alternatives have been proposed where the oxygen sensor spot is not coated onto a fibre that then needs to be introduced in the measurement chamber, but onto the inside of a glass respiration chamber, thus allowing measurements to be conducted from the outside through the chamber wall (Warkentin *et al*., 2007; Moodley *et al*., 2008).

Optodes are suited for experimentation under conditions in which conventional chemical analysis or use of polarographic electrodes is difficult, e.g. at high or variable pressure (Stokes & Somero, 1999) or at very low temperatures (Gatti *et al*., 2002). They have an acceptably rapid response time (in the order of seconds to minutes). Depending on the type of matrix in which the fluorophore is immobilised, autoclavation of optodes may be possible (Klimant *et al*., 1999; Voraberger *et al*., 2001). Similar devices exist for the determination of CO_2 (Weigl & Wolfbeis, 1995; DeGrandpre *et al.*, 1999; Mills & Eaton, 2000).

In ecology, optodes were first used for high-resolution mapping of oxygen distributions in sediments and biofilms. See, e.g. Glud *et al*. (1996, 1999b) for the use of planar optodes in determining two-dimensional oxygen distributions; and Glud *et al.* (1999a) and Wenzhofer *et al*. (2001) for the application of optodes to deep-sea sediments. More recently, optodes have become a more common tool at the specimen level too. Frederich and Portner (2000) determined hemolymph oxygenation levels in the spider crab *Maja squinado* under varying temperatures. Holst and Grunwald (2001) applied transparent oxygen optodes to a foraminifer with symbiotic diatoms. Gatti *et al*. (2002) determined respiration rates in Antarctic sponges, and Irwin *et al*. (2007) studied temperature and salinity effects on the respiration of *Artemia*.

Carbon dioxide production

Estimates of metabolic rates based on CO_2 measurements may deviate from those based on O_2 consumption, depending on the type of substrate used (lipid, carbohydrate, protein) and on the prevalence of alternative biochemical pathways (Braeckman *et al.*, 2001).

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CO_2 and O_2 concentrations in air can be measured simultaneously by gas chromatography (Mitchell, 1973; Abrams & Mitchell, 1978). Since most incubations with animals will take place in aqueous media, CO_2 concentration is then measured in a head space. A water sample is taken and transferred into a vial (pre-flushed with nitrogen) that is subsequently sealed with a septum containing cap, providing a head space in the vial. All the aqueous inorganic carbon is transferred to the head space by acidification of the sample through syringe addition of concentrated HCl or H_2PO_4. A sample can then be taken from the head space to measure air CO_2 concentration by gas chromatography. In case the sediment is rich in carbonates, there may be a high background that can strongly decrease the sensitivity of the method.

InfraRed Gas Analysis (IRGA) is an alternative, more sensitive and rapid means of detecting (changes in) CO_2 concentration in air samples. Many commercially available types of IRGA exist. For example, Van Voorhies (2000) and Van Voorhies and Ward (1999) used two different types of IRGA-based devices (the TR2 CO_2 gas respirometry system from Sable Systems and the LiCor 6251 CO_2 analyser) allowing reproducible measurements of CO_2 production of batches of as few as 50 specimens of the nematode *Caenorhabditis elegans*.

^{14}C or ^{13}C labelling

The respiration rate of unicellular organisms is so low that it can be measured only at the level of individuals in large species using diver techniques. For smaller species, several or even many individuals inevitably need to be lumped together, precluding direct assessment of, for example, body size or biomass to respiration allometries. The ^{14}C labelling technique feeds unicellular organisms with a ^{14}C-labelled food source during several subsequent generations (Stoecker & Michaels, 1991; Crawford *et al*., 1994). Protozoan cells are then assumed to have a specific activity that equals that of the radioactive food source (Crawford *et al*., 1994). Single cells are then incubated – after serial transfer through unlabelled medium – for variable, but mostly short periods of time, and the amount of ^{14}C released into the surrounding medium is determined. Results so obtained on a marine amoeba compared favourably to rates obtained from Cartesian diver measurements (Crawford *et al.*, 1994). A similar method, relying on uniformly labelled organisms, has been used to measure the energetic costs of feeding and foraging in a deposit-feeding gastropod (Forbes & Lopez, 1989). The radioactive ^{14}C isotope can be readily replaced by the stable ^{13}C isotope, which can be analysed mass spectrometrically.

Growth

Basically, the measurement of growth is straightforward and consists of repeated measurements of the mass of the whole body (or parts of it), for which the methodology has been discussed above. Nevertheless, a complicating factor is that for specific measurements, for example *AFDM* determination, the animal has to be killed.

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One might then obtain such figures indirectly through non-destructive methods (e.g. by measuring length growth and using a length–mass calibration curve), or alternatively by starting with a group of similar animals, which are subsequently sacrificed over time (see also Section 8.4).

Animals that produce skeletons may offer the possibility to use growth lines for estimating the age–size relationship over the entire previous lifespan of the collected individual. Such growth lines indicate periods of growth cessation, which may be due to internal, physiological or external, environmental forcing. If such cessations are formed at regular intervals, e.g. following a tidal, diurnal, lunar or annual cycle, they can be used to relate age to size. A crucial prerequisite is of course solid evidence that the growth lines in question are formed at regular intervals and not randomly (so-called disturbance rings; Richardson, 2001). Such proof can be obtained through controlled growth experiments or by profiling stable isotope ratios or trace element ratios that reflect environmental variability along the shell growth trajectory, e.g. the stable oxygen isotope ratio as a proxy of the seasonal temperature cycle (Carroll *et al*., 2009). Grounding and polishing a sectioned surface of, for example, a bivalve shell provide a much clearer picture of the growth lines than a visual inspection of the shell surface (Richardson, 1989).

Rhoads and Lutz (1980) provided the classical work on the use of skeletons in (mostly invertebrate) aquatic organisms. Although much attention was paid to molluscs (mainly bivalves and gastropods), growth patterns are also formed in corals, barnacle shells, polychaete jaws and echinoderm skeletons. Richardson (2001) reviewed the recent developments in studies of growth line formation and skeletal deposition in molluscs, but with an emphasis on the use of bivalve shells to reconstruct historical changes in the marine environment.

Reproductive output

Reproductive output is regarded as being part of the production that includes all energy and matter invested into sperm, eggs and material associated with it, such as egg capsules. Two main reasons to investigate energy investment in reproduction can be distinguished: constructing an energy budget of a species under varying environmental conditions or to determine the reproduction strategy. Clarke (1987) hypothesises two alternative strategies of reproductive investment in species living in areas of different latitudes. Brey (1995) reviewed the literature and compiled a data set to test this hypothesis. In this text we will discuss only the methods of measuring reproductive output and will not dwell upon reproduction strategies. Both laboratory and field methods are discussed. It should be noted that reproductive output as described below does not include possible ‘overhead’ costs associated with the production of reproductive material.

Laboratory methods

Most papers that estimated fecundity in the laboratory are based on research on the effects of concentration and quality of organic matter in sediment on growth and

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reproductive effort of deposit-feeding polychaetes, with the short-lived opportunistic *Capitella* sp. as a model species (Tenore, 1977; Grémare *et al*., 1989; Pechenik *et al*., 2000), or on the effects of environmental factors such as temperature and salinity on the reproduction of marine nematodes (Tietjen & Lee, 1977; Heip *et al.*, 1978, 1985; Vranken *et al*., 1986). Estimating fecundity in the laboratory can be roughly divided into three categories: (i) non-destructive techniques, (ii) dissection and (iii) biochemical analysis.

The non-destructive techniques include the frequently used method to count eggs – or juveniles in some ovoviviparous nematode species – under a microscope (Grémare *et al.*, 1989; Honkoop & van der Meer, 1998) and to determine egg sizes by calliper (Honkoop & van der Meer, 1998) or image analysis techniques (Clarke, 1993). Other reported methods include the collection of embryos from the parent with no apparent adverse effects for the parent (Bridges, 1996) or the collection of shed brood from the basin (Prevedelli & Vandini, 1998; Pechenik *et al*., 2000). Another indirect method, proposed by Crisp (1984), relies on the assumption that the energy content or mass of an animal before spawning minus that after spawning equals the energy content of reproductive output, as applied by Horn (1986). This method introduces some error since all other possible losses, such as respiration, are attributed to reproduction but it is a simple and widely applicable method. The main advantage of the non-destructive techniques is that animals can be studied after reproduction also.

Dissection of the animals to count egg numbers and measure egg sizes is also frequently reported (Sola, 1996; Linton & Taghon, 2000). This method implies that a suite of chemical analysis on the eggs becomes available such as caloric content (Clarke, 1993; Prevedelli & Vandini, 1998) and *C*/*N* analysis (Clarke, 1993), given that sufficient material can be collected.

A biochemical approach to quantify reproductive output based on the animal's lipid composition was proposed by Taghon *et al*. (1994). Levels of different classes of lipids and glycogen in a deposit-feeder were followed for one year. Most levels remained stable throughout this period except for levels of the lipid triacylglyceride that were elevated during oogenesis and it 'may represent a complementary method for measuring reproductive effort if these lipids are preferentially used to provision eggs'. Linton and Taghon (2000) reported the only additional test and they showed a correlation between triacylglyceride and numbers of eggs produced. However, the method still awaits rigorous testing.

Field methods

Estimating reproductive investment in the field requires extensive knowledge on natural spawning period(s) that last in the order of a few weeks and may shift from year to year depending on environmental conditions such as temperature and light. Generally, temperate areas host annual breeders, meaning that spawning occurs once a year. However, in warmer areas, reproduction can be a more or less continuous process, complicating the measurement of energy invested in

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reproduction. For many meiofauna, even in temperate areas, reproduction is assumed to be more or less continuous (Heip *et al*., 1985), but the evidence is equivocal. In macrofaunal-sized, slow-growing nematode species, regular field sampling has indicated annual reproduction cycles or two to three reproductive cycles per year (Skoolmun & Gerlach, 1971; Smol *et al.*, 1980).

The only method for *in situ* measurement of reproductive output of benthic marine species is described in Qian and Chia (1994), which appears to be labour-intensive. On an intertidal mudflat, experimental trays containing sieved sediment and a known amount of dyed *Capitella* sp. siblings from a single parent were deployed. Growth and death rates were followed during maturation (∼2.5 months) by regular collection and redeployment of the experimental trays. Eggs could relatively easily be counted and handpicked since the tube-dwelling *Capitella* sp. deposits eggs within the burrow. Egg numbers, sizes and energy content were determined. Reproductive output was comparable to the laboratory measurements but the observed variation was very large.

Alternatively, semi-field estimates are gained by collecting specimens at commencement of spawning in the field. Spawning can be spontaneous or induced shortly after arrival in the laboratory to reduce any bias from keeping animals under laboratory conditions. In this way the previously mentioned laboratory methods of egg counting, dissection or biochemical analysis become available. Spawning is induced by imposing an environmental shock after which eggs and sperm can be collected from the basin for further analysis, for example by pipetting (Honkoop & van der Meer, 1998). Reported effective environmental shocks comprise a temperature shock (Honkoop & van der Meer, 1998), injection of a KCl solution into the mantle of mussels (Honkoop & van der Meer, 1998) and addition of Prozac to the basin (Honkoop *et al.*, 1999).

Regeneration

Regeneration of body parts, following sublethal predation or other sorts of injuries, is a form of somatic production that should be taken into consideration when calculating secondary production of a benthic population. Examples of sublethal predation concern siphons in bivalves (Peterson & Quammen, 1982; De Goeij *et al*., 2001), feeding palps and posterior segments in polychaetes (De Vlas, 1979b; Zajac, 1995; Lindsay & Woodin, 1996) and arms in echinoderms (Lawrence & Vasquez, 1996; Skoeld & Rosenberg, 1996; Lawrence *et al.*, 1999). Injuries due to fights with conspecifics occur in crabs, which may lose chelipeds and walking limbs (McVean, 1976; McVean & Findlay, 1979; Abello *et al.*, 1994). In some species the contribution to overall secondary production can be considerable. In North Inlet, South Carolina, the trophic transfer related to arm regeneration of *Microphiopholis gracillima* ranged from 3.3 up to 9.7 g *AFDM* m^{-2} a^{-1}, an amount equivalent to total community macrobenthic secondary production in other systems (Pape-Lindstrom *et al*., 1997). In the Skagerak, South Sweden, about 13% (0.34 g

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AFDM m^{-2} a^{-1}) of the total production of an *Amphiura filiformis* population was due to arm regeneration (Skoeld *et al*., 1994).

Predators responsible for the sublethal predation are, for example, shorebirds, flatfish, crabs and shrimp (Peterson & Quammen, 1982; Lawrence & Vasquez, 1996; Skoeld & Rosenberg, 1996; De Goeij *et al*., 2001).

Methods for studying the importance of regeneration of body parts can be categorised in two ways. Firstly, the frequency at which body parts are lost must be estimated and the methods that have been applied are very similar to those that have been used in studying predation rates in general (discussed in the section entitled 'Ingestion and absorption by predators'): direct observation, encaging or excluding predators and gut content analysis (De Vlas, 1979a). Secondly, the regeneration rate should be assessed, which can easily be done by taking repeated measurements during regeneration, just as in normal growth studies. A complicating factor is of course that one might have to amputate the body part to enable a proper measurement. In that case one should start with a group of similar animals.

However, in some cases, an approach specific to sublethal predation can be used. Lugworms *Arenicola marina*, which normally live burrowed in the sediment at a depth where they are relatively safe from predators, expose the tip of their tail many times a day when they have to defaecate. During these short moments, the animals are vulnerable to sublethal predation. Predators such as flatfish may crop one or a few tail segments. The tail regenerates by lengthening the remaining segments, and no new segments are ever formed. This observation may imply that the number of segments in a field population can be used to estimates sublethal predation frequency (De Vlas, 1979b).

Finally, one should realise that the damage to body parts may also influence the feeding abilities of the organism, as the browsed tissue is often part of the feeding apparatus (Nilsson, 2000a, 2000b), and this may complicate the description of the energetic effects of sublethal predation (De Goeij & Luttikhuizen, 1998).

Product formation

Recently, considerable interest has arisen in the production rate of non-living body parts, such as calcareous shells. Due to increased anthropogenic CO_2 release, not only temperatures are changing but the ocean is acidifying as well (Solomon *et al.*, 2007). Lowering pH changes the carbonate equilibrium in seawater, and may lead to under-saturation for important calcium carbonate species such as aragonite and calcite, forming organisms' shells. There is thus a danger that calcareous shells dissolve or are difficult to construct for the organisms. Results on the effect of acidification on the rate of calcification in diverse groups of planktonic and benthic organisms are equivocal (Hendriks *et al*., 2010; Kroeker *et al.*, 2010). Calcium carbonate deposition is an active physiological process, influenced by the local pH at the place of deposition. The subsequent stability of the shells depends not only on the calcium carbonate chemistry but also on the degree of shielding of

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the carbonate structures from seawater by organic protection. Thus, acidification may increase the energetic cost of production of calcareous structures, without rendering this production impossible (Cummings *et al*., 2011).

Basically, there are two different ways to influence the pH of seawater: by increasing the Dissolved Inorganic Carbon (DIC) content while keeping Total Alkalinity (TA) constant, or by keeping DIC constant and changing TA. The first is achieved by bubbling the seawater with air containing elevated CO_2 concentrations, the second by adding acid (usually HCl) to the seawater. Hurd *et al*. (2009) discuss the methodological details in the context of phytoplankton studies, but this review is also very useful for study of other groups. Most experiments are conducted in the laboratory or in mesocosms. Campbell and Fourqurean (2011) describe an *in situ* incubation method that can keep pH lowered for extended periods of time in the field.

8.4 From the individual to the population

Basically, describing the flows of energy through a population is simply a matter of good bookkeeping of the flows of the constituent individuals. In practice, problems arise because not every single individual can be followed. In this section, we will discuss what can be said about energy flows through populations when only limited information on individuals is available.

Thus, if we want to know the exact fate of all individuals in a population, i.e. if we want to know their time of birth and death, and all the state variables (such as the size of their structural body and reserves) in relation to age, and all the relevant process rates (such as their absorption rate, respiration rate and gonad production rate) in relation to age or size, then the calculation of population sums such as, for example the overall population gonad production over any period of time, would be a straightforward and simple exercise. Such detailed information is of course rarely available. In the literature, different calculation methods have been proposed for different types of data.

Here we distinguish three types of data:

(1) Individuals can be aged by using growth lines. An example concerns many bivalve species that can be aged using annual rings in their shells. Since each individual animal can be aged, it can also be uniquely assigned to a specific cohort. A cohort is a group of individuals that are born at more or less the same time. Of course, the more precise the aging, the more precise the classification into cohorts. In practice, this means that animals with annual growth rings are classified into annual cohorts. Methods are applicable if at least one cohort has been sampled at regular intervals over its entire lifetime.
(2) Individuals cannot be aged, but if cohorts appear separated in time and if growth during the time until the arrival of the next cohort is relatively large compared to the initial size, then individuals can be statistically assigned to a specific

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cohort on the basis of their size. Various methods of statistically separating cohorts are described in the literature. These methods can be applied if the population has been sampled repeatedly and over a period longer or even much longer than the maximum lifespan of the individuals.

(3) Individuals cannot be aged, and cohorts cannot be separated.

Basically, the advantage of cohort data (the first two data types) is that they enable the estimation of both the initial size of the population, i.e. the recruitment, and the survival function. Normally, the (average) size–age relationship, or growth function, is also determined. As we will show below, these three elements are the essential ingredients for estimating the production of new somatic and reproductive material. The measurement of this so-called secondary production of animal populations is frequently one of the major objectives of descriptive studies of ecosystem energetics. Below we will discuss the estimation of secondary production in more detail. If data are also available on the relationship between size and the rate of some other process than growth, for example the relationship between size and respiration rate, then the results that we will obtain below on the estimation of secondary production can easily be translated into the estimation of the overall population process rate, e.g. overall population respiration. For populations that do not produce identifiable cohorts, i.e. data type (3), estimates of all these population figures are not easily obtained. Below we will first discuss the case of identifiable cohorts. But before we do that, we make a few remarks on (i) the concept of secondary production and (ii) statistical methods to separate cohorts.

Secondary production

Secondary production has been defined many times, with most definitions dating back to the pioneering work of Thienemann (1931), who stated that the production of a population for a known period of time is considered to be the sum of growth increments of all the individuals existing at the start of the investigated period and remaining to the end, as well as the growth of newly born individuals and of those individuals that, for various reasons, do not survive to form part of the final population. Two things should be kept in mind with regard to this definition. Firstly, production is apparently considered as a quantity and not as a rate, and although the exact physical dimension depends on the way of expressing the growth increments (this has been done, for example as dry mass (DM), number of carbon atoms or nitrogen), the unit time does not occur. Often, the term ‘productivity’ is specifically reserved for the rate of secondary production (Lindeman, 1942). However, some authors characterise production as a (mean) rate or as a rate per unit area. For example, Waters and Crawford (1973) define production as ‘that amount of tissue elaborated per unit time per unit area, regardless of its fate’. Macfadyen (1948) gives an early review of these definition problems. We regard the issue to be of minor importance, as long as authors and readers are aware that comparisons between

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different studies may be seriously hampered when measurements are made over periods of different length or at different times in the year. The second point that we want to draw attention to is the role of mass at birth. Thienemann (1931) referred only to the growth of newly born individuals and did not include their mass at birth. This must then imply that the production of progeny (or more generally gonads) should be assigned to the 'growth increment' of the parents.

Statistical methods to separate cohorts on the basis of size

ELEFAN (Pauly, 1987) was a computer programme widely used to separate cohorts. The algorithm identifies modes in the size-frequency distributions, connects them to cohorts and lets the cohorts progress through time and size. Similar to ELEFAN, Shepherd Length Composition Analysis (SLCA) is based on the goodness-of-fit of the location of modes calculated from a von Bertalanffy growth curve. It uses a different goodness-of-fit measure (Shepherd, 1987). Both ELEFAN and SLCA can be accomplished using the software FiSAT-II, which is part of the FAO package (Gayanilo *et al.*, 1996). McQuaid and Lindsay (2000) provide a comparison of growth parameters of molluscs estimated by SLCA with direct measurements of growth using tagged individuals. These direct measurements are based on shell marking (Ekaratne & Crisp, 1982) and growth band analysis using acetate peels (Pentilla *et al*., 1988; Richardson, 1989).

The programmes MULTIFAN (Fournier *et al.*, 1990, 1991) and MULTIFAN-CL (Fournier *et al*., 1997) are based on the same basic reasoning, but differ from ELEFAN in their estimation procedure. The method is very much tailored to fisheries' problems, and starts by specifying 'catch equations' that govern the number of fish in a particular age class in a particular year, as a function of background mortality, fisheries mortality, spatial movement, modelled as a diffusion process in Fournier *et al*. (1997). Constraints on the mortality parameters (e.g. natural mortality is independent of year and region, but varies with age) can be introduced into the equations. Similarly, assumptions regarding fisheries' mortality can be specified. The model specified in terms of age is transformed to a model as a function of length (most data sets in fisheries have length distributions, not age distributions) by assuming that the mean length of an age class follows a von Bertalanffy growth curve, that the lengths of fish in each age class are normally distributed, and that the standard deviations of these distributions are a linear function of the mean. Fournier *et al*. (1997) also give an option for density-dependent growth. With the model thus specified (including a specification of the error distributions), estimation of the parameters is done by maximum-likelihood estimation methods, using efficient numerical algorithms. The estimation procedure also yields confidence limits for the parameter estimates. Hypotheses on the processes involved are tested using a Bayesian approach. The method is computationally quite intensive. Interfaces in the software programme R are available for input of data and post-treatment of results (R Development Core Team, 2008).

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Bjorndal and Bolten (1995) compared ELEFAN, SLCA and MULTIFAN on a set of data on green turtles, where the output from the programmes could be compared to growth data obtained from tagging. They concluded that MULTIFAN obtained the best output, but that SLCA is useful to conduct prior estimates of the parameters that can be used as input to MULTIFAN.

Calculation of production of populations with identifiable cohorts

Crisp (1984) mentioned two different approaches in the measurement of the total secondary production of a cohort of animals and stated that these must be clearly distinguished. The first method, which is known as the *increment-summation* method (Winberg, 1971), is to add all the growth increments of all the members of the cohort as they occur during the period under consideration. The second method mentioned by Crisp, which is known as the *removal-summation* method, is to consider both the matter that leaves the cohort by mortality, and the difference between the total biomass of the cohort at the end of the observation period and at the start of the period. Adding these two terms gives the cohort production. Crisp (1984), as well as many others (Gillespie & Benke, 1979; Rigler & Downing, 1984), argued that the two methods are similar. Below we will first repeat Crisp's argument by using a simple illustrative example. We will proceed with a more formal treatise.

Consider at time t_1 a cohort with a total number of N_1 animals, each with individual mass w_1. Hence, the total biomass at the start is N_1w_1. A short period later, at time t_2, only N_2 animals are still alive, each with individual mass w_2. All other animals (i.e. $N_1 - N_2$) have died. For convenience, we suppose that they all died with a mass of $(w_1 + w_2)/2$. Using the *increment-summation* method, the production equals the sum of growth increments of all the individuals existing at the start of the investigated period and remaining to the end, i.e. $N_2(w_2 - w_1)$, plus the growth of those individuals that did not survive, that is $(N_1 - N_2)\,((w_1 + w_2))/2 - w_1)$. Alternatively, the *removal-summation method* equals the production to the matter that has left the population by mortality, that is $(N_1 - N_2)(w_1 + w_2))/2$, plus the difference between the total biomass of the population at the end of the period and at the start of the period, that is $N_2w_2 - N_1w_1$. Some simple algebraic manipulation shows that the two approaches do reveal exactly the same result, which also can be written as $(N_1 + N_2)(w_2 - w_1))/2$. An easy interpretation of the latter expression is that all the N_1 animals present at the start of the short period had a growth increment of at least $(w_2 - w_1))/2$, but that only the N_2 animals alive at the end of the period had an additional growth increment of again $(w_2 - w_1))/2$. This formulation forms the basis of the so-called *growth-survivorship* method, or *Allen-curve* method. The Allen curve gives the relationship between N and w, and the area under the curve equals the production (Fig. 8.3).

A more extensive and perhaps more insightful numerical example can be constructed when a cohort has been observed at multiple points in time, e.g. from its birth until all animals have died (Table 8.5; Fig. 8.4).

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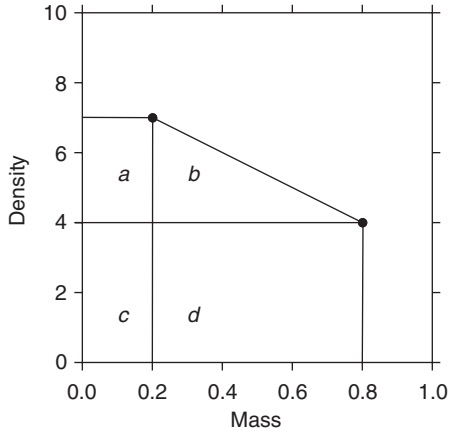

Fig. 8.3 Allen-plot. At the first sampling occasion, seven animals per unit area with a mass of 0.2 units of mass were observed. At the second occasion, four animals with mass 0.8. Secondary production over this period equals 3.3. *Increment-summation* method takes area *b* (which represents the growth increment of those animals that die) plus *d* (the growth increment of the survivors). *Removal-summation* method first takes area *a* (initial mass of those that die) plus *b*, and then adds *c* (initial mass of the survivors) plus *d* and subtracts *a* plus *c*, which also gives *b* plus *d*.

One complicating factor that should not be overlooked is the treatment of the mass at birth, or, more generally, the gonad production. Unfortunately, both Crisp (1984) and Rigler and Downing (1984), aiming to show the equivalence between the *removal-summation* and the *increment-summation* method, used examples where the birth mass was zero and thus did not emphasise this potential source of error. The standard approach is to add the mass of newly born individuals to the production of the parent. This implies that the total initial mass of a cohort should indeed be subtracted when using the *removal-summation* method. However, it also implies that the cumulative gonad production of each individual should be added to the mass at death. Using the *increment-summation* method, it implies that the increment

Table 8.5 Production calculations for the 1985 cohort of the bivalve *Macoma balthica* at Balgzand. See text for further explanation.

Year	Density (n)	Mass (w)	Δn	Δw	$\bar{n}$	$\bar{w}$	$\Delta n \cdot \bar{w}$	$\bar{n} \cdot \Delta w$
1986	101	0.12						
1987	59	1.43	42	1.31	80	0.775	32.55	104.8
1988	36	2.71	23	1.28	47.5	2.07	47.61	60.8
1989	26	4.35	10	1.64	31	3.53	35.3	50.84
1990	16	6.49	10	2.14	21	5.42	54.2	44.94
1991	9	9	7	2.51	12.5	7.745	54.215	31.375
1992	4	9	5	0	6.5	9	45	0
1993	0	9	4	0	2	9	36	0
Sum							304.875	292.755
Initial mass							−12.12	
Sum							292.755	

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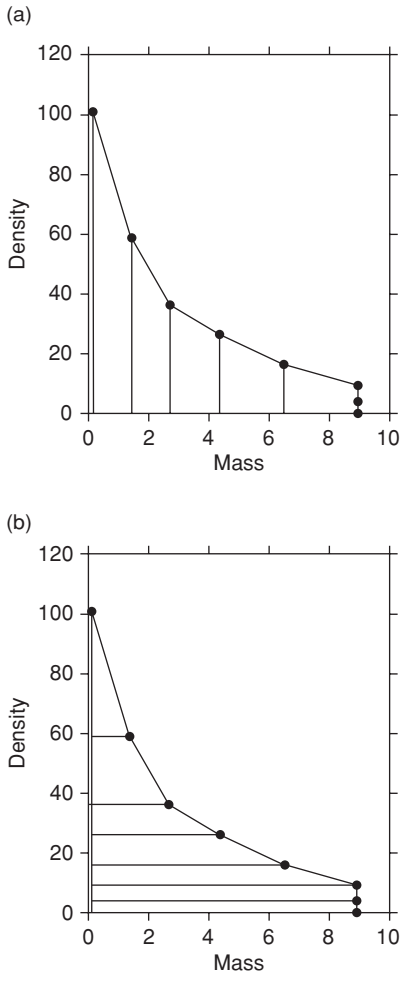

Fig. 8.4 (a) Allen-plot for the 1985 cohort of the bivalve *Macoma balthica* at Balgzand. (b) Allen-plot for the 1985 cohort of the bivalve *M. balthica* at Balgzand. Illustration of the *removal-summation* method.

should contain the gonad production. The alternative approach is to account the mass at birth to the newly born individual. In that case, the total initial mass of a cohort should not be subtracted when using the *removal-summation* method, and it should be added when using the *increment-summation* or *Allen-curve* method.

A more formal treatise goes as follows: in accordance with the definitions of Thienemann (1931) and others, the production P of a single cohort can be defined as the summed growth

$$P = N_0 \int_0^\infty S(x)\, g(x) \mathrm{dx}$$

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Here N_0 is the initial cohort size (or recruitment), $S(x)$ the fraction that is still alive at age x and $g(x)$ the growth rate at age x. Hence, this definition provides a theoretical justification for application of the *increment-summation* method for calculating production rate on the basis of knowledge on the age-structure of the population and the growth function. For each age class

$$\left\{x - \frac{\Delta x}{2},\ x + \frac{\Delta x}{2}\right\}$$

the total number, which approximately equals $N_0 S(x)$, is multiplied by the age-dependent growth $g(x)\Delta x$. Summation over all age classes leads to the following approximation for the production of the population:

$$P \approx N_0 \sum S\,(x)\, g\,(x)\, \Delta x$$

Partial integration of the integral provided above gives

$$P\,(t) = -N_0 G\,(0) - N_0 \int_0^{\infty} \frac{\mathrm{dS}\,(x)}{\mathrm{dx}} G\,(x)\,\mathrm{dx}$$

leading to the *removal-summation* method

$$P = -N_0 G\,(0) - N_0 \sum \Delta S\,(x) G\,(x)$$

where the term $N_0 \Delta S(x)$ gives the change in numbers (as a negative number) from one age class to the next, i.e. the numbers that die at a weight $G(x)$. The term $N_0 G(0)$ is the total initial mass of the cohort.

Calculation of production when cohorts cannot be identified

Often one is not able to follow identifiable cohorts through time. It is impossible to recognise age classes and the only type of field data available concern (repeated) observations of the mass distribution of the population. Fortunately, the production rate at any point in time can always be calculated directly from the age distribution or from the mass distribution provided that the age–mass relationship (the growth function) is known. As it is often rather difficult to measure the growth of soft-bodied benthic invertebrates directly in the field, the usual approach is to carry out additional growth measurements in the laboratory. Yet the use of such growth data to estimate production in the field should always be regarded with some suspicion, because it is extremely difficult to mimic natural field conditions, particularly concerning food supply, in the laboratory (Crisp, 1984).

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Without additional knowledge of the growth function it is impossible to estimate production. The size-frequency method of Hynes and Coleman (1968), later corrected by Hamilton (1969), has caused considerable confusion and debate in the literature (Fager, 1969; Benke & Waide, 1977; Benke, 1979). One of the reasons for this confusion is that it seems, at first sight, that no information on the growth function is needed or used. However, it can be shown mathematically that the method makes the implicit assumption of a linear and constant length growth rate throughout development time.

In order to obtain a production estimate over a specific period of time, say a year, repeated observations over time of the mass structure have to be made, and the calculated production rates should subsequently be integrated over time. A more fundamental approach is to explicitly model the three underlying processes (renewal or birth rate, growth rate and survival or mortality rate) that together determine population production rate, in terms of characteristics at the level of individuals. Hence, the basic problem we treat in the remaining part of this section is to what extent is it possible to estimate demographic parameters of the population (in particular: patterns of renewal or birth rate, growth rates and survival or mortality rates) from this type of observation. Knowledge of these rates will be needed for the production estimation. Moreover, the demographic parameters in themselves may be very relevant, e.g. as a means of comparing different populations or in the context of evolutionary studies.

The basic approach to the analysis of size or stage-frequency distributions is to construct a theoretical model of the population dynamics, in which the parameters are defined, and then to apply more or less sophisticated estimation methods to derive the values of these parameters from the available data. This modelling is illustrated in Fig. 8.5. Knowing the initial conditions (i.e. the age structure at time zero), the renewal or birth rate of the population as a function of time (in technical terms, the boundary condition at size zero) and the survival rate as a function of time and age, one can construct a surface in the age–time space (Fig. 8.5a). The height of the surface more or less describes the number of animals of a certain age present at a certain time. A group of animals born at the same moment into the population will describe a trajectory across this surface, which is constrained by the fact that their age will increase by exactly 1 d/d and that their number can only decline in the process. In Fig. 8.5a, where recruitment was quite variable (it was described by a moving average time series model), these trajectories can easily be recognised, as the peaks in the recruitment function follow an oblique course through the age–time space.

As the age of an individual is in most cases impossible to measure, we have to rely on size information. The age–time landscape is projected into a size–time landscape (Fig. 8.5b) by a growth function, in which, in general, the growth rate is also a function of age and time. The projection by a non-linear function results in a distortion of the general shape of the landscape. In general, it will tend to collapse the high-age part of the landscape, as growth is slow or absent at older

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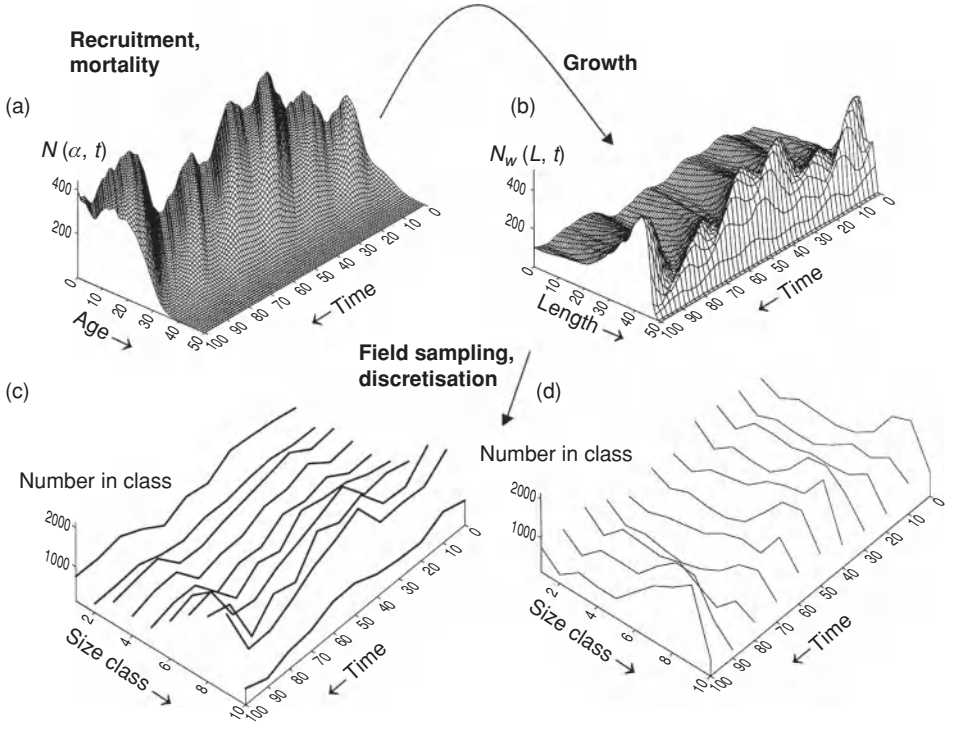

Fig. 8.5 Illustration of the basic approaches in the analysis of size- or stage-frequency distributions. The population can be described by a surface in the (age–time) space (a). The form of this curve depends on the recruitment boundary conditions (a moving average function in this example) and on the age- and time-dependent mortality function. Taking into account the (age- and time-dependent) growth function, the population surface in the age–time landscape can be transformed into a surface in a size–time landscape (b). When sampling the population in the field, and using discrete size and time intervals, the observable functions either give a picture of number of individuals in discrete size classes as a function of time (c), or number of individuals in different size classes at different moments in time (d). See the text for more details.

age, while it will extend the range and resolution in the low-age part where growth is fast.

Note that whereas for the sake of clarity Fig. 8.5 uses deterministic functions to model the population, in practice all processes depicted are subject to different sources of natural variability. Added to this are measurement errors, and therefore, real data may appear much more scattered than they do in Fig. 8.5. The structure of the natural variability in the recruitment, growth and mortality functions is a particularly complicated problem. Individual variation in the essential parameters of these rates may be considerable, and in addition the different functions can co-vary, when, for instance, fast-growing individuals suffer a different mortality rate than slow-growing ones.

The problem of estimation of demographic parameters from size or stage frequencies is the inverse problem of the construction of the 'field data' as was done in

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Fig. 8.5. The field sampling approach will furthermore limit our view of the entire landscape to a number of slices at different points in time, where classification of the data in size classes provides a number of discontinuous curves. These can be either time-courses of numbers in different classes (Fig. 8.5c), or size-frequency diagrams at different points in time (Fig. 8.5d). Three major functions were involved in the construction: (i) recruitment, (ii) mortality and (iii) growth. In addition, initial conditions were needed. In general, when a set of field data is available (which includes the initial conditions up to measurement error), there is more than one set of recruitment, mortality and growth functions that may (within measurement error) reproduce the field data. Even a perfect fit between model predictions and observed data does not prove that the model is a true representation of reality, but merely shows that further improvement or change of the model is not possible until more or different data are tested (Aksnes *et al*., 1997). Note that this 'perfect fit' is more of a theoretical than a real possibility. In order to lead to practical results, the general estimation problem as sketched above must be simplified by either of three approaches: (i) the use of independent auxiliary information, (ii) simplifying assumptions about the essential functions or (iii) both.

For the *recruitment* function, independent information is rarely available in benthic species. In zooplankton, and notably in the study of rotifers and daphnids, extensive research has been devoted to the so-called egg-ratio methods, dating back to Edmondson (1960). We do not discuss this approach in detail. Basic publications on the method are Paloheimo (1974), Seitz (1979) and Threlkeld (1979). In benthic research, many populations have peak recruitment in a short period, a situation that we categorised as data type (2) (see Section 8.4). Fig. 8.6 shows a simulation (with the same functions as used in Fig. 8.5) for a population where recruitment is non-zero only for a short period. The age–time and size–time diagrams clearly show the progression of the peak (mode) of the recruitment function through the size classes with time. Unfortunately, no simple alternative descriptions of the time-course of recruitment are available, if such recruitment peaks do not occur.

For the *growth* function, independent auxiliary information is often available from laboratory experiments or from experiments in the field (see the section entitled 'Growth'). As a simplifying assumption, a functional form for the growth function is often applied. In fisheries research, there is a long tradition of using the von Bertalanffy growth equation. This growth function is also derived by the DEB model (see Section 8.3) and should, with proper scaling, be useful in the study of many other populations. The application of a functional form to the growth function reduces its estimation to the estimation of a few parameters only. For longer lived populations, a seasonal form of the von Bertalanffy equation is often used, allowing for slower growth in the cold season.

For the *mortality* function, neither external information nor functional representation offers great possibilities. Typically, mortality does not directly depend on the size of the individual, but on factors outside the population under study: presence of predators, occurrence of diseases, food shortage, etc. An exception where external

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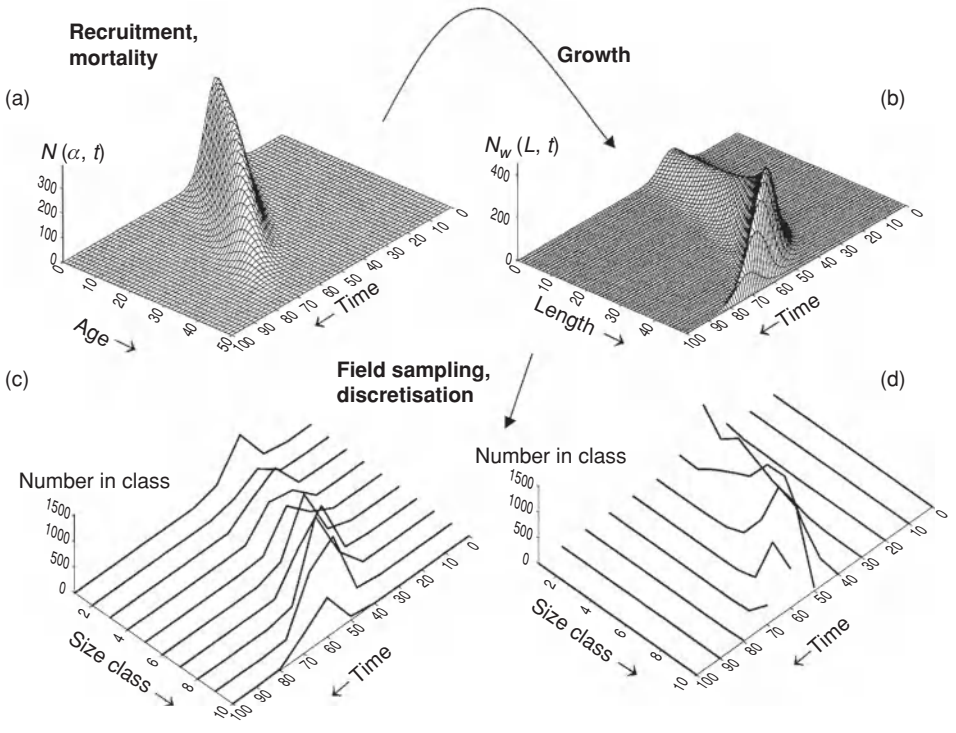

Fig. 8.6 As Fig. 8.5, but illustrating the case of a population with one cohort. Recruitment in this population is restricted to a short period of time compared with the lifetime of the cohort, which greatly simplifies the interpretation of the resulting graphs.

information is available is fishery mortality, which is often included as a separate factor in fisheries models, added to 'natural' mortality. It can often be estimated based on fisheries statistics. Another exception is the use of shed ostracod shells conveying information on the time when animals died in the sediment (Herman *et al.*, 1983). Simplifying assumptions on the mortality function are often included. Some methods (notably theoretical studies investigating stationary populations) assume mortality rate to be constant throughout life. Other methods (see below) require that mortality rate is a smooth function of time and age (Wood, 1994; Manly, 1997). This may reduce the number of parameters to be estimated, while still allowing representing variable mortality functions through life.

Parallel scientific traditions have developed in the estimation of demographic parameters from size-frequency and from stage-frequency data. The former notably developed in fisheries research, where the bulk of the data is in the form of length-frequency and catch number data, and where a (seasonally adjusted) von Bertalanffy growth curve can generally be used to link size to age. Fisheries biologists also have reference data sets based on otoliths, with which they can check the age–length relationships estimated from the field data. Stage-frequency analysis

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methods have been developed mainly in the context of crustacean zooplankton research. Further discussions on the estimation of population parameters from repeated size-frequency or stage-frequency data can be found in Manly (1990), Wood (1994) and Aksnes *et al*. (1997).

Production to biomass ratios and the turnover of individuals

Sometimes biologists wish to estimate production for a particular population in a very simplified way, which means that they hope to avoid the laborious task of obtaining information on the recruitment function, and the growth and survival curves. One popular approach is to use published production to biomass ratios (*P*/*B*), and to combine this information with knowledge of the biomass of the study population. A biomass estimate is of course much easier to obtain than a production figure. The underlying idea of this approach is that populations of the same species or of species with the same ecology or physiology must have similar *P*/*B* ratios. Some authors have tried to construct empirical relationships between published *P*/*B* ratios and physiological or ecological characteristics of the species: body size (Banse & Mosher, 1980), lifespan (Robertson, 1979), temperature (Tumbiolo & Downing, 1994), food availability, etc. Some studies specifically dealt with marine macrobenthos populations (Brey, 1990; Tumbiolo & Downing, 1994). However, the unexplained variation that is left after fitting these empirical relationships is still so large that only rough approximations can be expected when using an externally derived *P*/*B* ratio (Banse & Mosher, 1980). Rigler and Downing (1984) conclude that when genuine production estimates are needed to test specific hypotheses, rough approximations are not very useful. Therefore, they do not recommend the use of this simplified method. We do not object to this point of view. Nevertheless, the use of *P*/*B* ratios is still fairly standard for obtaining production estimates of meiobenthos, since data on recruitment, growth and survival of most meiofauna are lacking. Waters (1969) proposed an average life cycle turnover of 3.5 for fresh water invertebrates, with a fairly narrow range (2.5–5). Empirical work supporting that meiofauna fit into this range comes from lab studies on some nematode and harpacticoid copepod species (Herman *et al*., 1984; Heip *et al*., 1985). To obtain estimates of yearly production, this life cycle turnover needs to be multiplied by the annual number of generations. Based on a laboratory study of two nematode species, Gerlach (1971) proposed a number of ca. three generations per year, to be multiplied with a life cycle turnover of three yielding an annual *P*/*B* ratio of 9, a value that has since been widely adopted for meiofauna in general. However, we do not agree with the use of such generalised values, since empirical studies have shown that the number of generations may vary from less than one to well over twenty (Heip *et al*., 1978, 1985; Vranken *et al*., 1986).

The ratio of production rate (note that annual production divided by one year can be regarded as an average rate) to biomass has the dimension one over time. For stationary populations, it can be interpreted as the turnover rate of biomass. Many

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authors and some textbooks on production (Winberg, 1971) have erroneously stated that for stationary populations the turnover rate of biomass (i.e. the *P*/*B* ratio) equals the turnover rate of individuals. The latter rate equals one over the mean lifespan (Bartlett, 1970). Consequently, production has often been calculated as the total biomass divided by mean lifespan. Though this approach seems to be in agreement with the previously mentioned empirical observation by Robertson (1979) that the *P*/*B* ratio was approximately inversely related to maximum lifespan, Rigler and Downing (1984) showed that the turnover rate of biomass does not always equal the turnover rate of individuals. However, they appear to have missed the critical condition. This condition was pointed out by Van Straalen (1985), who showed that the turnover rate of biomass equals the turnover rate of individuals, corrected for the ratio of mean mass at death to mean mass of live individuals. The latter ratio equals 1 if the age-specific per capita mortality rate is constant, in other words, if lifespan is exponentially distributed. Unfortunately, no shorthand rule exists to assess this ratio of mean mass at death to mean mass of live individuals in the case of age-dependent mortality.

8.5 Community-level measurements and modelling

The methods described earlier consider measurements at the level of the organism or population. Several experimental methods are also available that focus more on the activity by or on the mass transfer through the whole benthic community.

Community-level activity

The oxygen uptake rate of the seafloor is the most widely used measure of benthic mineralisation of organic matter. Different terms exist for the community-level respiration rate of the benthic community: sediment oxygen consumption, sediment community oxygen consumption and Total Oxygen Uptake (TOU). Yet these three terms denote the same process, which is the total oxygen flux into the sediment and typically expressed as mmol O_2 m^{-2} d^{-1}. What is further apparent in this terminology is that these measures aim to give information on organic matter mineralisation (CO_2 production), but are described in terms of oxygen (O_2 consumption or uptake). Oxygen microelectrodes and oxygen optodes (see the section entitled 'Optodes') give a quick and almost continuous record of oxygen measurements, whereas carbon dioxide measurements require sampling a volume of water and are more tedious to conduct. Oxygen uptake/consumption is often coupled to carbon dioxide production by using a stoichiometric conversion coefficient from O_2 consumption to CO_2 production equal to one. However, degradation of more complex compounds and chemoautotrophy (e.g. nitrifying bacteria) produce variations in this coefficient. Though depending on the setting, a conversion coefficient of O_2 consumption to CO_2 production of 0.8–1.2 is common (Glud, 2008).

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The classical way to measure this oxygen flux is by means of chamber incubations, in which a sediment core is enclosed and the oxygen concentration is monitored in the overlying water with an oxygen electrode or optode. Several points need to be taken into account in this approach: (i) the water volume in the core should be accurately measured to ensure that the oxygen concentration can be converted into oxygen uptake; (ii) it is important to make sure that there are no air bubbles in the enclosed core; (iii) the overlying water should be stirred to ensure that the sensor monitors the oxygen concentration in the water representatively. The oxygen consumption is finally derived by inferring the slope of the oxygen decrease over time. Full methodological descriptions can be found in Glud (2008).

In recent years it has become clear that, especially in more open sandy sediments, the enclosure of sediment reduces the oxygen consumption in the sediment. Sandy sediments have coarser grains and, therefore, are more permeable to water flow than muddy sediments. This higher permeability opens the possibility of advective transport through sediments as opposed to diffusion-dominated muddy sediments. Advective transport is induced by wave action and water flow over a non-flat seafloor topography and results in an entrapment of labile organic matter from the water column (Rusch & Huettel, 2000) and enhanced solute exchange (D'Andrea *et al.*, 2002). Hence, despite their low organic carbon content, sandy sediments may mineralise an equivalent amount of organic matter as compared to their more organic-rich muddy sediments (e.g. D'Andrea *et al*., 2002; de Beer *et al*., 2005). Standard core incubations impede the advective transport processes, and therefore, the oxygen uptake and carbon mineralisation rates inferred by closed core incubations may be an under-estimate of the true *in situ* rates.

To overcome these limitations, a non-invasive eddy correlation technique has been developed that measures oxygen uptake rates without disturbance of the seafloor (Berg *et al.*, 2003). The principle is that all O_2 transported vertically towards or away from the sediment surface is facilitated by turbulent motions. The vertical flux of oxygen can be derived by analysing high-frequency time series of both concentration (oxygen microelectrode) and vertical velocity (acoustic Doppler velocimeter) obtained at approximately 10–50 cm above the seafloor. From the correlation between the vertical flux and the oxygen concentration, the net oxygen flux of the sediment surface can be inferred. This method has great advantages, since the natural transport processes that control the oxygen uptake rates are left undisturbed. A limited amount of comparative studies have been carried out, but they show that the eddy-correlation technique gave 1.5–4 times higher oxygen uptake rates than traditional core incubations (Berg *et al*., 2003; Berg & Huettel, 2008). Recent applications in the deep sea show that the method is also able to measure small oxygen fluxes in the order of about 1 mmol O_2 m^{-2} d^{-1} (Berg *et al*., 2009). The footprint on the seafloor that gives rise to the measured oxygen flux is located upstream of the measuring equipment and depends on the distance that the sensor is located above the sediment surface. A typical footprint area is

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40 m long and 1 m wide (Berg *et al*., 2007). One research field in which the eddy-correlation technique may be successfully applied is on seafloors with more complex morphological structures such as seagrass beds, sponge beds, oyster or mussel reefs or cold-water coral communities.

The TOU is the sum of three components: (i) Diffusive Oxygen Uptake (DOU), (ii) Advective Oxygen Uptake (AOU) and (iii) Faunal Oxygen Uptake (FOU). The DOU is defined as the oxygen uptake that occurs through the sediment surface as a result of diffusion into the sediment. Oxygen is consumed in the top millimetres or centimetres of the sediment and, hence, there exists an oxygen gradient from the overlying water through the benthic boundary layer into the sediment. This gradient drives the diffusive uptake of oxygen by the sediment. Oxygen microelectrodes have a very small tip (10–30 μm) and can be stepwise lowered through the benthic boundary layer into the sediment to accurately measure the oxygen gradient at a resolution of about 100 μm. Different methods based on diffusion laws are then available to infer total oxygen flux into the sediment from the steepness of the gradient in the benthic boundary layer and/or sediment. We refer to Glud (2008) for an informative and detailed overview of the different methodologies.

The FOU has principally two components. First is the direct oxygen consumption through respiration of benthic fauna. The second component relates to the enhanced oxygen consumption of the sediment as a result of bioirrigation and other activities by benthic fauna. Bioirrigation is known to stimulate oxygen uptake through enhanced solute exchange and oxygenation of deeper layers of the sediment (Kristensen & Holmer, 2001) and may thus be an important component of the FOU. Quantifying the FOU can be done in different ways. The most straightforward is probably to measure TOU and DOU simultaneously and infer the FOU from the difference (e.g. Archer & Devol, 1992; Glud *et al*., 2003). This approach is only valid in low permeable sediments where the advective component of oxygen uptake (AOU) can justifiably be neglected (Glud *et al*., 2003). As an alternative, a dedicated mesocosm study can be set up in which the different components can be teased apart by following oxygen consumption in different faunal exclusion/inclusion treatments and explicit measurement of faunal respiration. Kristensen and Mikkelsen (2003) executed a mesocosm study in which the faunal contribution to the degradation of different ^{14}C-labelled detritus types was disentangled in great detail. The authors found a stimulus of the degradation of the labelled detritus due to the presence of the bioirrigator *Nereis diversicolor* that was in excess of its contribution to respiration.

Vertical exchange of food to suspension-feeders has been the subject of several studies. Early experiments used tunnels or field mesocosms, measuring the difference in concentrations of chlorophyll *a* or nutrients in inflowing and outflowing water, e.g. Dame *et al*. (1991).The methodology has improved by the use of *in situ* fluorimetry, e.g. Grizzle *et al*. (2008), and by the connection of hydrodynamic measurements with concentration measurements (Jones *et al*., 2009).

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Community-level mass transfer

What benthic organisms feed upon in the sedimentary environment is a particularly difficult question to address, because it is impossible to observe directly what organisms are feeding on because of the presence of the sedimentary matrix. Moreover, smaller macrofauna and meiofauna are too small to allow direct observation of feeding behaviour. One way to investigate feeding relations is to use the principle 'you are what you eat'. This principle has been successfully applied with the use of stable isotopes. Elements may have different isotopes that differ only in the number of neutrons. The isotopes that are not susceptible to radioactive decay are called stable isotopes. The element carbon, for example, occurs in the stable forms of ^{12}C and ^{13}C, nitrogen as ^{14}N and ^{15}N and sulphur as ^{32}S, ^{33}S, ^{34}S and ^{36}S. In this notation, the superscripted number denotes the number of neutrons. The lightest stable isotope is strongly dominant in nature (between 95% and 99%).

For convenience, isotope values are presented in a delta notation, by means of the pro mille deviation from a reference material. The δ^{13}C notation is, for example, used for carbon

$$\delta^{13}\mathrm{C} = \left(\frac{R_S}{R_r} - 1\right) \times 1000$$

in which R_S and R_r are the ^{13}C/^{12}C ratio of the sample and the reference material, respectively.

The isotope values of producers and consumers in a food web are determined by sampling biomass of the respective producers and consumers in the system under study by conventional sampling techniques such as box cores or filtration of water samples. Larger samples are dried and ground with a pestle and mortar, and can be sent to a commercial laboratory for isotope analysis on an Isotope Ratio Mass Spectrometer (IRMS). Note that analysis of δ^{13}C values requires that carbonate shells be removed because the light isotope value of the carbonate disturbs the δ^{13}C values of the flesh. Smaller organisms, such as small macrobenthic or meiobenthic specimens, can be directly transferred to small silver measuring boats that can be directly transferred into the IRMS. However, it is practically impossible to sort microbes from environmental samples in order to measure their isotope composition in a similar way. However, microbes are at the base of many aquatic food webs so that it is important to include their stable isotope composition in food web studies. Hamilton *et al*. (2005) describe a method based on density separation in colloidal silica in which phytoplankton or microphytobenthos can be separated from detritus after which the isotope values can be measured. Though the method did not completely separate the algal from the detrital fraction, nor could it separate different algal species or bacteria, nevertheless, a reasonable efficacy could be achieved. Another method is the use of biomarkers in which biomarkers

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are specific compounds (e.g. specific fatty acids or amino acids) that can be linked to a group or class of microbes. Boschker and Middelburg (2002) describe the use of Phospholipid-Derived Fatty Acids (PLFA) as biomarkers and Veuger *et al.* (2005) use specific amino acids as biomarkers.

The isotopes of three elements are mostly used in food web research: (i) carbon, (ii) nitrogen and, to a lesser extent, (iii) sulphur. The mass differences between isotopes, due to the difference in the number of neutrons, will result in partial separation of the light isotopes from the heavy isotopes during chemical reactions and during physical processes – a process called isotope fractionation. Isotope fractionation has important advantages for the use of stable isotopes in food web research, because an important series of events that foster isotope fractionation occur during a trophic transfer in the food web. Fractionation of ^{13}C and ^{34}S is comparatively limited during trophic transfer and, therefore, the $\delta^{13}C$ and $\delta^{34}S$ signal at the base of the food web is retained through successive transfers in the food web. However, fractionation of ^{15}N is more pronounced and results in a trophic fractionation factor of 2–3.4% (Minagawa & Wada, 1984; Post, 2002; McCutchan *et al*., 2003). Therefore, the increase in $\delta^{15}N$ with respect to the primary resource at the base of the food web is an indication of the trophic position of the organism. Hence, when stable isotope signatures are gathered of the primary producers and consumers in the food web, it becomes feasible to decipher the importance of different producers in a consumers' diet and their trophic position. The stable isotope approach has allowed Peterson and Howarth (1987) to demonstrate that macrobenthos in a salt marsh depended mostly on phytoplankton and *Spartina* detritus, whereas sulphur-oxidising bacteria and terrestrial organic matter were much less utilised. Herman *et al.* (2000) used the $\delta^{13}C$ difference between phytoplankton and microphytobenthos to estimate dependence of an intertidal flat community on both resources. Finally, Rossi *et al*. (2004) used $\delta^{13}C$ and $\delta^{15}N$ values to demonstrate an ontogenetic shift in the life cycle of the bivalve *Macoma balthica*.

The above qualitative reasoning based on isotope values can be formalised by using a mixing model (e.g. Phillips *et al*., 2005). The basic assumption is 'you are what you eat', so that the isotope signature of a consumer is the result of contributions of sources with a different base isotope signatures. Here we follow the notation of Phillips *et al*. (2005) and write a mixing model of two isotopes (with isotope values δ^1 and δ^2) for consumer m that consumes three food sources a, b and c (whose fractions in the diet are denoted by the symbols f_a, f_b and f_c):

$$\begin{aligned}\delta^1_m &= f_a\delta^1_a + f_b\delta^1_b + f_c\delta^1_c\\ \delta^2_m &= f_a\delta^2_a + f_b\delta^2_b + f_c\delta^2_c\\ 1 &= f_a + f_b + f_c\end{aligned}$$

Note that it is assumed that there are only three food sources and hence the fractions must sum to one. The above model requires that the isotope values of the

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consumer *m* and of the three food resources *a*, *b* and *c* are known and on the basis of this information it predicts the diet composition of the consumer. It is clear that the number of food sources that can be distinguished is limited by the number of isotopes that are included in the study. In general, with *n* different isotopes it is possible to determine the contributions of $n + 1$ resources. If the number of sources exceeds $n + 1$, then the model can only return a range of values for the fractions rather than a unique solution (Phillips & Gregg, 2003). The authors Phillips *et al*. (2005) have written the simple and useful software tool IsoSource (http://www.epa.gov/wed/pages/models/stableIsotopes/isosource/isosource.htm; accessed 6 November 2012) that allows diet fractions to be inferred from isotope signatures based on the theory of mixing models. Since then, this software tool has been used in many food web studies of benthic communities (e.g. Kang *et al*., 2007; Jaschinski *et al*., 2008; McLeod & Wing, 2009).

There are several disadvantages to the use of mixing models based on natural abundance isotope values. Sometimes the natural abundance isotope values of some food sources may not be distinct enough to separate food sources sufficiently to allow a mixing model approach. Moreover, in benthic food webs in deep sediments it is impossible to separate the detritus input from the water column from the ambient pool so that it is impossible to determine the dependence on fresh phytodetritus. One way around these problems is to manipulate the isotope signature of a specific food source. The isotope manipulation will propagate through the food web and give important insights into the transfer of this food source through the food web.

Middelburg *et al*. (2000), for example, sprayed ^{13}C-enriched bicarbonate on the surface of an intertidal mudflat during low tide. The microphytobenthos quickly assimilated the inorganic ^{13}C and the authors demonstrated the rapid transfer to nematodes and macrobenthic grazers in the food web. Bacteria also quickly incorporated the ^{13}C probably after assimilating organic excretion products from the microphytobenthos. Herman *et al*. (2000) similarly enriched the microphytobenthos through spraying with ^{13}C-enriched bicarbonate in a flume that was placed on pre-cored box cores. Field-collected water was pre-labelled with ^{15}N-ammonium and was later added to the flume. The differential uptake of ^{13}C and ^{15}N by macrobenthic species clearly separated dependence on benthic versus pelagic algae.

Another application of isotope tracer studies has been applied in deep-sea sediments, where algae are labelled with ^{13}C in laboratory cultures. The algae are collected by centrifugation and stored frozen or freeze-dried as ^{13}C-enriched phytodetritus. This phytodetritus can then be added to deep-sea sediments by submersible (Blair *et al*., 1996) or can be injected into cores during *in situ* (Moodley *et al*., 2002, 2005; Witte *et al*., 2003) or *ex situ* (Woulds *et al*., 2009) incubations. Notwithstanding the difficulty of carrying out such experiments in the deep sea, they have provided valuable information regarding the timing and magnitude of transfer of phytodetritus in deep-sea sediments.

Finally, the isotope tracer methodology has also been used to study the importance of bacteria in the benthic food webs. Pascal *et al*. (2008) cultured bacteria in

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the laboratory and enriched them by using ^{15}N-ammonium in the culture medium. The enriched bacteria were mixed with the top layer of the sediment of an intertidal flat and traced into the nematode community to determine rates of bacterivory. An alternative method to study bacterivory was employed by Van Oevelen *et al*. (2006a) who injected ^{13}C-glucose into the top centimetres of an intertidal flat. Biomarker analysis showed an immediate enrichment of the bacterial community. Subsequent transfer of the ^{13}C from bacteria to the meiobenthic and macrobenthic community was monitored and analysed with an isotope tracer model.

Community-level modelling

The types of measurements that have been discussed in this chapter range from the individual organism through the population to the community level and in fact all address components of the benthic food web albeit with a different level of magnification. In this final paragraph, we present an inverse modelling methodology that allows the integration of different pieces of information on the benthic food web structure. Inverse analysis, or more generally linear inverse modelling, has been developed in the marine sciences to quantify the fluxes between food web components based on incomplete and uncertain data sets (Klepper & Van de Kamer, 1987; Vézina & Platt, 1988). Since then, it has mainly been used in marine (e.g. Jackson & Eldridge, 1992; Niquil *et al*., 1998; Richardson *et al*., 2004; Van Oevelen *et al*., 2006b) and fresh water (Diffendorfer *et al*., 2001; Gaedke *et al*., 2002) sciences.

Recently, several introductory (Soetaert & Van Oevelen, 2009) and overview (Niquil *et al.*, in press) papers have been published that deal in detail with the methodology of linear inverse modelling. Therefore, we limit our methodological discussion here to the basics, but focus on the applicability of the method.

A Linear Inverse Model (LIM) is based on (i) the topological food web model and (ii) the empirical data. The *topological model* is the food web representation in terms of food web compartments and the fluxes between the compartments. Food web compartments in a benthic food web can, for example, be heterotrophic bacteria, deposit-feeders, microphytobenthos and detritus. The food web fluxes involve, among others, deposition of detritus from the water column, grazing of microphytobenthos by deposit-feeders and uptake of detritus by heterotrophic bacteria. The fluxes that are depicted in a food web define the mass balance for each compartment j as

$$\frac{\mathrm{d}C_j}{\mathrm{dt}} = \sum_i F_{ij} - \sum_k F_{jk}$$

in which the accumulation of compound C over time in compartment j is expressed as the difference between sums of the incoming fluxes F_{ij} and that of the outgoing fluxes F_{jk}. A mass balance is said to be in steady state when dC/dt is zero for all

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compartments. In many food web LIMs, the compartments states (mmol C m^{-2}) and fluxes (mmol C m^{-2} d^{-1}) are expressed in units of carbon.

Without any quantitative input to the topological food web model, the fluxes are unconstrained and can in theory range from –infinity to + infinity. In order to constrain this range into more realistic values, empirical data need to be applied to the model. These data can be obtained from the food web under study, but data from the literature can also be used. Van Oevelen *et al*. (2010) define five categories of empirical data: (i) *flux measurements*, (ii) *biomass data*, (iii) *conversion efficiencies*, (iv) *stoichiometry* and (v) *stable isotope signatures*. Flux measurements involve direct information on the magnitude of a flow or a combination of flows. Biomass or stock data designate the amount of carbon present in a compartment and can be used in combination with biomass-specific rate constants such as respiration or production rates to constrain the flux values. Conversion efficiencies relate to the physiology of the organism and typical examples are the assimilation efficiency and net growth efficiency. Stoichiometric data are only used when food web fluxes are resolved in more elements (e.g. C and N) to couple the different element flows. Stable isotope signatures are used to constrain the relative importance of different food sources in the diet of consumer.

The topological food web and five categories of empirical data are cast in a LIM in a linear equality matrix equation $Ax = b$ and a linear inequality matrix equation $Gx \geq h$, in which x is a vector with the food web fluxes. The equality equation expresses the set of mass balances, where the vector b gives the accumulation terms for each mass balance or the measured value in case of empirical data. The inequality equation is used to place upper and/or lower bounds on single flows or combinations of flows. A default set of constraint that is always imposed is that $Ix \geq 0$, so that the fluxes cannot become negative and hence are given a direction. To account for the fact that data that are gathered from the literature may not directly translate to the food web under study, one may implement data in the form of an upper and lower limit (i.e. in the inequality equation) rather than as a fixed equality. For example, one may use the information that the assimilation efficiency of a deposit-feeder ranges between 40% and 75%. Site-specific data can on the other hand also be phrased in terms of an equality equation; the sum of the respiration fluxes in the model can, for example, be equalled to the measured community respiration (TOU; see the section entitled 'Community-level activity'). Soetaert and Van Oevelen (2009) and Van Oevelen *et al*. (2010) provide a detailed explanation and example models on how to implement mass balances and various empirical data in an LIM.

After the topological model and empirical data have been framed in the matrix equation, the model has to be solved. Soetaert and Van Oevelen (2009) discuss three ways to solve the model: (i) by calculating a single solution to the model, (ii) by calculating the flux ranges that are compatible with the model and (iii) by performing a sampling procedure so that a whole spectrum of different, but compatible, solutions can be calculated. Selecting a single solution has been customary

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since the early development of LIM in ecology, but selection criteria that are based on ecological theory are still in development (Vézina *et al*., 2004; Niquil *et al*., in press). Flux ranges provide a means to quantify the uncertainty that is associated with each flux. The sampling methodology is a more sophisticated way of solving the LIM, because it creates a probability distribution for each flux and reveals correlation between food web fluxes. The model set-up and above-mentioned solution procedures can all be performed and triggered within the package LIM (Soetaert & Van Oevelen, 2008) that runs in the software R (R Development Core Team, 2008).

The basic advantage of linear inverse modelling is the combination of various empirical data sources that allows a complete food web structure to be inferred from a comparatively limited set of data. Eldridge and Jackson (1993), for example, obtained detailed information on the carbon and nitrogen fluxes in the food webs of two deep sediments that differed in the oxygenation of the bottom water based on a handful of flux measurements (e.g. O_2 consumption) and standing stock data and a substantial set of literature data. Leguerrier *et al*. (2003) developed a benthic–pelagic coupled model of carbon fluxes in an Atlantic mudflat ecosystem and showed that the benthic food web was mostly driven by primary production of microphytobenthos. Van Oevelen *et al*. (2006b) quantified fluxes in the food web of an intertidal mudflat using an extensive set of empirical data consisting of flux measurements, standing stocks, natural abundance $\delta^{13}C$ signatures and ^{13}C isotope tracer data. An important conclusion was that despite the fact that deposit feeding was a common feeding mode in the benthic community, faunal detritivory was negligible due to this selective feeding and they concluded that the herbivorous and detrital-microbial pathways functioned more or less autonomously, with limited interaction.

In all, linear inverse modelling provides an effective means to gain insight in the structure of benthic food web based on an incomplete and uncertain data set, which is all too often the situation in benthic ecosystem research. However, the methodology allows the uncertainty (i.e. flux ranges) that is associated with the model solution to be quantified. This uncertainty information can be used to the researcher's advantage by using the uncertainty to identify data gaps that can be filled in by dedicated sampling or monitoring.

References

Abello, P., Warman, C.G., Reid, D.G. & Naylor, E. (1994) Chela loss in the shore crab *Carcinus maenas* (Crustacea: Brachyura) and its effect on mating success. *Marine Biology*, **121**, 247–252.

Abrams, B.I. & Mitchell, M.J. (1978) Role of oxygen in affecting survival and activity of *Pelodera-punctata* (Rhabditidae) from sewage sludge. *Nematologica*, **24**, 456–462.

Ahrens, M.J., Hertz, J., Lamoureux, E.M., Lopez, G.R., McElroy, A.E. & Brownawell, B.J. (2001) The effect of body size on digestive chemistry and absorption efficiencies of food

https://telegram.me/Geologybooks

and sediment-bound organic contaminants in *Nereis succinea* (Polychaeta). *Journal of Experimental Marine Biology and Ecology*, **263**, 185–209.

Aksnes, D.L., Miller, C.B., Ohman, M.D. & Wood, S.N. (1997) Estimation techniques used in studies of copepod population dynamics – a review of underlying assumptions. *Sarsia*, **82**, 279–296.

Anderson, L.G., Haraldsson, C. & Lindegren, R. (1992) Gran linearization of potentiometric Winkler titration. *Marine Chemistry*, **37**, 179–190.

Archer, D. & Devol, A. (1992) Benthic oxygen fluxes on the Washington shelf and slope – a comparison of *in situ* microelectrode and chamber flux measurements. *Limnology and Oceanography*, **37**, 614–629.

Atkinson, C.L., First, M.R., Covich, A.P., Opsahl, S.P. & Golladay, S.W. (2011) Suspended material availability and filtration-biodeposition processes performed by a native and invasive bivalve species in streams. *Hydrobiologia*, **667**, 191–204. doi: 10.1007/s10750-011-0640-5.

Banse, K. & Mosher, S. (1980) Adult body mass and annual production/biomass relationships of field populations. *Ecological Monographs*, **50**, 355–379.

Bartlett, M.S. (1970) Age distributions. *Biometrics*, **26**, 377–383.

Bayne, B.L. (1998) The physiology of suspension feeding bivalve molluscs: an introduction to the Plymouth 'TROPHEE' workshop. *Journal of Experimental Marine Biology and Ecology*, **219**, 1–19.

Bayne, B.L. (2004) Comparisons of measurements of clearance rates in bivalve molluscs. *Marine Ecology-Progress Series*, **276**, 305–306.

Beninger, P.G. (2000) A critique of premises and methods in a recent study of particle capture mechanisms in bivalves. *Limnology and Oceanography*, **45**, 1196–1199.

Beninger, P.G. & St-Jean, S.D. (1997) The role of mucus in particle processing by suspension-feeding marine bivalves: unifying principles. *Marine Biology*, **129**, 389–397.

Benke, A.C. (1979) A modification of the Hynes method for estimating secondary production with particular significance for multivoltine populations. *Limnology and Oceanography*, **24**, 168–171.

Benke, A.C. & Waide, J.B. (1977) In defense of average cohorts. *Freshwater Biology*, **7**, 61–63.

Berg, P. & Huettel, M. (2008) Monitoring the seafloor using the noninvasive eddy correlation technique: integrated benthic exchange dynamics. *Oceanography*, **21**, 164–167.

Berg, P., Roy, H. & Wiberg, P.L. (2007) Eddy correlation flux measurements: the sediment surface area that contributes to the flux. *Limnology and Oceanography*, **52**, 1672–1684.

Berg, P., Roy, H., Janssen, F., Meyer, V., Jorgensen, B.B., Huettel, M. & de Beer, D. (2003) Oxygen uptake by aquatic sediments measured with a novel non-invasive eddy-correlation technique. *Marine Ecology Progress Series*, **261**, 75–83.

Berg, P., Glud, R.N., Hume, A., Stahl, H., Oguri, K., Meyer, V. & Kitazato, H. (2009) Eddy correlation measurements of oxygen uptake in deep ocean sediments. *Limnology and Oceanography: Methods*, **7**, 576–584.

Berner, R.A. (1982) Burial of organic carbon and pytite sulfur in the modern ocean: its geochemical and environmental significance. *American Journal of Science*, **282**, 451–473.

Beukema, J.J. (1997) Caloric values of marine invertebrates with an emphasis on the soft parts of marine bivalves. *Oceanography and Marine Biology: An Annual Review*, **35**, 387–414.

https://telegram.me/Geologybooks

Bjorndal, K.A. & Bolten, A.B. (1995) Comparison of length-frequency analyses for estimation of growth parameters for a population of green turtles. *Herpetologica*, **51**, 160–167.

Blair, N.E., Levin, L.A., DeMaster, D.J. & Plaia, G. (1996) The short-term fate of fresh algal carbon in continental slope sediments. *Limnology and Oceanography*, **41**, 1208–1219.

Bligh, E.G. & Dyer, W. (1959) A rapid method for total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, **37**, 911–917.

Bos, O.G., Hendriks, I.E., Strasser, M., Dolmer, P. & Kamermans, P. (2006) Estimation of food limitation of bivalve larvae in coastal waters of north-western Europe. *Journal of Sea Research*, **55**, 191–206. doi: 10.1016/j.seares.2005.10.006.

Boschker, H.T.S. & Middelburg, J.J. (2002) Stable isotopes and biomarkers in microbial ecology. *FEMS Microbiology Ecology*, **40**, 85–95.

Bourles, Y., Alunno-Bruscia, M., Pouvreau, S., Tollu, G., Leguay, D., Arnaud, C., Goulletquer, P. & Kooijman, S. (2009) Modelling growth and reproduction of the Pacific oyster *Crassostrea gigas*: advances in the oyster-DEB model through application to a coastal pond. *Journal of Sea Research*, **62**, 62–71. doi: 10.1016/j.seares.2009.03.002.

Braeckman, B.P., Houthoofd, K. & Vanfleteren, J.R. (2001) Insulin-like signaling, metabolism, stress resistance and aging in *Caenorhabditis elegans*. *Mechanisms of Ageing and Development*, **122**, 673–693.

Brey, T. (1986) Formalin and formaldehyde-depot chemicals: effects on dry weight and ash-free dry weight of 2 marine bivalve species. *Meeresforschung*, **31**, 52–57.

Brey, T. (1990) Estimating productivity of macrobenthic invertebrates from biomass and mean individual weight. *Meeresforschung*, **32**, 329–343.

Brey, T. (1995) Temperature and reproductive metabolism in macrobenthic populations. *Marine Ecology Progress Series*, **125**, 87–93.

Brey, T. (1999) A collection of empirical relations for use in ecological modelling. *Naga*, **22**, 24–28.

Brey, T., Rumohr, H.I. & Ankar, S. (1988) Energy content of macrobenthic invertebrates: general conversion factors from weight to energy. *Journal of Experimental Marine Biology and Ecology*, **117**, 271–278.

Brey, T., Muller-Wiegmann, C., Zittier, Z.M.C. & Hagen, W. (2010) Body composition in aquatic organisms – a global data bank of relationships between mass, elemental composition and energy content. *Journal of Sea Research*, **64**, 334–340. doi: 10.1016/j.seares.2010.05.002.

Bridges, T.S. (1996) Effects of organic additions to sediment, and maternal age and size, on patterns of offspring investment and performance in two opportunistic deposit-feeding polychaetes. *Marine Biology*, **125**, 345–357.

Brockington, S. & Clarke, A. (2001) The relative influence of temperature and food on the metabolism of a marine invertebrate. *Journal of Experimental Marine Biology and Ecology*, **258**, 87–99.

Buffan-Dubau, E. & Carman, K.R. (2000) Diel feeding behavior of meiofauna and their relationships with microalgal resources. *Limnology and Oceanography*, **45**, 381–395.

Buffan-Dubau, E., deWit, R. & Castel, J. (1996) Feeding selectivity of the harpacticoid copepod *Canuella perplexa* in benthic muddy environments demonstrated by HPLC analyses of chlorin and carotenoid pigments. *Marine Ecology-Progress Series*, **137**, 71–82. doi: 10.3354/meps137071.

https://telegram.me/Geologybooks

Calow, P. & Fletcher, C.R. (1972) A new radiotracer technique involving ^{14}C and ^{51}Cr for estimating the assimilation efficiency of aquatic primary producers. *Oecologia*, **9**, 155–170.

Cammen, L.M. (1980) A method for measuring ingestion rate of deposit feeders and its use with the polychaete *Nereis succinea*. *Estuaries*, **3**, 55–60.

Campbell, J.E. & Fourqurean, J.W. (2011) Novel methodology for *in situ* carbon dioxide enrichment of benthic ecosystems. *Limnology and Oceanography: Methods*, **9**, 97–109. doi: 10.4319/lom.2011.9.97.

Campos, J., van der Veer, H.W., Freitas, V. & Kooijman, S. (2009) Contribution of different generations of the brown shrimp *Crangon crangon* (L.) in the Dutch Wadden Sea to commercial fisheries: a dynamic energy budget approach. *Journal of Sea Research*, **62**, 106–113. doi: 10.1016/j.seares.2009.07.007.

Cardoso, J., van der Veer, H.W. & Kooijman, S. (2006) Body-size scaling relationships in bivalve species: a comparison of field data with predictions by the Dynamic Energy Budget (DEB) theory. *Journal of Sea Research*, **56**, 125–139. doi: 10.1016/j.seares.2006.05.001.

Carpenter, J.H. (1965) The Chesapeake Bay Institute technique for the Winkler dissolved oxygen method. *Limnology and Oceanography*, **10**, 141–143.

Carroll, M.L., Johnson, B.J., Henkes, G.A., McMahon, K.W., Voronkov, A., Ambrose, W.G., Jr & Denisenko, S.G. (2009) Bivalves as indicators of environmental variation and potential anthropogenic impacts in the southern Barents Sea. *Marine Pollution Bulletin*, **59**, 193–206. doi: 10.1016/j.marpolbul.2009.02.022.

Cartes, J.E. & Maynou, F. (1998) Food consumption by bathyal decapod crustacean assemblages in the western Mediterranean: predatory impact of megafauna and the food consumption – food supply balance in a deep-water food web. *Marine Ecology Progress Series*, **171**, 233–246.

Chapelle, A., Menesguen, A., Deslous-Paoli, J.M., Souchu, P., Mazouni, N., Vaquer, A. & Millet, B. (2000) Modelling nitrogen, primary production and oxygen in a Mediterranean lagoon. Impact of oysters farming and inputs from the watershed. *Ecological Modelling*, **127**, 161–181.

Charles, F., Gremare, A. & Amouroux, J.M. (1995) Utilization of C-14 formaldehyde to infer ingestion rates and absorption efficiencies by benthic deposit-feeders. *Marine Ecology Progress Series*, **127**, 121–129.

Clark, L.C. (1956) Monitor and control of blood and tissue oxygen tensions. *Transactions American Society for Artificial Internal Organs*, **2**, 41–48.

Clarke, A. (1987) Temperature, latitude and reproductive effort. *Marine Ecology Progress Series*, **38**, 89–99.

Clarke, A. (1993) Egg size and egg composition in polar shrimps (Caridea, Decapoda). *Journal of Experimental Marine Biology and Ecology*, **168**, 189–203.

Clarke, A. & Prothero-Thomas, E. (1997) The influence of feeding on oxygen consumption and nitrogen excretion in the Antarctic nemertean *Parborlasia corrugatus*. *Physiological Zoology*, **70**, 639–649.

Conover, R.J. (1966) Assimilation of organic matter by zooplankton. *Limnology and Oceanography*, **11**, 338–345.

Cortés, E. (1996) A critical review of methods of studying fish feeding based on analysis of stomach contents: application to elasmobranch fishes. *Canadian Journal of Fisheries and Aquatic Sciences*, **54**, 726–738.

https://telegram.me/Geologybooks

Cote, J., Rakocinski, C.F. & Randall, T.A. (2001) Feeding efficiency by juvenile blue crabs on two common species of micrograzer snails. *Journal of Experimental Marine Biology and Ecology*, **264**, 189–208.

Cowie, G.L. & Hedges, J.I. (1992) Improved amino-acid quantification in environmental samples – charge-matched recovery standards and reduced analysis time. *Marine Chemistry*, **37**, 223–238.

Craig, J.F., Kenley, M.J. & Talling, J.F. (1978) Comparative estimations of the energy content of fish tissue from bomb calorimetry, wet oxidation and proximate analysis. *Freshwater Biology*, **8**, 585–590. doi: 10.1111/j.1365-2427.1978.tb01480.x.

Cranford, P.J. & Hargrave, B.T. (1994) In situ time-series measurement of ingestion and absorption rates of suspension-feeding bivalves: *Placopecten magellanicus*. *Limnology and Oceanography*, **39**, 730–738.

Cranford, P.J., Emerson, C.W., Hargrave, B.T. & Milligan, T.G. (1998) In situ feeding and absorption responses of sea scallops *Placopecten magellanicus* (Gmelin) to storm-induced changes in the quantity and composition of the seston. *Journal of Experimental Marine Biology and Ecology*, **219**, 45–70.

Crawford, D.W., Rogerson, A. & Laybourn-Parry, J. (1994) Respiration of the marine amoeba *Trichosphaerium sieboldi* determined by ^{14}C labelling and Cartesian diver methods. *Marine Ecology Progress Series*, **112**, 135–142.

Crisp, D.J. (1984) Energy flow measurements. In: *Methods for the Study of Marine Benthos* (eds N.A. Holme & A.D. McIntyre), Blackwell Publishing Ltd., Oxford, pp 284–372.

Crompton, T.R. (1989) *Analysis of Seawater*. Butterworths, London.

Cummings, V., Hewitt, J., Van Rooyen, A., Currie, K., Beard, S., Thrush, S., Norkko, J., Barr, N., Heath, P., Halliday, N.J., Sedcole, R., Gomez, A., McGraw, C. & Metcalf, V. (2011) Ocean acidification at high latitudes: potential effects on functioning of the antarctic bivalve *Laternula elliptica*. *Plos One*, **6**, e16069. doi: 10.1371/journal.pone.0016069.

D'Andrea, A.F., Aller, R.C. & Lopez, G.R. (2002) Organic matter flux and reactivity on a South Carolina sandflat: the impacts of porewater advection and macrobiological structures. *Limnology and Oceanography*, **47**, 1056–1070.

Dahlhoff, E.P., Buckley, B.A. & Menge, B.A. (2001) Physiology of the rocky intertidal predator *Nucella ostrina* along an environmental stress gradient. *Ecology*, **82**, 2816–2829.

Dame, R., Dankers, N., Prins, T., Jongsma, H. & Smaal, A. (1991) The influence of mussel beds on nutrients in the western Wadden Sea and eastern Scheldt Estuaries. *Estuaries*, **14**, 130–138.

Danovaro, R., Dell'Anno, A., Martorano, D., Parodi, P., Marrale, N.D. & Fabiano, M. (1999) Seasonal variation in biochemical composition of deep-sea nematodes: bioenergetic and methodological considerations. *Marine Ecology Progress Series*, **179**, 273–283.

Davies, M.S. & Williams, G.A. (1995) Pedal mucus of a tropical limpet, *Cellana grata* (Gould): energetics, production and fate. *Journal of Experimental Marine Biology and Ecology*, **186**, 77–87.

Davies, M.S. & Hawkins, S.J. (1998) Mucus from marine molluscs. *Advances in Marine Biology*, **34**, 1–71.

Davies, M.S. & Beckwith, P. (1999) Role of mucus trails and trail-following in the behaviour and nutrition of the periwinkle *Littorina littorea*. *Marine Ecology Progress Series*, **179**, 247–257.

https://telegram.me/Geologybooks

de Beer, D., Wenzhofer, F., Ferdelman, T.G., Boehme, S.E., Huettel, M., van Beusekom, J.E.E., Bottcher, M.E., Musat, N. & Dubilier, N. (2005) Transport and mineralization rates in North Sea sandy intertidal sediments, Sylt-Romo Basin, Wadden Sea. *Limnology and Oceanography*, **50**, 113–127.

De Goeij, P. & Luttikhuizen, P.C. (1998) Deep-burying reduces growth in intertidal bivalves: field and mesocosm experiments with *Macoma balthica*. *Journal of Experimental Marine Biology and Ecology*, **228**, 327–337.

De Goeij, P., Luttikhuizen, P.C., van der Meer, J. & Piersma, T. (2001) Facilitation on an intertidal mudflat: the effect of siphon nipping by flatfish on burying depth of the bivalve *Macoma balthica*. *Oecologia*, **126**, 500–506.

De Troch, M., Houthoofd, L., Chepurnov, V. & Vanreusel, A. (2006) Does sediment grain size affect diatom grazing by harpacticoid copepods? *Marine Environmental Research*, **61**, 265–277. doi: 10.1016/j.marenvres.2005.10.004.

De Vlas, J. (1979a) Annual food intake by plaice and flounder in a tidal flat area in the Dutch Wadden Sea, with special reference to consumption of regenerating parts of macrobenthic prey. *Netherlands Journal of Sea Research*, **13**, 117–153.

De Vlas, J. (1979b) Secondary production by tail regeneration in a tidal flat population of lugworms (*Arenicola marina*), cropped by flatfish. *Netherlands Journal of Sea Research*, **13**, 362–393.

Debovee, F. & Labat, J.P. (1993) A simulation-model of a deep meiobenthic compartment – a preliminary approach. *Marine Ecology (Pubblicazioni Della Stazione Zoologica Di Napoli I)*, **14**, 159–173.

DeGrandpre, M.D., Baehr, M.M. & Hammar, T.R. (1999) Calibration-free optical chemical sensors. *Analytical Chemistry*, **71**, 1152–1159.

Diffendorfer, J.E., Richards, P.M., Dalrymple, G.H. & DeAngelis, D.L. (2001) Applying linear programming to estimate fluxes in ecosystems or food webs: an example from the herpetological assemblage of the freshwater Everglades. *Ecological Modelling*, **144**, 99–120.

Duchemin, M.B., Wessel, N., Fournier, M. & Auffret, M. (2008) Flow cytometric measurement of the clearance rate in the blue mussel *Mytilus edulis* and the development of a new individual exposure system for aquatic immunotoxicological studies. *Environmental Pollution*, **153**, 492–496. doi: 10.1016/j.envpol.2007.08.017.

Edmondson, T.W. (1960) Reproductive rates of rotifers in natural populations. *Memorie dell'Istituto Italiano di Idrobiologia*, **12**, 21–77.

Ejdung, G., Byrén, L. & Elmgren, R. (2000) Benthic predator-prey interactions: evidence that adult *Monoporeia affinis* (Amphipoda) eat postlarvel *Macoma balthica* (Bivalvia). *Journal of Experimental Marine Biology and Ecology*, **253**, 243–251.

Ekaratne, S.U.K. & Crisp, D.J. (1982) Tidal micro-growth bands in intertidal gastropod shells, with an evaluation of band-dating techniques. *Proceedings of the Royal Society of London, Series B*, **214**, 305–323.

Eldridge, P.M. & Jackson, G.A. (1993) Benthic trophic dynamics in California coastal basin and continental slope communities inferred using inverse analysis. *Marine Ecology Progress Series*, **99**, 115–135.

Elliot, J.M. & Davison, W. (1975) Energy equivalents of oxygen consumption in animal energetics. *Oecologia*, **19**, 195–201.

Enminger, A. (1995) *The Concise Encyclopedia of Foods and Nutrition*. Taylor & Francis (CRC Press), Boca Raton, FL.

https://telegram.me/Geologybooks

Fager, E.W. (1969) Production of stream benthos: a critique of the method of assessment proposed by Hynes and Coleman (1968). *Limnology and Oceanography*, **14**, 766–770.

Famme, P., Riisgård, H.U. & Jørgensen, C.B. (1986) On direct measurements of pumping rates in the mussel *Mytilus edulis*. *Marine Biology*, **92**, 323–327.

Feller, R.J. & Warwick, R.M. (1988) Energetics. In: *Introduction to the Study of Meiofauna* (eds R. P. Higgins & H. Thiel), Smithsonian Institute, Washington, DC, pp 181–196.

Filgueira, R., Labarta, U. & Fernandez-Reiriz, M.J. (2006) Flow-through chamber method for clearance rate measurements in bivalves: design and validation of individual chambers and mesocosm. *Limnology and Oceanography: Methods*, **4**, 284–292.

Finlay, B.J. & Uhlig, G. (1981) Calorific and carbon values of marine and fresh-water protozoa. *Helgolander Meeresuntersuchungen*, **34**, 401–412. doi: 10.1007/bf01995913.

Forbes, T.L. & Lopez, G.R. (1990) Ontogenic changes in individual growth and egestion rates in the deposit-feeding polychaete *Capitella* sp 1. *Journal of Experimental Marine Biology and Ecology*, **143**, 209–220.

Forbes, V.E. & Lopez, G.R. (1989) The role of sediment particle-size in the nutritional energetics of a surface deposit-feeder. 1. Ingestion and absorption of sedimentary microalgae by *Hydrobia truncata (vanatta)*. *Journal of Experimental Marine Biology and Ecology*, **126**, 181–192.

Forseth, T., Jonsson, B., Neumann, R. & Ugedal, O. (1992) Radioisotope method for estimating food consumption by brown trout (*Salmo trutta*). *Canadian Journal of Fisheries and Aquatic Sciences*, **49**, 1328–1335.

Fournier, D.A., Sibert, J.R. & Terceiro, M. (1991) Analysis of length frequency samples with relative abundance data for the Gulf of Maine northern shrimp (*Pandalus borealis*) by the MULTIFAN method. *Canadian Journal of Fisheries and Aquatic Sciences*, **48**, 591–598.

Fournier, D.A., Hampton, J. & Sibert, J.R. (1997) MULTIFAN-CL: a length-based, age-structured model for fisheries stock assessment, with application to South Pacific albacore, *Thunnus alalunga*. *Canadian Journal of Fisheries and Aquatic Sciences*, **55**, 2105–2116.

Fournier, D.A., Sibert, J.R., Majkowski, J. & Hampton, J. (1990) MULTIFAN: a likelihood-based method for estimating growth parameters and age composition from multiple length frequency data sets illustrated using data for southern bluefin tuna (*Thunnus maccoyii*). *Canadian Journal of Fisheries and Aquatic Sciences*, **47**, 301–317.

Fraschetti, S., Robertson, M., Albertelli, G., Capelli, R. & Eleftheriou, A. (1994) Calorimetry: use of the Philipson microbomb. *Oebalia*, **20**, 117–127.

Frederich, M. & Portner, H.O. (2000) Oxygen limitation of thermal tolerance defined by cardiac and ventilatory performance in spider crab, *Maja squinado*. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology*, **279**, R1531–R1538.

Freitas, V., Cardoso, J., Lika, K., Peck, M.A., Campos, J., Kooijman, S. & van der Veer, H.W. (2010) Temperature tolerance and energetics: a dynamic energy budget-based comparison of North Atlantic marine species. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **365**, 3553–3565. doi: 10.1098/rstb.2010.0049.

Freitas, V., Cardoso, J., Santos, S., Campos, J., Drent, J., Saraiva, S., Witte, J.I.J., Kooijman, S. & van der Veer, H.W. (2009) Reconstruction of food conditions for Northeast Atlantic bivalve species based on Dynamic Energy Budgets. *Journal of Sea Research*, **62**, 75–82. doi: 10.1016/j.seares.2009.07.004.

https://telegram.me/Geologybooks

Frost, B.W. (1972) Effects of size and concentration of food particles on feeding behavior of marine planktonic copepod *Calanus pacificus*. *Limnology and Oceanography*, **17**, 805–815.

Gaedke, U., Hochstadter, S. & Straile, D. (2002) Interplay between energy limitation and nutritional deficiency: empirical data and food web models. *Ecological Monographs*, **72**, 251–270.

Gallucci, F., Steyaert, M. & Moens, T. (2005) Can field distributions of marine predacious nematodes be explained by sediment constraints on their foraging success? *Marine Ecology-Progress Series*, **304**, 167–178. doi: 10.3354/meps304167.

Gatti, S., Brey, T., Muller, W.E.G., Heilmayer, O. & Holst, G. (2002) Oxygen microoptodes: a new tool for oxygen measurements in aquatic animal ecology. *Marine Biology*, **140**, 1075–1085. doi: 10.1007/s00227-002-0786-9.

Gayanilo, F.C., Sparre, P. & Pauly, D. (1996) The FAO-ICLARM stock assessment tools (FiSAT) user's guide. *FAO Computerized Information Series*, **6**, 1–186.

Gerchacov, S.M. & Hatcher, P.G. (1972) Improved technique for analysis of carbohydrates in sediments. *Limnology and Oceanography*, **17**, 938–943.

Gerlach, S.A. (1971) Importance of marine meiofauna for benthos communities. *Oecologia (Berlin)*, **6**, 176–190. doi: 10.1007/bf00345719.

Gillespie, D.M. & Benke, A.C. (1979) Methods of calculating cohort production from field data – some relationships. *Limnology and Oceanography*, **24**, 171–176.

Gingras, J. & Boisclair, D. (2000) Comparison between consumption rates of yellow perch (*Perca flavescens*) estimated with a digestive tract model and with a radioisotope approach. *Canadian Journal of Fisheries and Aquatic Sciences*, **57**, 2547–2557.

Glud, R.N. (2008) Oxygen dynamics of marine sediments. *Marine Biology Research*, **4**, 243–289. doi: 10.1080/17451000801888726.

Glud, R.N., Ramsing, N.B., Gundersen, J.K. & Klimant, I. (1996) Planar optrodes: a new tool for fine scale measurements of two-dimensional O_2 distribution in benthic communities. *Marine Ecology Progress Series*, **140**, 217–226.

Glud, R.N., Kuhl, M., Kohls, O. & Ramsing, N.B. (1999a) Heterogeneity of oxygen production and consumption in a photosynthetic microbial mat as studied by planar optodes. *Journal of Phycology*, **35**, 270–279.

Glud, R.N., Gundersen, J.K., Roy, H. & Jorgensen, B.B. (2003) Seasonal dynamics of benthic O-2 uptake in a semienclosed bay: importance of diffusion and faunal activity. *Limnology and Oceanography*, **48**, 1265–1276.

Glud, R.N., Klimant, I., Holst, G., Kohls, O., Meyer, V., Kühl, M. & Gundersen, J.K. (1999b) Adaption, test and in situ measurements with O_2 microopt(r)odes on benthic landers. *Deep-Sea Research Part I: Oceanographic Research Papers*, **46**, 171–183.

Gnaiger, E. & Bitterlich, G. (1984) Proximate biochemical composition and caloric content calculated from elemental CHN analysis: a stoichiometric concept. *Oecologia*, **62**, 289–298.

Grasshoff, K., Ehrhardt, M. & Kremling, K. (1983) *Method of Seawater Analysis*, 2nd edn. Verlag Chemie, Weinheim.

Grémare, A., Marsh, A.G. & Tenore, K.R. (1989) Fecundity and energy partitioning in *Capitella capitata* type I (Annelida: Polychaeta). *Marine Biology*, **100**, 365–371.

Grizzle, R.E., Greene, J.K. & Coen, L.D. (2008) Seston removal by natural and constructed intertidal eastern oyster (Crassostrea virginica) reefs: a comparison with previous

https://telegram.me/Geologybooks

laboratory studies, and the value of in situ methods. *Estuaries and Coasts*, **31**, 1208–1220. doi: 10.1007/s12237-008-9098-8.

Haller, T., Ortner, M. & Gnaiger, E. (1994) A respirometer for investigating oxidative cell-metabolism – toward optimization of respiratory studies. *Analytical Biochemistry*, **218**, 338–342.

Hamburger, K. (1981) A gradient diver for measurement of respiration in individual organisms from the micro- and meiofauna. *Marine Biology*, **61**, 179–183.

Hamilton, A.L. (1969) On estimating annual production. *Limnology and Oceanography*, **14**, 771–782.

Hamilton, S.K., Sippel, S.J. & Bunn, S.E. (2005) Separation of algae from detritus for stable isotope or ecological stoichiometry studies using density fractionation in colloidal silica. *Limnology and Oceanography: Methods*, **3**, 149–157.

Harley, M.B. (1950) Occurrence of a filter-feeding mechanism in the polychaete *Nereis diversicolor*. *Nature*, **165**, 734–735.

Hartree, E.F. (1972) Determination of proteins: a modification of the Lowry method that gives a linear photometric response. *Analytical Biochemistry*, **48**, 422–427.

Hatcher, A. (1994) Nitrogen and phosphorus turnover in some benthic marine invertebrates: implication for the use of C:N ratios to assess food quality. *Marine Biology*, **121**, 161–166.

Hawkins, A.J.S., Smith, R.F.M., Bayne, B.L. & Héral, M. (1996) Novel observations underlying the fast growth of suspension-feeding shellfish in turbid environments: *Mytilus edulis*. *Marine Ecology Progress Series*, **131**, 179–190.

Hawkins, A.J.S., Bayne, B.L., Bougrier, S., Héral, M., Iglesias, I.P., Navarro, E., Smith, R.F.M. & Urrutia, M.B. (1998) Some general relationships in comparing the feeding physiology of suspension-feeding bivalve molluscs. *Journal of Experimental Marine Biology and Ecology*, **219**, 87–103.

Heip, C., Smol, N. & Absillis, V. (1978) Influence of temperature on reproductive potential of *Oncholaimus oxyuris* (Nematoda Oncholaimidae). *Marine Biology*, **45**, 255–260. doi: 10.1007/bf00390608.

Heip, C., Vincx, M. & Vranken, G. (1985) The ecology of marine nematodes. *Oceanography and Marine Biology*, **23**, 399–489.

Hendriks, I.E., Duarte, C.M. & Alvarez, M. (2010) Vulnerability of marine biodiversity to ocean acidification: a meta-analysis. *Estuarine Coastal and Shelf Science*, **86**, 157–164. doi: 10.1016/j.ecss.2009.11.022.

Herman, P.M.J. & Vranken, G. (1988) Studies of the life-history and energetics of marine and brackish-water nematodes. *Oecologia*, **77**, 457–463.

Herman, P.M.J., Heip, C. & Vranken, G. (1983) The production of *Cyprideis torosa* Jones 1850 (Crustacea, Ostracoda). *Oecologia*, **58**, 326–331.

Herman, P.M.J., Vranken, G. & Heip, C. (1984) Problems in meiofauna energy-flow studies. *Hydrobiologia*, **118**, 21–28. doi: 10.1007/bf00031784.

Herman, P.M.J., Middelburg, J.J., Widdows, J., Lucas, C.H. & Heip, C.H.R. (2000) Stable isotopes as trophic tracers: combining field sampling and manipulative labelling of food resources for macrobenthos. *Marine Ecology Progress Series*, **204**, 79–92.

Hiddink, J.G., ter Hofstede, R. & Wolff, W.J. (2002) Predation of intertidal infauna on juveniles of the bivalve *Macoma balthica*. *Journal of Sea Research*, **47**, 141–159.

https://telegram.me/Geologybooks

Hildreth, D.I. & Crisp, D.J. (1976) Corrected formula for calculation of filtration-rate of bivalve mollusks in an experimental flowing system. *Journal of the Marine Biological Association of the United Kingdom*, **56**, 111–120.

Holst, G. & Grunwald, B. (2001) Luminescence lifetime imaging with transparent oxygen optodes. *Sensors and Actuators B – Chemical*, **74**, 78–90.

Holter, H. & Zeuthen, E. (1966) Manometric techniques for single cells. In: *Physical Techniques in Biological Research* (eds G. Oster & A. W. Pollister), Academic Press, New York, pp 251–317.

Honkoop, P.J.C. & van der Meer, J. (1998) Experimentally induced effects of water temperature and immersion time on reproductive output of bivalves in the Wadden Sea. *Journal of Experimental Marine Biology and Ecology*, **220**, 227–246.

Honkoop, P.J.C., Luttikhuizen, P.C. & Piersma, T. (1999) Experimentally extending the spawning season of a marine bivalve using temperature change and fluoxetine as synergistic triggers. *Marine Ecology Progress Series*, **180**, 297–300.

Horn, P.L. (1986) Energetics of *Chiton pelliserpentis* (Quoy & Gaimard, 1835) (Mollusca: Polyplacophora) and the importance of mucus on its energy budget. *Journal of Experimental Marine Biology and Ecology*, **101**, 119–141.

Hurd, C.L., Hepburn, C.D., Currie, K.I., Raven, J.A. & Hunter, K.A. (2009) Testing the effects of ocean acidification on algal metabolism: considerations for experimental designs. *Journal of Phycology*, **45**, 1236–1251. doi: 10.1111/j.1529-8817.2009.00768.x.

Hynes, H.B.N. & Coleman, M.J. (1968) A simple method of assessing the annual production of stream benthos. *Limnology and Oceanography*, **13**, 569–573.

Irwin, S., Wall, V. & Davenport, J. (2007) Measurement of temperature and salinity effects on oxygen consumption of *Artemia franciscana K.*, measured using fibre-optic oxygen microsensors. *Hydrobiologia*, **575**, 109–115. doi: 10.1007/s10750-006-0358-y.

Jackson, G.A. & Eldridge, P.M. (1992) Food web analysis of a planktonic system off Southern California. *Progress in Oceanography*, **30**, 223–251.

Jaschinski, S., Brepohl, D.C. & Sommer, U. (2008) Carbon sources and trophic structure in an eelgrass *Zostera marina* bed, based on stable isotope and fatty acid analyses. *Marine Ecology-Progress Series*, **358**, 103–114. doi: 10.3354/meps07327.

Jensen, P. (1984) Measuring carbon content in nematodes. *Helgoländer Meeresuntersuchungen*, **38**, 83–86.

Jones, H.D., Richards, O.G. & Southern, T.A. (1992) Gill dimensions, water pumping rate and body size in the mussel *Mytilus edulis*. *Journal of Experimental Marine Biology and Ecology*, **155**, 213–237.

Jones, N.L., Thompson, J.K., Arrigo, K.R. & Monismith, S.G. (2009) Hydrodynamic control of phytoplankton loss to the benthos in an estuarine environment. *Limnology and Oceanography*, **54**, 952–969.

Jørgensen, C.B. (1966) *Biology of Suspension Feeding*. Pergamon Press, Oxford.

Jørgensen, C.B. (1996) Bivalve filter feeding revisited. *Marine Ecology Progress Series*, **142**, 287–302.

Kang, C.K., Choy, E.J., Paik, S.K., Park, H.J., Lee, K.S. & An, S. (2007) Contributions of primary organic matter sources to macroinvertebrate production in an intertidal salt marsh (*Scirpus triqueter*) ecosystem. *Marine Ecology-Progress Series*, **334**, 131–143.

Kautsky, H. (1939) Quenching of luminescence by oxygen. *Transactions of the Faraday Society*, **35**, 216–219.

https://telegram.me/Geologybooks

Kim, S.B., Cho, H.C., Cha, G.S. & Nam, H. (1998) Microtiter plate-format optode. *Analytical Chemistry*, **70**, 4860–4863.

Kiørboe, T. & Møhlenberg, F. (1981) Particle selection in suspension-feeding bivalves. *Marine Ecology Progress Series*, **5**, 291–296.

Klekowski, R.Z. (1971) Cartesian diver microrespirometry for aquatic animals. *Polskie Archiwum Hydrobiologii*, **18**, 93–144.

Klepper, O. & Van de Kamer, J.P.G. (1987) The use of mass balances to test and improve the estimates of carbon fluxes in an ecosystem. *Mathematical Biosciences*, **85**, 37–49.

Klimant, I., Meyer, V. & Kühl, M. (1995) Fiber-optic oxygen microsensors, a new tool in aquatic biology. *Limnology and Oceanography*, **40**, 1159–1165.

Klimant, I., Ruckruh, F., Liebsch, G., Stangelmayer, C. & Wolfbeis, O.S. (1999) Fast response oxygen micro-optodes based on novel soluble ormosil glasses. *Mikrochimica Acta*, **131**, 35–46.

Kofoed, L., Forbes, V.E. & Lopez, G. (1989) Time-dependent absorption in deposit feeders. In: *Ecology of Marine Deposit Feeders* (eds G.R. Lopez, G.L.Taghon & J.S. Levinton), Springer-Verlag, New York, pp 129–148.

Kohls, O. & Scheper, T. (2000) Setup of a fiber optical oxygen multisensor-system and its applications in biotechnology. *Sensors and Actuators B - Chemical*, **70**, 121–130.

Kooijman, S. (2006) Pseudo-faeces production in bivalves. *Journal of Sea Research*, **56**, 103–106. doi: 10.1016/j.seares.2006.03.003.

Kooijman, S.A.L.M. (2010) *Dynamic Energy Budget Theory for Metabolic Organization*, 3rd edn. Cambridge University Press, Cambridge.

Kristensen, E. & Holmer, M. (2001) Decomposition of plant materials in marine sediment exposed to different electron accepters (O_2, NO_3^-, and SO_4^{2-}), with emphasis on substrate origin, degradation kinetics, and the role of bioturbation. *Geochimica Et Cosmochimica Acta*, **65**, 419–433.

Kristensen, E. & Mikkelsen, O.L. (2003) Impact of the burrow-dwelling polychaete *Nereis diversicolor* on the degradation of fresh and aged macroalgal detritus in a coastal marine sediment. *Marine Ecology-Progress Series*, **265**, 141–153.

Kroeker, K.J., Kordas, R.L., Crim, R.N. & Singh, G.G. (2010) Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. *Ecology Letters*, **13**, 1419–1434. doi: 10.1111/j.1461-0248.2010.01518.x.

Kryger, J. & Riisgård, H.U. (1988) Filtration rate capacities in 6 species of European freshwater bivalves. *Oecologia*, **77**, 34–38.

Lasserre, P. (1976) Metabolic activities of benthic microfauna and meiofauna: recent advances and suitable methods of analysis. In: *The Benthic Boundary Layer* (ed. I.N. McCave), Plenum Publishing Corporation, New York, pp 95–142.

Lavrentyev, P.J., Gardner, W.S. & Yang, L.Y. (2000) Effects of the zebra mussel on nitrogen dynamics and the microbial community at the sediment-water interface. *Aquatic Microbial Ecology*, **21**, 187–194.

Lawrence, J.M. & Vasquez, J. (1996) The effect of sublethal predation on the biology of echinoderms. *Oceanologica Acta*, **19**, 431–440.

Lawrence, J.M., Byrne, M., Harris, L., Keegan, B., Freeman, S. & Cowell, B. (1999) Sublethal arm loss in *Asterias amurensis*, *A. rubens*, *A. vulgaris* and *A. forbesi* (Echinodermata: Asterioidea). *Vie et Milieu*, **49**, 69–73.

https://telegram.me/Geologybooks

Leguerrier, D., Niquil, N., Boileau, N., Rzeznik, J., Sauriau, P.G., Le Moine, O. & Bacher, C. (2003) Numerical analysis of the food web of an intertidal mudflat ecosystem on the Atlantic coast of France. *Marine Ecology Progress Series*, **246**, 17–37.

Lehtonen, K.K. (1995) Geographical variability in the bioenergetic characteristics of *Monoporeia/Pontoporeia* spp. populations from the northern Baltic Sea, and their potential contribution to benthic nitrogen mineralization. *Marine Biology*, **123**, 555–564.

Li, Y.Q., Veilleux, D.J. & Wikfors, G.H. (2009) Particle removal by Northern bay scallops *Argopecten irradians irradians* in a semi-natural setting: application of a flow-cytometric technique. *Aquaculture*, **296**, 237–245. doi: 10.1016/j.aquaculture.2009.08.026.

Lindeman, R.L. (1942) The trophic-dynamic aspect of ecology. *Ecology*, **23**, 399–418.

Lindroth, P. & Mopper, K. (1979) High performance liquid chromatographic determination of subpicomole amounts of amino acids by precolumn fluoresence derivatization with o-phthaldialdehyde. *Analytical Chemistry*, **51**, 1667–1674.

Lindsay, S.M. & Woodin, S.A. (1996) The effect of palp loss on feeding behavior of two spionid polychaetes: changes in exposure. *Biological Bulletin*, **183**, 440–447.

Linton, D.L. & Taghon, G.L. (2000) Feeding, growth, and fecundity of *Abarenicola pacifica* in relation to sediment organic concentration. *Journal of Experimental Marine Biology and Ecology*, **254**, 85–107.

Lopez, G. & Elmgren, R. (1989) Feeding depths and organic absorption for the deposit-feeding benthic amphipods *Pontoporeia affinis* and *Pontoporeia femorata*. *Limnology and Oceanography*, **34**, 982–991.

Lopez, G.R. & Crenshaw, M.A. (1982) Radiolabelling of sedientary organic matter with C-14 formaldehyde: preliminary evaluation of a new technique for use in deposit-feeding studies. *Marine Ecology Progress Series*, **8**, 283–289.

Lopez, G.R. & Cheng, I.J. (1983) Synoptic measurements of ingestion rate, ingestion selectivity, and absorption efficiency of natural foods in the deposit-feeding molluscs *Nucula annulata* (Bivalvia) and *Hydrobia totteni* (Gastropoda). *Marine Ecology Progress Series*, **11**, 55–62.

Lopez, G.R. & Levinton, J.S. (1987) Ecology of deposit-feeding animals in marine sediments. *Quarterly Review of Biology*, **62**, 235–260.

Lopez, G.R., Tantichodok, P. & Cheng, I.J. (1989) Radiotracer methods for determining utilization of sedimentary organic matter by deposit feeders. In: *Ecology of Marine Deposit Feeders* (eds G. Lopez, G. Taghon & J.S. Levinton), Springer-Verlag, Berlin, pp 149–170.

Low, B. & Richards, F. (1952) Determination of protein crystal densities. *Nature*, **170**, 412–413.

Maar, M., Bolding, K., Petersen, J.K., Hansen, J.L.S. & Timmermann, K. (2009) Local effects of blue mussels around turbine foundations in an ecosystem model of Nysted off-shore wind farm, Denmark. *Journal of Sea Research*, **62**, 159–174. doi: 10.1016/j.seares.2009.01.008.

Macdonald, B.A. & Nodwell, L.M. (2003) A portable and practical method to monitor bivalve feeding activity in the field using time-lapse video technology. *Journal of Shellfish Research*, **22**, 209–212.

Macdonald, B.A., Robinson, S.M.C. & Barrington, K.A. (2009) Evaluating the use of exhalent siphon area in estimating feeding activity of blue mussels, *Mytilus edulis*. *Journal of Shellfish Research*, **28**, 289–297.

https://telegram.me/Geologybooks

Macfadyen, A. (1948) The meaning of productivity in biological systems. *Journal of Animal Ecology*, **17**, 75–80.

Maire, O., Amouroux, J.M., Duchene, J.C. & Gremare, A. (2007) Relationship between filtration activity and food availability in the Mediterranean mussel *Mytilus galloprovincialis*. *Marine Biology*, **152**, 1293–1307. doi: 10.1007/s00227-007-0778-x.

Majdi, N., Tackx, M., Traunspurger, W. & Buffan-Dubau, E. (2012) Feeding of biofilm-dwelling nematodes on microalgae examined using HPLC-analysis of gut pigment contents. *Hydrobiologia*, **680** (1), 219–232.

Manahan, D.T., Wright, S.H. & Stephens, G.C. (1983) Simultaneous determination of net uptake of 16 amino acids by a marine bivalve. *American Journal of Physiology*, **244**, 832–838.

Manahan, D.T., Wright, S.H., Stephens, G.C. & Rice, M.A. (1982) Transport dissolved amino acids by the mussel, *Mytilus edulis*: demonstration of net uptake from natural seawater. *Science*, **215**, 1253–1255.

Manly, B.F.J. (1990) *Stage-Structured Populations: Sampling, Analysis and Simulation*. Chapman and Hall, London.

Manly, B.F.J. (1997) A method for the estimation of parameters for natural stage-structured populations. *Researches on Population Ecology*, **39**, 101–111.

Marsh, J.B. & Weinstein, W.J. (1966) A simple charring method for determination of lipids. *Journal of Lipid Research*, **7**, 574–576.

Mascaró, M. & Seed, R. (2001) Foraging behavior of juvenile *Carcinus maenas* (L.) and *Cancer pagurus* L. *Marine Biology*, **139**, 1135–1145.

Mayer, L.M., Jumars, P.A., Taghon, G.L., Macko, S.A. & Trumbore, S. (1993) Low-density particles as potential nitrogenous foods for benthos. *Journal of Marine Research*, **51**, 373–389.

McCutchan, J.H., Lewis, W.M., Kendall, C. & McGrath, C.C. (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos*, **102**, 378–390.

McLeod, R.J. & Wing, S.R. (2009) Strong pathways for incorporation of terrestrially derived organic matter into benthic communities. *Estuarine Coastal and Shelf Science*, **82**, 645–653. doi: 10.1016/j.ecss.2009.02.025.

McQuaid, C.D. & Lindsay, T.L. (2000) Effect of wave exposure on growth and mortality rates of the mussel *Perna perna*: bottom-up regulation of intertidal populations. *Marine Ecology Progress Series*, **206**, 147–154.

McVean, A. (1976) The incidence of autotomy in *Carcinus maenas* (L.). *Journal of Experimental Biology and Ecology*, **24**, 177–187.

McVean, A. & Findlay, I. (1979) The incidence of autotomy in an estuarine population of the crab *Carcinus maenas*. *Journal of the Marine Biological Association of the United Kingdom*, **59**, 341–354.

Middelburg, J.J., Barranguet, C., Boschker, H.T.S., Herman, P.M.J., Moens, T. & Heip, C.H.R. (2000) The fate of intertidal microphytobenthos: an *in situ* ^{13}C labelling study. *Limnology and Oceanography*, **45**, 1224–1234.

Migne, A. & Davoult, D. (1997) Ammonium excretion in two benthic cnidarians: *Alcyonium digitatum* (Linnaeus, 1758) and *Urticina felina* (Linnaeus, 1767). *Journal of Sea Research*, **37**, 101–107.

Mills, A. & Eaton, K. (2000) Optical sensors for carbon dioxide: an overview of sensing strategies past and present. *Quimica Analitica*, **19**, 75–86.

https://telegram.me/Geologybooks

Minagawa, M. & Wada, E. (1984) Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between $\delta^{15}N$ and animal age. *Geochimica et Cosmochimica Acta*, **48**, 1135–1140.

Mitchell, M.J. (1973) An improved method for microrespirometry using gas chromatography. *Soil biology and biochemistry*, **5**, 271–274.

Moens, T. & Vincx, M. (1997) Observations on the feeding ecology of estuarine nematodes. *Journal of the Marine Biological Association of the United Kingdom*, **77**, 211–227.

Moens, T., Vierstraete, A. & Vincx, M. (1996) Life strategies in two bacterivorous marine nematodes: preliminary results. *Marine Ecology (Pubblicazioni Della Stazione Zoologica Di Napoli I)*, **17**, 509–518.

Moens, T., Verbeeck, L. & Vincx, M. (1999a) Preservation- and incubation time-induced bias in tracer-aided grazing studies with meiofauna. *Marine Biology*, **133**, 69–77.

Moens, T., Verbeeck, L. & Vincx, M. (1999b) Feeding biology of a predatory and a facultatively predatory nematode (*Enoploides longispiculosus* and *Adoncholaimus fuscus)*. *Marine Biology*, **134**, 585–593. doi: 10.1007/s002270050573.

Moens, T., Herman, P.M.J., Verbeeck, L., Steyaert, M. & Vinckx, M. (2000) Predation rates and prey selectivity in two predacious estuarine nematode species. *Marine Ecology Progress Series*, **205**, 185–193.

Moens, T., dos Santos, G.A.P., Thompson, F., Swings, J., Fonseca-Genevois, V., Vincx, M. & De Mesel, I. (2005) Do nematode mucus secretions affect bacterial growth? *Aquatic Microbial Ecology*, **40**, 77–83. doi: 10.3354/ame040077.

Møhlenberg, F. & Riisgård, H.U. (1979) Filtration rate, using a new indirect technique, in thirteen species of suspension-feeding bivalves. *Ophelia*, **17**, 239–246.

Montagna, P.A. (1983) Live controls for radioisotope tracer food-chain experiments using meiofauna. *Marine Ecology-Progress Series*, **12**, 43–46. doi: 10.3354/meps012043.

Montagna, P.A. & Bauer, J.E. (1988) Partitioning radiolabeled thymidine uptake by bacteria and meiofauna using metabolic blocks and poisons in benthic feeding studies. *Marine Biology*, **98**, 101–110. doi: 10.1007/bf00392664.

Moodley, L., Middelburg, J.J., Soetaert, K., Boschker, H.T.S., Herman, P.M.J. & Heip, C.H.R. (2005) Similar rapid response to phytodetritus deposition in shallow and deep-sea sediments. *Journal of Marine Research*, **63**, 457–469.

Moodley, L., Middelburg, J.J., Boschker, H.T.S., Duineveld, G.C.A., Pel, R., Herman, P.M.J. & Heip, C.H.R. (2002) Bacteria and foraminifera: key players in a short-term deep-sea benthic response to phytodetritus. *Marine Ecology Progress Series*, **236**, 23–29.

Moodley, L., Steyaert, M., Epping, E., Middelburg, J.J., Vincx, M., van Avesaath, P., Moens, T. & Soetaert, K. (2008) Biomass-specific respiration rates of benthic meiofauna: demonstrating a novel oxygen micro-respiration system. *Journal of Experimental Marine Biology and Ecology*, **357**, 41–47. doi: 10.1016/j.jembe.2007.12.025.

Morissette, S. & Himmelman, J.H. (2000) Subtidal food thieves: interactions of four invertebrate kleptoparasites with the sea star *Leptasterias polaris*. *Animal Behaviour*, **60**, 531–543.

Nehring, S., Jensen, P. & Lorenzen, S. (1990) Tube-dwelling nematodes - tube construction and possible ecological effects on sediment-water interfaces. *Marine Ecology-Progress Series*, **64**, 123–128. doi: 10.3354/meps064123.

https://telegram.me/Geologybooks

Nieuwenhuize, J., Maas, Y.E.M. & Middelburg, J.J. (1994) Rapid analysis of organic-carbon and nitrogen in particulate materials. *Marine Chemistry*, **45**, 217–224.

Nilsson, H.C. (2000a) Effects of hypoxia and organic enrichment on growth of the brittle star *Amphiura filiformis* (O.F. Mueller) and *Amphiura chiajei* Forbes. *Journal of Experimental Biology and Ecology*, **237**, 11–30.

Nilsson, H.C. (2000b) Interaction between water flow and oxygen deficiency on gowth in the infaunal brittle star *Amphiura filiformis* (Echinodermata: Ophiuroidea). *Journal of Sea Research*, **44**, 233–241.

Niquil, N., Jackson, G.A., Legendre, L. & Delesalle, B. (1998) Inverse model analysis of the planktonic food web of Takapoto Atoll (French Polynesia). *Marine Ecology Progress Series*, **165**, 17–29.

Niquil, N., Soetaert, K., Johnson, G.A., van Oevelen, D., Bacher, C., Saint-Béat, B. & Vézina, A.F. (in press) Inverse modelling in modern ecology and application to coastal ecosystems. In: *Treatise on Estuarine and Coastal Science* (eds E.Wolanski, D.D. McLusky), *Estuarine and Coastal Ecosystem Modelling*, Vol. 9 (eds D. Baird & A. Mehta). Elsevier, Oxford.

Paloheimo, J.E. (1974) Calculation of instantaneous birth rate. *Limnology and Oceanography*, **19**, 692–694.

Pamatmat, M.M. (1978) Oxygen-uptake and heat-production in a metabolic conformer (*Littorina irrotata*) and a metabolic regulator (*Uca pugnax*). *Marine Biology*, **48**, 317–325.

Pamatmat, M.M. (1983) Measuring aerobic and anaerobic metabolism of benthic infauna under natural conditions. *Journal of Experimental Zoology*, **228**, 405–413.

Pape-Lindstrom, P.A., Feller, R.J., Stancyk, S.E. & Woodin, S.A. (1997) Sublethal predation: field measurements of arm tissue loss from the ophiurid *Microphiopholis gracillima* and immunochemical identification of its predators in North Inlet, South Carolina, USA. *Marine Ecology Progress Series*, **156**, 131–140.

Pascal, P.Y., Dupuy, C., Richard, P., Rzeznik-Orignac, J. & Niquil, N. (2008) Bacterivory of a mudflat nematode community under different environmental conditions. *Marine Biology*, **154**, 671–682. doi: 10.1007/s00227-008-0960-9.

Pauly, D. (1987) A review of the ELEFAN system for analysis of length-frequency data in fish and aquatic invertebrates. In: *Length-Based Methods in Fisheries Research* (eds D. Pauly & G. R. Morgan), International Center for Aquatic Living Resources Management, Manila, pp 7–34.

Pechenik, J.A., Berard, R. & Kerr, L. (2000) Effects of reduced salinity on survival, growth, reproductive success, and energetics of the euryhaline polychaete *Capitella* sp I. *Journal of Experimental Marine Biology and Ecology*, **254**, 19–35.

Peck, L.S., Prothero-Thomas, E. & Hough, N. (1993) Pedal mucus production by the Antartic limpet *Nacella concinna* (Strebel, 1908). *Journal of Experimental Marine Biology and Ecology*, **174**, 177–192.

Penry, D.L. (1998) Applications of efficiency measurements in bioaccumulation studies: definitions, clarifications, and a critique of methods. *Environmental Toxicology and Chemistry*, **17**, 1633–1639.

Pentilla, J., Jearld, A. & Clark, S. (1988) Age determination methods for Northwest Atlantic species. *NOAA Technical Report, US Department of Commerce*, **72**, 3–135.

https://telegram.me/Geologybooks

Petersen, J.K. (2004) Methods for measurement of bivalve clearance rate – hope for common understanding. *Marine Ecology-Progress Series*, **276**, 309–310.

Petersen, J.K., Bougrier, S., Smaal, A.C., Garen, P., Robert, S., Larsen, J.E.N. & Brummelhuis, E. (2004) Intercalibration of mussel *Mytilus edulis* clearance rate measurements. *Marine Ecology-Progress Series*, **267**, 187–194.

Peterson, B.J. & Howarth, R.W. (1987) Sulfur, carbon, and nitrogen isotopes used to trace organic matter flow in the salt-marsh estuaries of Sapelo Island, Georgia. *Limnology and Oceanography*, **32**, 1195–1213.

Peterson, C.H. & Quammen, M.L. (1982) Siphon nipping: its importance to small fishes and its impact on the growth of the bivalve *Prothothaca staminea* (Conrad). *Journal of Experimental Marine Biology and Ecology*, **63**, 249–268.

Phillips, D.L. & Gregg, J.W. (2003) Source partitioning using stable isotopes: coping with too many sources. *Oecologia*, **136**, 261–269.

Phillips, D.L., Newsome, S.D. & Gregg, J.W. (2005) Combining sources in stable isotope mixing models: alternative methods. *Oecologia*, **144**, 520–527.

Phillipson, J. (1964) A miniature bomb calorimeter for small biological samples. *Oikos*, **15**, 130–139.

Pomeroy, L.R., Sheldon, J.E. & Sheldon, W.M. (1994) Changes in bacterial numbers and leucine assimilation during estimations of microbial respiratory rates in seawater by the Precision Winkler Method. *Applied and Environmental Microbiology*, **60**, 328–332.

Post, D.M. (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology*, **83**, 703–718.

Poulsen, E., Riisgård, H.U. & Mohlenberg, F. (1982) Accumulation of cadmium and bioenergetics in the mussel *Mytilus edulis*. *Marine Biology*, **68**, 25–29.

Prevedelli, D. & Vandini, R.Z. (1998) Effect of diet on reproductive characteristics of *Ophryotrocha labronica* (Polychaeta: Dorvilleidae). *Marine Biology*, **132**, 163–170.

Qian, P.Y. & Chia, F.S. (1994) *In situ* measurement of recruitment, mortality, growth, and fecundity of *Capitella* sp. (Annelida: Polycheata). *Marine Ecology Progress Series*, **111**, 53–62.

R Development Core Team (2008) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna.

Ren, J.S. (2009) Effect of food quality on energy uptake. *Journal of Sea Research*, **62**, 72–74. doi: 10.1016/j.seares.2008.11.002.

Ren, J.S. & Ross, A.H. (2001) A dynamic energy budget model of the Pacific Oyster *Crassostrea gigas*. *Ecological Modelling*, **142**, 105–120.

Ren, J.S. & Schiel, D.R. (2008) A dynamic energy budget model: parameterisation and application to the Pacific oyster *Crassostrea gigas* in New Zealand waters. *Journal of Experimental Marine Biology and Ecology*, **361**, 42–48. doi: 10.1016/j.jembe.2008.04.012.

Rhoads, D.C. & Lutz, R.A. (1980) *Skeletal Growth of Aquatic Organisms*. Plenum Press, New York.

Rice, D.L. (1982) The detritus nitrogen problem: new observations and perspectives from organic geochemistry. *Marine Ecology Progress Series*, **9**, 153–162.

Richardson, C.A. (1989) An analysis of the microgrowth bands in the shell of the common mussel *Mytilus edulis*. *Journal of the Marine Biological Association of the United Kingdom*, **69**, 477–491.

https://telegram.me/Geologybooks

Richardson, C.A. (2001) Molluscs as archives of environmental change. *Oceanography and Marine Biology: An Annual Review*, **39**, 103–164.

Richardson, T.L., Jackson, G.A., Ducklow, H.W. & Roman, M.R. (2004) Carbon fluxes through food webs of the eastern equatorial Pacific: an inverse approach. *Deep-Sea Research Part I: Oceanographic Research Papers*, **51**, 1245–1274.

Riemann, F. & Schrage, M. (1978) Mucus-trap hypothesis on feeding of aquatic nematodes and implications for biodegradation and sediment texture. *Oecologia*, **34**, 75–88. doi: 10.1007/bf00346242.

Rigler, R.H. & Downing, J.A. (1984) The calculation of secondary productivity. In: *A Manual on Methods for the Assessment of Secondary Productivity in Fresh Waters* (eds J.A. Downing & R.H. Rigler), Blackwell Publishing Ltd., Oxford, pp 19–58.

Riisgård, H.U. (1977) On measurements of the filtration rates of suspension feeding vivalves in a flow system. *Ophelia*, **16**, 167–173.

Riisgård, H.U. (1989) Properties and energy-cost of the muscular piston pump in the suspension feeding polychaete *Chaetopterus variopedatus*. *Marine Ecology Progress Series*, **56**, 157–168.

Riisgård, H.U. (1991) Supension feeding in the polychaete *Nereis diversicolor*. *Marine Ecology Progress Series*, **70**, 29–37.

Riisgård, H.U. (2001) On measurement of filtration rates in bivalves - the stony road to reliable data: review and interpretation. *Marine Ecology Progress Series*, **211**, 275–291.

Riisgård, H.U. (2004) Intercalibration of methods for measurement of bivalve filtration rates - a turning point. *Marine Ecology-Progress Series*, **276**, 307–308.

Riisgård, H.U. & Mohlenberg, F. (1979) An improved automatic recording apparatus for determining the filtration rate of *Mytilus edulis* as a function of size and algal concentration. *Marine Biology*, **52**, 61–67.

Riisgård, H.U. & Randløv, A. (1981) Energy budgets, growth and filtration rates in *Mytilus edulis* at different algal concentration. *Marine Biology*, **61**, 227–234.

Riisgård, H.U. & Ivarsson, N.M. (1990) The crown-filament pump of the suspension-feeding polychaete *Sabella penicillus* - filtration, effects of temperature, and energy-cost. *Marine Ecology Progress Series*, **62**, 249–257.

Riisgård, H.U. & Larsen, P.S. (1995) Filter-feeding in marine macro-invertebrates: pump characteristics, modelling and energy cost. *Biological Reviews*, **70**, 67–106.

Riisgård, H.U. & Larsen, P.S. (2000) A comment on experimental techniques for studying particle capture in filter-feeding bivalves. *Limnology and Oceanography*, **45**, 1192–1195.

Robertson, A.I. (1979) Relationship between annual production - biomass ratios and lifespans for marine macrobenthos. *Oecologia*, **38**, 193–202.

Rossi, F., Herman, P.M.J. & Middelburg, J.J. (2004) Inter- and intra-specific variation of d13C and d15N in deposit- and suspension-feeding bivalves (*Macoma balthica* and *Cerastoderma edule*): evidence of ontogenetic changes in feeding mode of *Macoma balthica*. *Limnology and Oceanography*, **49**, 408–414.

Rowan, D.J. & Rasmussen, J.B. (1994) Bioaccumulation of radiocesium by fish: influence of physico-chemical factors and trophic structure. *Canadian Journal of Fisheries and Aquatic Sciences*, **51**, 2388–2410.

Rusch, A. & Huettel, M. (2000) Advective particle transport into permeable sediments - evidence from experiments in an intertidal sandflat. *Limnology and Oceanography*, **45**, 525–533.

https://telegram.me/Geologybooks

Sadava, D. & Orians, G.H. (2000) *Life: The Science of Biology*. W.H. Freeman and Co., New York.

Sanchez-Pedreno, C., Ortuno, J.A., Albero, M.I., Garcia, M.S. & de las Bayonas, J.C.G. (2000) A new procedure for the construction of flow-through optodes. Application to determination of copper (II). *Fresenius Journal of Analytical Chemistry*, **366**, 811–815.

Santos, P.J.P., Castel, J. & Souza-Santos, L.P. (1995) Microphytobenthic patches and their influence on meiofaunal distribution. *Cahiers De Biologie Marine*, **36**, 133–139.

Saraiva, S., van der Meer, J., Kooijman, S. & Sousa, T. (2011) Modelling feeding processes in bivalves: a mechanistic approach. *Ecological Modelling*, **222**, 514–523. doi: 10.1016/j.ecolmodel.2010.09.031.

Saurel, C., Gascoigne, J.C., Palmer, M.R. & Kaiser, M.J. (2007) *In situ* mussel feeding behavior in relation to multiple environmental factors: regulation through food concentration and tidal conditions. *Limnology and Oceanography*, **52**, 1919–1929.

Scheu, S. (1991) Mucus excretion and carbon turnover of endogeic earthworms. *Biology and Fertility of Soils*, **12**, 217–220.

Schmidt, O., Scrimgeour, C.M. & Curry, J.P. (1999) Carbon and nitrogen stable isotope ratios in body tissue and mucus of feeding and fasting earthworms (*Lumbricus festivus*). *Oecologia*, **118**, 9–15.

Schraer, W.D. & Stoltze, H.J. (1999) *Biology: The Study of Life*. Prentice Hall, New York.

Seitz, A. (1979) On the calculation of birth rates and death rates in fluctuating populations with continuous recruitment. *Oecologia*, **41**, 343–360.

Shepherd, J.G. (1987) A weakly parametric method for estimating growth parameters from length composition data. In: *Length-Based Methods in Fisheries Research* (eds D. Pauly & G.R. Morgan), International Center for Living Aquatic Resources Management, Manila, pp 113–119.

Shick, J.M., De Zwaan, A. & De Bont, A.M.T. (1983) Anoxic metabolic rate in the mussel *Mytilus edulis* L. estimated by simultaneous direct calorimetry and biochemical analysis. *Physiological Zoology*, **56**, 56–63.

Sikora, J.P., Sikora, W.B., Erkenbrecher, C.W. & Coull, B.C. (1977) Significance of ATP, carbon, and caloric content of meiobenthic nematodes in partitioning benthic biomass. *Marine Biology*, **44**, 7–14. doi: 10.1007/bf00386899.

Silverman, H., Lynn, J.W. & Dietz, T.H. (2000) *In vitro* studies of particle capture and transport in suspension-feeding bivalves. *Limnology and Oceanography*, **45**, 1199–1203.

Skoeld, M. & Rosenberg, R. (1996) Arm regeneration frequency in eight species of Ophiuroidea (Echinodermata) from European sea areas. *Journal of Sea Research*, **35**, 353–362.

Skoeld, M., Loo, L.-O. & Rosenberg, R. (1994) Production, dynamics and demography of an *Amphiura filiformis* population. *Marine Ecology Progress Series*, **103**, 81–90.

Skoolmun, P. & Gerlach, S.A. (1971) Jahreszeitliche Fluctuationen der Nematoden im Gezeitenbereich des Weser-Ästuars (Deutsche Bucht). *Veröffentlichungen des Instituts für Meeresforschungen in Bremerhaven*, **12**, 119–138.

Smaal, A.C. & Vonck, A.P.M.A. (1997) Seasonal variation in C, N and P budgets and tissue composition of the mussel *Mytilus edulis*. *Marine Ecology Progress Series*, **153**, 167–179.

Smol, N., Heip, C. & Govaert, M. (1980) The life cycle of *Oncholaimus oxyuris* (Nematoda) in its habitat. *Annales de la Société Royale Zoologique de Beligique*, **110**, 87–103.

https://telegram.me/Geologybooks

Soetaert, K. & Van Oevelen, D. (2008) LIM: linear inverse model examples and solution methods. R package version 1.2.

Soetaert, K. & Van Oevelen, D. (2009) Modeling food web interactions in benthic deep-sea ecosystems: a practical guide. *Oceanography*, **22**, 130–145.

Sola, J.C. (1996) Population dynamics, reproduction, growth, and secondary production of the mud-snail *Hydrobia ulvae* (Pennant). *Journal of Experimental Marine Biology and Ecology*, **205**, 49–62.

Solomon, S., Qin, D., Manning, M., Alley, R.B., Berntsen, T., Bindoff, N.L., Chen, Z., Chidthaisong, A., Gregory, J.M., Hegerl, G.C., Heimann, M., Hewitson, B., Hoskins, B.J., Joos, F., Jouzel, J., Kattsov, V., Lohmann, U., Matsuno, T., Molina, M., Nicholls, N., Overpeck, J., Raga, G., Ramaswamy, V., Ren, J., Rusticucci, M., Somerville, R., Stocker, T.F., Whetton, P., Wood, R.A. & Wratt, D. (2007) Technical summary. In: *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (eds S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor & H.L. Miller). Cambridge University Press, Cambridge, New York.

Solórzano, L. (1969) Determination of ammonia in natural waters by the phenolhypochlorite method. *Limnology and Oceanography*, **14**, 799–800.

Sommer, U., Meusel, B. & Stielau, C. (1999) An experimental analysis of the importance of body-size in the seastar-mussel predator-prey relationship. *Acta Oecologica*, **20**, 81–86.

Souza-Santos, L.P., Castel, J. & Santos, P.J.P. (1995) Feeding rate cycle of the epibenthic harpacticoid copepod *Harpacticus flexus*: laboratory experiments using fecal pellet counts. *Vie et Milieu*, **45**, 75–83.

Stephens, D. & Krebs, J.R. (1986) *Foraging Theory*. Princeton University Press, Princeton, NJ.

Stoecker, D.K. & Michaels, A.E. (1991) Respiration, photosynthesis and carbon metabolism in planktonic ciliates. *Marine Biology*, **108**, 441–447.

Stokes, M.D. & Somero, G.N. (1999) An optical oxygen sensor and reaction vessel for high-pressure applications. *Limnology and Oceanography*, **44**, 189–195.

Strickland, J.D.H. & Parsons, T.R. (1972) *A Practical Handbook of Seawater Analysis*. Fisheries Research Board of Canada.

Taghon, G. & Greene, R.R. (1998) Utilization of deposited and suspended particulate matter by benthic 'interface' feeders. *Limnology and Oceanography*, **37**, 1370–1391.

Taghon, G.L. (1988) The benefits and costs of deposit feeding in the polychaete *Abarenicola pacifica*. *Limnology and Oceanography*, **33**, 1166–1175.

Taghon, G.L., Prahl, F.G., Sparrow, M., & Fuller, C.M. (1994) Lipid class and glycogen content of the lugworm *Abarenicola pacifica* in relation to age, growth rate and reproductive condition. *Marine Biology*, **120**, 287–295.

Taylor, B.N. (1995) *Guide for the Use of the International System of Units (SI)*. Government Printing Office, Washington, DC.

Tenore, K.R. (1977) Growth of *Capitella capitata* cultured on various levels of detritus derived from different sources. *Limnology and Oceanography*, **22**, 936–941.

Thienemann, A. (1931) Productionsbegrift in der Biologie. *Archiv für Hydrobiologie*, **22**, 616–621.

Threlkeld, S. (1979) Estimating cladoceran birth rates: the importance of egg mortality and the egg age distribution. *Limnology and Oceanography*, **24**, 601–612.

https://telegram.me/Geologybooks

Tietjen, J.H. & Lee, J.J. (1977) Life history of marine nematodes. Influence of temperature and salinity on the reproductive potential of *Chromadorina germanica* Bütschli. *Mikrofauna Meeresboden*, **15**, 263–270.

Troost, K., Stamhuis, E.J., van Duren, L.A. & Wolff, W.J. (2009) Feeding current characteristics of three morphologically different bivalve suspension feeders, *Crassostrea gigas, Mytilus edulis* and *Cerastoderma edule*, in relation to food competition. *Marine Biology*, **156**, 355–372. doi: 10.1007/s00227-008-1088-7.

Tumbiolo, M.L. & Downing, J.A. (1994) An empirical model for the prediction of secondary production in marine benthic invertebrate populations. *Marine Ecology Progress Series*, **114**, 165–174.

Underwood, G.J.C. & Kromkamp, J. (1999) Primary production by phytoplankton and microphytobenthos in estuaries. *Advances in Ecological Research*, **29**, 93–153.

Urrutia, M.B., Navarro, E., Ibarrola, I. & Iglesias, J.I.P. (2001) Preingestive selection processes in the cockle *Cerastoderma edule*: mucus production related to rejection of pseudofaeces. *Marine Ecology Progress Series*, **209**, 177–187.

van der Meer, J. (2006) An introduction to Dynamic Energy Budget (DEB) models with special emphasis on parameter estimation. *Journal of Sea Research*, **56**, 85–102. doi: 10.1016/j.seares.2006.03.001.

van der Meer, J. & Piersma, T. (1994) Invited perspectives in physiological zoology: physiologically inspired regression models for estimating and predicting nutrient stores and their composition in birds. *Physiological Zoology*, **67**, 305–329.

van der Veer, H.W., Kooijman, S. & van der Meer, J. (2003) Body size scaling relationships in flatfish as predicted by Dynamic Energy Budgets (DEB theory): implications for recruitment. *Journal of Sea Research*, **50**, 255–270. doi: 10.1016/j.seares.2003.05.001.

Van Oevelen, D., Moodley, L., Soetaert, K. & Middelburg, J.J. (2006a) The trophic significance of bacterial carbon in a marine intertidal sediment: results of an in situ stable isotope labeling study. *Limnology and Oceanography*, **51**, 2349–2359.

Van Oevelen, D., Van den Meersche, K., Meysman, F., Soetaert, K., Middelburg, J.J. & Vézina, A.F. (2010) Quantitative reconstruction of food webs using linear inverse models. *Ecosystems*, **13**, 32–45.

Van Oevelen, D., Soetaert, K., Middelburg, J.J., Herman, P.M.J., Moodley, L., Hamels, I., Moens, T. & Heip, C.H.R. (2006b) Carbon flows through a benthic food web: integrating biomass, isotope and tracer data. *Journal of Marine Research*, **64**, 1–30.

Van Straalen, N.M. (1985) Production and biomass turnover in stationary stage-structured populations. *Journal of Theoretical Biology*, **113**, 331–352.

Van Voorhies, W.A. (2000) Broad oxygen tolerance in the nematode *Caenorhabditis elegans*. *American Zoologist*, **40**, 1044–1045.

Van Voorhies, W.A. & Ward, S. (1999) Genetic and environmental conditions that increase longevity in *Caenorhabditis elegans* decrease metabolic rate. *Proceedings of the National Academy of Sciences of the United States of America*, **96**, 11399–11403.

Veuger, B., Middelburg, J.J., Boschker, H.T.S. & Houtekamer, M. (2005) Analysis of ^{15}N incorporation into D-alanine: a new method for tracing nitrogen uptake by bacteria. *Limnology and Oceanography: Methods*, **3**, 230–240.

Vézina, A.F. & Platt, T. (1988) Food web dynamics in the ocean. I. Best-estimates of flow networks using inverse methods. *Marine Ecology Progress Series*, **42**, 269–287.

https://telegram.me/Geologybooks

Vézina, A.F., Berreville, F. & Loza, S. (2004) Inverse reconstructions of ecosystem flows in investigating regime shifts: impact of the choice of objective function. *Progress in Oceanography*, **60**, 321–341.

Vogel, S. (1994) *Life in Moving Fluids. The Physical Biology of Flow*. Princeton University Press, Princeton, NJ.

Voraberger, H.S., Kreimaier, H., Biebernik, K. & Kern, W. (2001) Novel oxygen optrode withstanding autoclavation: technical solutions and performance. *Sensors and Actuators B - Chemical*, **74**, 179–185.

Vranken, G., Herman, P.M.J., Vincx, M. & Heip, C. (1986) A reevaluation of marine nematode productivity. *Hydrobiologia*, **135**, 193–196. doi: 10.1007/bf00006530.

Ward, J.E., Sanford, L.P., Newel, R.I.E. & MacDonals, B.A. (2000) The utility of *in vivo* observations for describing particle capture processes in suspension-feeding bivalve molluscs. *Limnology and Oceanography*, **45**, 1203–1210.

Warkentin, M., Freese, H.M., Karsten, U. & Schumann, R. (2007) New and fast method to quantify respiration rates of bacterial and plankton communities in freshwater ecosystems by using optical oxygen sensor spots. *Applied and Environmental Microbiology*, **73**, 6722–6729. doi: 10.1128/aem.00405-07.

Warwick, R.M. & Price, R. (1979) Ecological and metabolic studies on free-living nematodes from an estuarine mudflat. *Estuarine and Coastal Marine Science*, **9**, 257–271.

Warwick, R.M. & Gee, J.M. (1984) Community structure of estuarine meiobenthos. *Marine Ecology Progress Series*, **18**, 97–111.

Waters, T.F. (1969) Turnover ratio in production ecology of freshwater invertebrates. *American Naturalist*, **103**, 173–185. doi: 10.1086/282591.

Waters, T.F. & Crawford, G.W. (1973) Annual production of a stream mayfly population: a comparison of methods. *Limnology and Oceanography*, **18**, 286–296.

Weigl, B.H. & Wolfbeis, O.S. (1995) New hydrophobic materials for optical carbon-dioxide sensors based on ion-pairing. *Analytica Chimica Acta*, **302**, 249–254.

Wenzhofer, F., Holby, O. & Kohls, O. (2001) Deep penetrating benthic oxygen profiles measured i*n situ* by oxygen optodes. *Deep-Sea Research Part I: Oceanographic Research Papers*, **48**, 1741–1755.

Wetherill, G.B. (1986) *Regression Analysis with Applications*. Chapman and Hall, London.

Wheatcroft, R.A., Starczak, V.R. & Butman, C.A. (1998) The impact of population abundance on the deposit-feeding rate of a cosmopolitan polychaete worm. *Limnology and Oceanography*, **43**, 1948–1953.

Widbom, B. (1984) Determination of average individual dry weights and ash-free dry weights in different sieve fractions of marine meiofauna. *Marine Biology*, **84**, 101–108.

Wieser, W. (1960) Benthic studies in Buzzards Bay. II. The meiofauna. *Limnology and Oceanography*, **5**, 121–137.

Winberg, G.G. (1971) *Methods for the Estimation of Production of Aquatic Animals*. Academic Press, London.

Winter, J.E. (1973) The filtration rate of *Mytilus edulis* and its dependence on algal concentration, measured by continuous atuomatic recording apparatus. *Marine Biology*, **22**, 317–328.

Witte, U., Wenzhofer, F., Sommer, S., Boetius, A., Heinz, P., Aberle, N., Sand, M., Cremer, A., Abraham, W.R., Jorgensen, B.B. & Pfannkuche, O. (2003) *In situ* experimental

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evidence of the fate of a phytodetritus pulse at the abyssal sea floor. *Nature*, **424**, 763–766.

Wood, S.N. (1994) Obtaining birth and mortality patterns from structured population trajectories. *Ecological Monographs*, **64**, 23–44.

Woombs, M. & Laybournparry, J. (1984) Feeding biology of *Diplogasteritus nudicapitatus* and *Rhabditis curvicaudata* (Nematoda) related to food concentration and temperature, in sewage-treatment plants. *Oecologia*, **64**, 163–167. doi: 10.1007/bf00376865.

Woulds, C., Andersson, J.H., Cowie, G.L., Middelburg, J.J. & Levin, L.A. (2009) The short-term fate of organic carbon in marine sediments: comparing the Pakistan margin to other regions. *Deep-Sea Research Part II: Topical Studies in Oceanography*, **56**, 393–402. doi: 10.1016/j.dsr2.2008.10.008.

Yahel, G., Marie, D. & Genin, A. (2005) InEx – a direct *in situ* method to measure filtration rates, nutrition, and metabolism of active suspension feeders. *Limnology and Oceanography: Methods*, **3**, 46–58.

Zajac, R.N. (1995) Sublethal predation on *Polydora cornuta* (Polychaeta: Spionidae): patterns of tissue loss in a field population, predator functional response and potential demographic impacts. *Marine Biology*, **123**, 531–541.

Zaldivar, J.M., Marinov, D., Dueri, S., Castro-Jimenez, J., Micheletti, C. & Worth, A.P. (2011) An integrated approach for bioaccumulation assessment in mussels: towards the development of Environmental Quality Standards for biota. *Ecotoxicology and Environmental Safety*, **74**, 244–252. doi: 10.1016/j.ecoenv.2010.10.025.

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Chapter 9
Phytobenthos Techniques

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Abstract

The vegetation substrates of the euphotic coastal zone being one of the most productive zones of the ocean have historically been the focus of many years of investigations. Included are up-to-date descriptions of techniques and methods used in the study of the macroscopic plant communities of the intertidal and the subtidal zones, to a depth of 30 m. Depending on the scope of the investigations, many different approaches have been adopted. As there is no single common method in use, this chapter comprises a comparative methodology relevant to the study of phytobenthic communities. Hands-on techniques, such as visual observations, transect intercept methods, cover estimates by means of frames or destructive sampling are discussed. A description of a quick, reliable SCUBA divers' method of monitoring along a transect line, the species depth distribution and coverage, is given. Remote techniques have been developed in recent decades that, given good conditions, can reveal large-scale distribution of the phytobenthic communities. These remote techniques range from satellite imaging, aerial photography, laser techniques (LIDAR), to ship-borne echo-sounding, multi-beam and sonar techniques. A brief review of methods used for monitoring in different countries is given.

Keywords phytobenthic communities, SCUBA transect techniques, continuous observation methods, free estimates, manta tow, diver propulsion, video techniques, ROV, echo-sounding, satellite, aerial photography

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9.1 Introduction

For many years, phytobenthic plant and animal communities have been extensively studied for species distribution and experimental studies of enclosures, manipulative studies of harvesting or introducing species or changing the environmental conditions by shading, fertilising, etc. There is an abundance of literature on the subject to which, depending on the scope of the scientific question, many different

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techniques have been applied. There is no single common method that is in use, and that causes problems when trying to draw comparisons between different authors and different methods. There is, therefore, is a need for a description of relevant and comparable methods for phytobenthic communities, for instance, in the context of the Water Framework Directive (WFD) (De Jonge *et al.*, 2006).

Therefore, the aim of this chapter is to harmonise the methods used when checking experimental studies performed in the laboratory or manipulations in the field (cages, species manipulation, etc.) against actual observations of real situations. Suggestions are made concerning appropriate techniques as well as which parameters should be included, depending on the scientific objective of the programmes in question. The methods described here are also applicable in contexts such as surveys, EU-funded programmes (NATURA 2000 biotope (habitat) descriptions), manipulative studies, exclusion/inclusion experiments, etc.: in other words, all scientific work that is based on field observations.

The present chapter includes descriptions of surveying techniques for the study of the macroscopic plant and animal communities of the intertidal and subtidal zone on hard to soft substrates down to 30 m depth (see also Chapter 4). The lower limit is not fixed but should be within the limits of safe diving and should to some extent coincide with the lower limit of macroscopic, non-crustose plants.

The general purpose of such programmes is to provide a description of the communities of interest and their changes over time. This can be achieved either by observations of the whole community using different scales of resolution, or by taking samples from which the true community composition can be estimated. The former is a matter of scale and the latter is subject to statistical considerations. As each approach is relevant, the choice must depend on the purpose of the study. At present, the use of fixed size frames (quadrats) is the most commonly method employed. In spite of its advantage when using traditional statistical methods, this method has several drawbacks: different sizes of frame give different results, the number of frames determines the level of confidence of the estimate and the area covered is usually extremely small compared to the community studied. To compensate for these drawbacks, the use of a substantial amount of frames has to be balanced with the resultant increased time spent in the field, which increases costs.

This chapter mentions some of the benefits that accrue from having a broader view of the community, rather than one limited by the use of given frames of fixed size. Though emphasis is placed on the methods used to survey and monitor shoreline phytobenthic plant and animal communities, a short overview of other methods is also included, depending on the scope of the investigation, as these methods may be more appropriate. A common method proposed by the International Council for the Exploration of the Sea (ICES), as well as certain variations, in use by several countries in the ICES area, is described.

The chapter also includes the use of video techniques as a substitute for divers when, for example, there are large areas to be surveyed and/or the distribution of

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a few conspicuous species is being investigated. Other surveying and monitoring methods for phytobenthos used in different parts of the world are also mentioned and literature with more detailed descriptions is cited.

9.2 Phytobenthic communities

In this chapter phytobenthic communities are defined as the vegetation-covered substrates of the euphotic zone, which includes all types of substrates ranging from hard, sandy, mixed, to soft sediments. However, in the Baltic Sea, hard substrates below the euphotic zone are also included. The lower limit is set at 30 m depth because of SCUBA diving safety regulations.

Historically, different nomenclatures have been used to describe the phytobenthic zone (see the classical works of Lewis, 1964; Stephenson & Stephenson, 1972; Schwenke, 1974; Lüning, 1985). A simple yet relevant subdivision is the separation of intertidal and subtidal zone as found in most American literature. The EUNIS (European University Information System) classification system that can be accessed at http://eunis.eea.europa.eu/habitats.jsp (14 November 2012) defines the phytobenthic communities in the littoral zone (including the supra- and eulittoral zones) and also the sub-littoral zone (including the infra- and circa-littoral zones). A EUNIS classification system relevant for the Baltic Sea area is under development.

The species richness of the phytobenthic community provides a multitude of plant and animal species sensitive to different pollutants and/or eutrophication. Many of the species included in a monitoring programme of the phytobenthic communities are attached and perennial, and, therefore, are capable of integrating the environmental load over a longer period of time. This, taken in conjunction with the relative ease of observing these communities, means that they are important indicators of the quality of the water body; as a result, they are included in several national monitoring programmes, for instance the EU WFD (Selig *et al.*, 2007, 2008), following the effects of eutrophication (e.g. Krause-Jensen *et al.*, 2008). Observations can also be made over longer time intervals to detect change, which makes the study of these communities cost-efficient.

Guidelines for the study of phytobenthic communities

For the study of phytobenthic communities there are some existing recommendations and standards. For example, in the Baltic Sea region there are the HELCOM Guidelines for monitoring of phytobenthic plant and animal communities (HELCOM, 1999). The ISO standard ISO-19493: 2007 'Water quality – guidance on marine biological surveys on hard-substrate communities' also includes general recommendations and is valid throughout the ICES area. The OSPAR documents on quality assurance (Rees, 2009) give a comprehensive review of methods for the study of epibenthic communities. Rees (2009) mainly describes methods used

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Table 9.1 Overview of methods available for the sampling of phytobenthos.

Approach	Methods
Walking (intertidal, shallow water), snorkelling	Direct observations
Diver	Transects Frames Destructive sampling
Satellite	Images
Aerial photograph (true colour, filtered, etc.)	Images
From ship	Echo-sounder, sidescan sonar Video hanger Remotely Operated Vehicle (ROV)
From boat	Aquascope Video hanger Pavane (manta tow)

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on soft sediments, but hard substrates and the photic zone are also briefly mentioned. In addition, there are several relevant descriptions of methods available for general as well as for specific habitats, such as those given by the Joint Nature Conservation Committee (JNCC, 2001). Hiscock (1998) reviewed methods for detecting change in phytobenthic communities, as did Davies *et al.* (2001). The JAMP Eutrophication Monitoring Guidelines (updated; ICES, 2009) also contain useful information. Seagrass beds and their monitoring are described by a number of authors, e.g. Borum *et al*. (2004). General descriptions and guidelines are found at web references[1], the JNCC handbooks being the most comprehensive. A detailed description of relevant methods is given in Kautsky (2013).

Statistical texts can help with planning the monitoring/sampling and analysis of data, e.g. Murray *et al*. (2006) and Underwood (1997). A brief review of methods adopted and practised worldwide can be found at the end of the chapter.

Data collection

The collection of data depends on the purpose of the study and also its scale (Table 9.1). If the scope is to draw national maps of the extension of complex phytobenthic communities (e.g. of a kelp bed), a rough grid of observation points is necessary. If the scope is to follow a single species distribution and temporal change, then a detailed, high-resolution scale of observation is necessary. The

[1]
http://www.ospar.org/ (accessed 7 November 2012)
http://www.jncc.gov.uk/marine/ (accessed 18 November 2012)
http://www.biomareweb.org/ (accessed 18 November 2012)
http://water.epa.gov/type/oceb/ (accessed 18 November 2012)
www.ospar.org/documents/dbase/decrecs/agreements/97-02e.doc (accessed 18 November 2012)

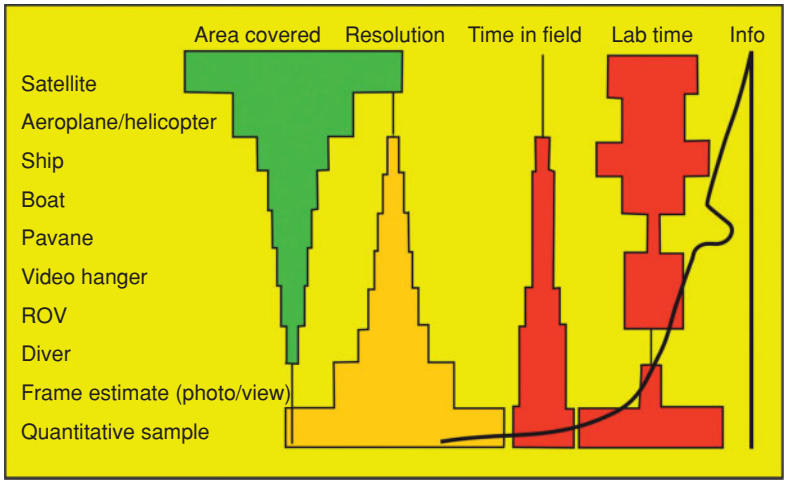

Fig. 9.1 Comparison of the features of the various general methods available.

broadest view (>100 m^2 area) is obtained by remote techniques, a more detailed one (down to somewhat less than 1 m^2) by direct observations. If the scale is down to fractions of mm, samples have to be collected and analysed in the laboratory. All methods have their advantages and disadvantages and may be appropriate for a given situation. There will be some degree of compromise between the area of coverage and the detail of observation, and the cost of the various methods varies when going from video hanger to Remotely Operated Vehicle (ROV) and ending with divers' observations (Fig. 9.1). The highest resolution and information are gained by quantitative sampling, either by frame pictures or destructive sampling. Destructive sampling is by far the most expensive method as the samples require time-consuming sorting in the laboratory. The more generally applied methods are discussed in more detail below.

9.3 Overview of methods for sampling phytobenthos

There is a wide range of indirect as well as direct methods available for sampling the phytobenthos.

Indirect methods, such as satellite, echo-sounder and sidescan sonar give all background data and indirect indications of the species distribution. These methods are recommended for large-scale mapping and for finding suitable stations for more detailed studies of the plant and animal communities that are to be followed over time (i.e. monitoring) and evaluated according to recommended criteria, etc. Other methods give the species geographical distribution directly and/or detailed occurrence on sites.

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When selecting the method(s) to be used for a survey it is important to consider, *inter alia*:

- the size of the area to be surveyed;
- the desired spatial resolution of the data;
- the amount of field time available or required;
- the laboratory processing requirements;
- the amount of information generated by the specific method.

It is apparent that for most methods there is an inverse relationship between the area covered and the resolution and field time required. The costs are variable but some of the current more synoptic methods can generate large quantities of data that require specialised software to access and manipulate.

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9.4 Transect line techniques

Overview of transect techniques

The transect method is one of the most widely used methods in order to observe community change with depth and time. There are several ways of locating and collecting information along transects from the whole shore, from the intertidal down to 30 m depth. SCUBA diving is usually carried out when doing transects in the subtidal zone. The application of transect lines and their variations is based on visual observation and assessment of these communities.

The transects can either be placed horizontally, i.e. parallel to the depth gradient, or vertically, perpendicular to the depth curve. The use of horizontally placed transect lines has mostly been practised in tropical waters (see, e.g. English *et al*., 1994) where the scope was to describe the community composition. In the context of monitoring ecological status and as data for the WFD, vertical transects are used to obtain data of species depth distribution.

The observations along the vertical transect line are made in several ways, either in frames placed along the line using replicates or single frames at regular distances. For monitoring purposes the frames are usually placed at fixed depth intervals in order to follow long-term changes in the communities. The use of fixed frames is a standard procedure in the intertidal zone but it has also been applied in the subtidal zone by Lundälv (1985) and Sandnes and Gulliksen (1980) (see Beuchel *et al*., 2006). Several investigations use fixed depths where the frames are placed (Karlsson *et al*., 1992) or at a regular distance along the transect line.

Its simplest form is the intercept method, where transects of a given length are placed parallel to the shoreline at defined or randomly determined tidal levels of depths, with every transect representing a replicate. The observations are made under the transect line, preferably having a metre scale, at given intervals, usually every metre. To obtain a quantitative measure the extension under the transect line

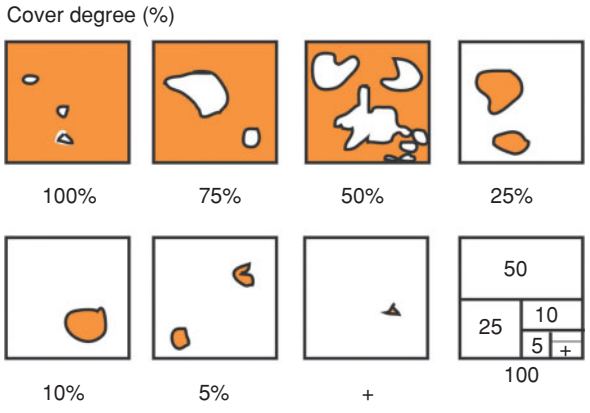

Fig. 9.2 The percentage coverage used. In the lower right corner the classical subdivision used by Braun-Blanquet is shown (slightly modified, as every lower percentage scale should be half of the former).

of the species can be noted, obtaining a measure of its relative abundance compared to other species or distance covered (distribution) (English *et al.*, 1994).

Another method consists of placing frames, usually of 0.5 or 1 m side length, along the transect line and making observations within the frame. The frames can be placed at random or at given intervals along the transect line. A special case occurs when frames are placed as a ladder side by side, and thus, observations are made continuously along the transect line. In the extreme case, frames are not used but instead observations are made in a corridor either defined by parallel lines at a given distance from the transect line or visualised and judged as being at a given distance from the transect line (e.g. 3–5 m, see the section entitled 'Recommended ICES method').

The transect lines can be placed horizontally, but if the scope is to see the tidal level or depth distribution of the plant species, the transect should be placed perpendicular to the depth gradient. The latter approach, with a vertical transect and observations made in a visualised corridor of approximately 6–10 m width, is the standard method for monitoring vegetation-covered substrates. It is quick and accurate and gives the tidal/depth distribution and estimated coverage (e.g. 7-grade scale) of the substrate of the conspicuous species (Fig. 9.2). The transects can easily be documented by overview photos when diving is carried out.

Recommended ICES method

This method (ICES BEWG, 2008) was designed for trend analysis of the benthic plant and animal communities in a given area, where species depth distribution is used as one of the criteria in the analysis. It has been in use in the Baltic Sea area since 1974 (Jansson & Kautsky, 1977; Jansson *et al.*, 1985; Kautsky *et al.*, 1986; 1988, 1999; Kautsky, 1989, 1992, 1993, 1995) and in other European seas (Hiscock,

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1998; Davies *et al*., 2001), and also in Australia (English *et al.*, 1994; Kingsford & Battershill, 1998). The main principles have been adopted by HELCOM (1999).

Sampling strategy hierarchy

In monitoring, the goal is to find temporal variation that can be attributed to environmental change, for instance an increase or decrease of eutrophicating substances or temperature shifts due to climate change. Therefore, environmental parameters not relevant for this purpose should be kept to a minimum. As phytobenthic communities are heterogeneous, merely relocating the transect 30 to 40 m along the shoreline may result in completely new communities as a result of the changes in wave exposure and type of substrate. The existence of a steep gradient can give rise to a new community at almost every successive metre depth. Shallow, intertidal communities are entirely different from those found deeper. If the purpose is not to mirror the geographic heterogeneity of the area and the depth gradient, the location of the transects and where to collect samples must be strictly determined and this hierarchy has to be taken into account in the interpretation of data. The comparisons in a given area or time span should follow a hierarchy (Fig. 9.3) where

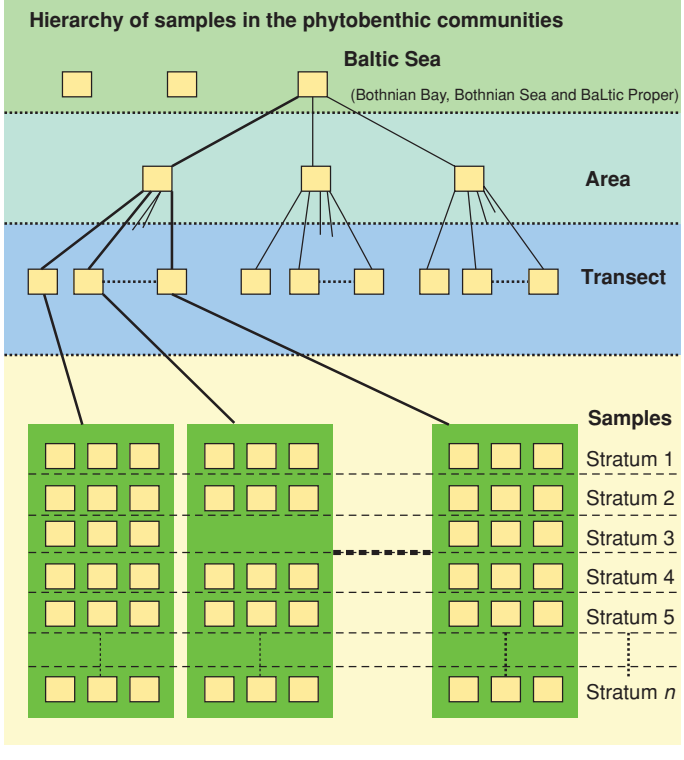

Fig. 9.3 Hierarchical tree of observations (quantitative samples). Starting from the base with strata defined by species depth distribution, repeated by transects within the same area to repeated areas in a region to whole water bodies. (For a colour version of this figure, see Plate 9.1.)

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samples/observations from the same depth/stratum are compared with a given area of similar environmental conditions, i.e. similar wave exposure or salinity.

Suggested hierarchical strategy:

- Comparison of samples from the same stratum;
- Comparison with samples from same type of sub-area (e.g. inner archipelago);
- Comparison of transects from the same area (at least three).

Changes noted may be either depth-dependent, e.g. the deepest finding of a given species, or may be dependent on the sub-area, or may be a change in the whole area. The final hierarchy will cover an entire region, for example the Baltic proper, the North Sea, etc.

Quantitative samples

When quantitative samples are taken, the statistical prerequisites in line with all sampling techniques should be carried out. The number of replicates has to be sufficient for valid statistical analysis, i.e. at least three samples per stratum (depth interval). To minimise the effort, it is advisable to apply a hierarchic sampling design. Location of samples should reflect the different belts as fully as possible. Therefore, the initial choice of sampling depth should be done in a hierarchical way, taking into account the existing zonation pattern (belts). Once randomly determined within a zone, the depth should be kept constant as depth is a major factor determining species distribution in the phytobenthic zone. When randomly taking samples in a wide zone (e.g. the *Fucus* belt in the Baltic Sea ranging from 0.5 m to 6–7 m depth) in one year, they may reflect luxuriant growth close to the surface and the following year all show sparse growth at maximum depth of the belt. Thus, in that case, the observed change can reflect only the difference in depth, determining species distribution.

In monitoring, a time series should be kept as long as possible, and in the case of zonal shift (Fig. 9.4) the samples should be collected continuously at the original depth and, when possible, a series of samples at a new depth that reflects the belt (stratum) of interest should be added.

For biodiversity studies, quantitative samples have to be collected. An easy, well-proven way is to use frames of a size appropriate to the communities collected. In the Baltic Sea, standard frames of 0.5 m × 0.5 m side length are used to collect samples from the *Fucus* communities and some seaweed communities. If *Fucus* is collected only the *Fucus* plants attached within the frame are picked. For the remaining communities a frame (0.2 m × 0.2 m) is placed on the substrate and everything within the frame is scraped into a mesh bag attached to one side of the frame (Fig. 9.5).

Choice of station/transect

Depending on the aim and the finances of the survey the starting point is either chosen or randomly placed in the environment. With some knowledge of the

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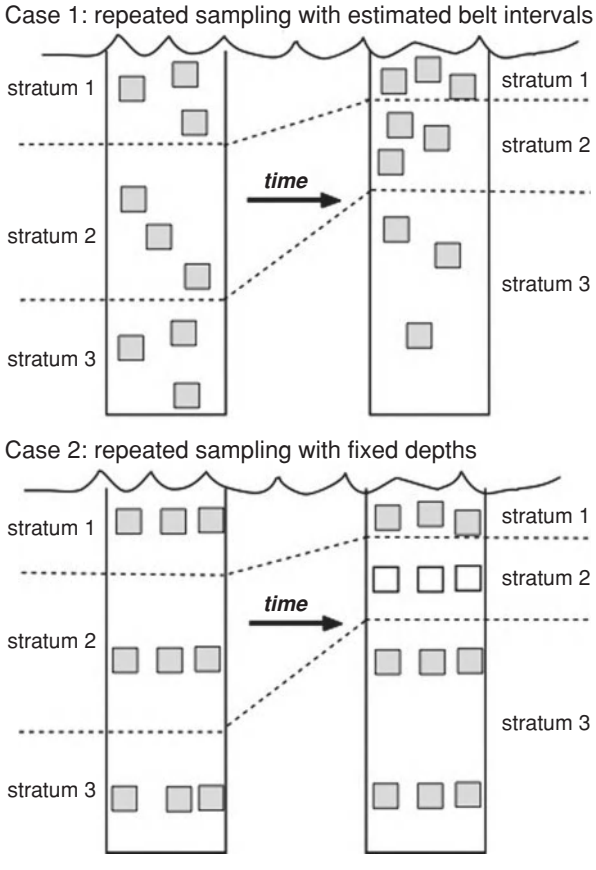

Fig. 9.4 The placement of quantitative samples either by random within a defined community (belt) or at fixed depth. When within fixed depth it might be necessary to include a new series if the belt has moved beyond the original fixed depth chosen. No series is interrupted.

geographic area it is better to use stratified sampling to obtain at least three replicates within the same type of area. In the area under investigation, it is recommended that samples should not be taken evenly in the area (Fig. 9.6a). Otherwise, when comparing different transects, they will be from different types of environmental conditions and, therefore, will be different. The same species will have a different maximum depth distribution, which will have nothing to do with any environmental changes. The transects should be placed in such a way that a certain number will be positioned in an inner part, and the same number in the middle and outer parts of the area (Fig. 9.6b). The choice of the number of transects in each sub-area should be made taking into consideration the homogeneity of the area, i.e. in the area under investigation it is quite likely that the inner and middle transects will differ from each other whereas the outer area is fairly similar. Therefore, less effort should be put in the most wave-exposed area. To give an example: four transects

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Fig. 9.5 Frames used within the Swedish monitoring programme and a picture of some modifications (second from left is the original). Four different designs of 'Kautsky' frame used in different Baltic laboratories (recommended by HELCOM COMBINE Guidelines).

are made in the inner and middle areas, respectively, and three in the outer. Then the stations from the sub-areas can be compared and any general trend in change of the plant and animal communities in the area may be detected.

When applied in the right way this sampling hierarchy makes it possible to separate the changes from the different sub-areas as well as the changes occurring at different depths.

In order to see changes at the temporal level, the results are analysed hierarchically: change within a given depth, within a transect, within a sub-area, within the area, within the region. A congruent change observed within one stratum validates progress to the next step in the hierarchy. It is recommended that for monitoring at the temporal level, the transect should be fixed with a permanent starting point and fixed compass direction. It is not possible to ascertain whether the observed changes are due to spatial differences or if they actually come from temporal change. Thus, the ability to make general statements concerning the changes within an area depends entirely on having enough replicates of transects in the area. With only one transect per area only changes on that transect can be observed, and even then only changes within a given depth. But if several transects within a sub-area or area show congruent development, we may predict general statements of the change in that area.

SCUBA transect estimates (ICES method)

When studies are carried out in the subtidal zone, SCUBA divers have to be used in spite of all the acknowledged drawbacks of time limitation and cost. As a standard

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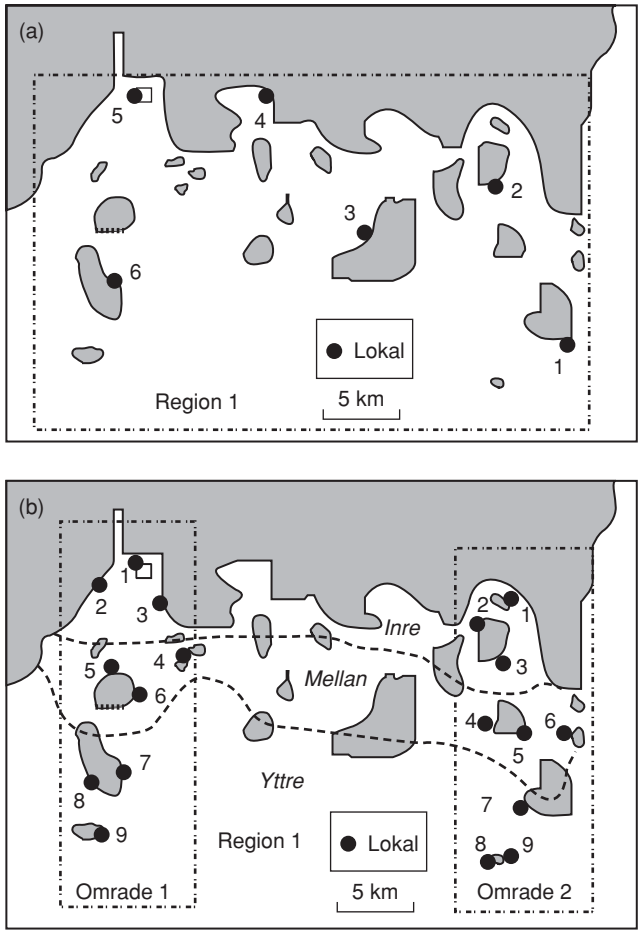

Fig. 9.6 (a & b) The choice of stations (transects). A random placement will inevitably lead to differences between stations caused by natural environmental factors, e.g. wave exposure (a). A hierarchical placement of the stations in, e.g. inner, intermediate and outer areas (or in any known gradient), will enable comparisons of stations within a given zone of similar environmental conditions.

diving procedure, two divers make independent observations in parallel along the transect line. Notes are made under water directly on site as the observed parameter occurs. For practical reasons it is essential that all the parameters investigated during a SCUBA divers' transect can be done by the divers in a replicable way. When compared, the two observations should contain the same information with only minor deviations, thus demonstrating that the method is independent of the observer. This is achieved by training and intercalibration. To keep costs at an acceptable level all the work has to be done within the limited time a diver can be exposed to a depth without more sophisticated diver routines (no forced decompression stop, etc.).

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Field Protocol

A field protocol must always contain the transect (station) number, full date including year when the observation was done and by whom. In the field diary or on the protocol, the position of the station (transect) is also noted, the divers, their starting time, end time and maximum depth, for divers' security. Additional observations regarding parameters such as salinity, Secchi depth, pH, temperature, as well as other relevant observations (compass direction, total length and maximum depth) should also be noted. A plastic writing board or slate can be used by the diver, with a sheet of inexpensive plasticised paper fixed on the slate, together with a compass, a depth gauge and a pencil tied to the slate by means of a cord.

SCUBA divers swim in a given compass direction, perpendicular to the depth curve, putting out a metre-marked line (standard measuring tape) until the end of the photic zone is reached. Then they turn and swim towards the shore, noting simultaneously *in situ*, on a writing slate, their observations on parameters such as the distance from shore, depth, type of substrate and its coverage, siltation and the species present and their coverage. These observations are made in a corridor 3–5 m wide on either side of the metre-marked transect line. Its width is dependent on the site. The divers note whenever there is a change in the substrate, a new species occurs and/or the coverage of the species changes. If none of the parameters have changed, no new notes should be taken. However, it is recommended that everything be written down anew when any new observation is made.

Position

The GPS position of the starting point of the transect on a station is always documented. The WGP84-system should be chosen as standard and positions given in grades and decimal grade – this facilitates the processing of results into a database. If the transect starts from the shore it should be photo-documented. If the transect is used for monitoring purposes, it should be marked permanently in an appropriate way (e.g. by means of a hole in the rock). As the transects may be very long in shallow areas, they can be subdivided in such a way that the entire depth range is covered, e.g. a part of the transect, for instance, 30–50 m long should cover 1–2 m depth, the next should cover 3–6 m depth, and so on depending on the topography of the area. In this case, it is essential to mark each starting point. The endpoint should in general be possible to calculate by the length of the sub-transect and its compass direction.

Distance and compass direction

Usually the starting point of a transect can only be established accurately if there is a hole in the rock, or by means of a DGPS position if the transect starts in mid-water. To revisit the exact spot one has to know the distance from the starting point along with its compass direction. From personal experience, from one year

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to another, marking bricks placed under the transect line at 2 and 5 m depth are usually within 1 m of the transect line. If the transect corridor is 10 m wide, divers will cover the same substrate as the previous year. However, changing currents may force the diver to deviate from the correct position. This can be minimised by placing permanent marks along the transect or noting conspicuous landmarks under water. If possible, iron bars may also be used, being bolted into the cliff at regular distances.

Distance from shore

The distance from shore is essential when repeating the sampling in, for example, a monitoring programme for detecting temporal change. The distance must always be the same from a given starting point. The depth may change both downwards and upwards with the distance from shore depending on the topography of the area. When repeated quantitative sampling is carried out, for instance by photogrammetric methods of a fixed area, or when using destructive sampling techniques, recording distance is the only way to ensure a return to exactly the same spot as before. The distance also gives the spatial range (width) of the species found along the transect.

Depth

Depth is an essential environmental parameter, easily measured with calibrated divers' depth gauges, giving an accuracy of 0.2 m. The phytobenthic communities are generally not affected by the water pressure increasing with depth, but other environmental parameters such as light and water movement will decrease with increased depth. The depth noted during the dive has to be calibrated to mean depth when recorded in the database. Therefore, the water level must always be noted (e.g. by noting the time of the dive and then by consulting tidal tables). When the depth distribution of species is the aim of the study, a good strategy is to sample several stations deeper than the expected deepest distribution range of the species being investigated.

Light

All plants are dependent on the light climate. In clear water the light decreases exponentially with depth. The change of water quality change is reflected in the depth distribution of the plants. There is a strong correlation between the maximum depth of plants and the Secchi disk depth, reflecting the light climate of the area. As a routine, whenever possible (this requires the use of a boat), the easily established Secchi depth should be measured at every station. The Secchi depth is bound to change quickly depending on the weather conditions at the moment of measuring. For monitoring purposes, the average change in the Secchi depth over the years is

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Table 9.2 The types of substrates and their codes used in databases. The ICES codes also contain SH, shells; CM, common mussels; OT, other.

Description	Substrate local SE	Code ICES	Rock (RK)	Boulders (BD)	Stones (ST)	Gravel (GR)	Sand (SD)	Soft (SS)
Hard	1	RK						
	2	BD						
	3	BD						
	4	ST						
Mixed	5	MX						
	6	ST					(Including, if only stones)	
	7	MX						
	8	GR						(Including, if only gravel)
	9	MX						
Sand	10	SD						
Soft	11	SD						
	12	SS						
Others hard	13	MX/OT						(Alternative also soft – SS)
	14	MX						(Alternative also soft – SS)

of major interest. Therefore, the changes in Secchi depth should be measured at least by other programmes in the area. The measure done during the dives may be used to compare the different sub-areas visited.

Substrate types

The type of substrate has to be recorded as different plant and animal communities have different capacities, both to occur and to remain on different types of substrate. The proportion of mixed and soft substrates normally increases with depth. Frequently, it is the type of substrate that sets the limit of species distribution with depth, rather than light. To describe the biodiversity of an area, as many of the different substrates as possible should be incorporated. Algal communities usually dominate on hard and mixed substrates, while seaweeds may dominate mixed and soft substrates.

The substrate noted *in situ* is divided into rock, boulders, stones, gravel, sand and soft sediment (Table 9.2). Each type of substrate is given a coverage, e.g. rock (100%) contains scattered boulders (10%) and stones (25%). The substrate can then be classified in the database as hard, mixed or soft substrates.

Percentage cover

It is essential to record the percentage cover of the different substrates as, for instance, where a large area along the transect is covered by seemingly depleted sand or soft substrate, or where on its scattering of boulders there occurs a luxuriant

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growth of plants that may cover the boulders completely. However, as the boulders have only a given low occurrence (i.e. percentage cover), the total coverage of the plants will be low in the area.

The percentage coverage is 'directly' estimated by the divers by looking around in the transect corridor and making a general description of the depth interval. The method relies on observation of the conditions, and not necessarily on the sampling of the area, which then has to be statistically interpreted. This seemingly subjective method is fully replicable by any skilled diver and thus is quite objective (for further discussion of this point see Dethier *et al*. (1993) and below).

The scale of the percentage cover is interpreted as: 100 = almost all substrate is covered (small holes are accepted); 75 = less than whole area but significantly more than 50%; 50 = around half of the substrate is covered; 25 = clearly less than 50% is covered. Then + is used for occurrence; 5 if there are more specimens and 10 if the species are seen to cover parts of the substrate. The estimate is also made by noting the absence of the species ('empty space') and the two should sum up to about 100. The estimates sound subjective, but there is a high replicability between observers and divers once the method has been learned (95–100%, the same as on average 12–15 zones with 5–10 species).

The depth distribution and coverage of biota

The purpose of the method is to establish the depth distribution of the animal and plant communities in the phytobenthic zone. The distance and depth is noted as well as the name of the species and an estimate of coverage using the seven-point scale previously described. For every observed species the coverage is estimated. Estimates should be made for all species, including the epiphytes. As the communities inhabit different strata (a bottom layer, shrub layer and canopy with epiphytes) the total coverage can often be considerably over 100%.

Loose plants should also be included. Problems caused by loose and decaying filamentous algal mats on shallow substrates have been discussed (Schramm & Nienhuis, 1996; Schiewer, 2008). Loose plants should be treated in the same way as the attached plants; where possible, they should be determined to species and given a percentage cover of the substrate. In several areas, loose plants may form natural, living communities, e.g. extensive *Furcellaria lumbricalis*-dominated communities outside the coast of the eastern Baltic States and *Fucus vesiculosus* communities in shallow bays of the Swedish Baltic coast. However, the deepest occurrence of a species should be based on recordings of attached specimens.

Wave exposure

Wave exposure is an essential environmental factor that together with the geological prerequisites of the site rules the composition of the substrate. It also directly influences the species composition and biomass. The wave exposure of a site

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should always be measured or estimated. ICES gives some direction of how this can be carried out in an easy way by using the longest fetch distance. More sophisticated methods using GIS applications were developed by Isaeus (2004). Reliable measurements of wave exposure giving an integrated value over a longer period of time have yet to be developed.

Frame size

The size of the frame has to be appropriate for the purpose of the investigation and for the species collected. It is strongly recommended to keep the use of different frame sizes to a minimum. The frame size may be one reason why there is an observed difference in the results. The problem is of course resolved by using just one frame size. However, a simple cost-benefit analysis will show that this is not always practical. It takes too long to process a 0.5 m × 0.5 m quantitative frame if the entire community is sampled. Therefore, in Sweden, a 0.2 m × 0.2 m frame is used as standard.

Sample processing

As it is not always easy to identify species underwater, samples are collected in numbered meshed bags, together with distance and depth, for later identification on land or in the laboratory. Samples are transferred to appropriately marked plastic bags and deep frozen for later sorting in the laboratory (see sampling gear, Fig. 9.7). Deep freezing is an efficient conservation method if species are later to be sorted and dried for dry weight. In the laboratory, the samples are sorted to species or the nearest higher taxon or to the level decided by the needs of the investigation. The animal species are counted, and each species is dried separately in 60°C to constant weight, i.e. for at least two weeks. In the Swedish monitoring programme the biomass is given in g dry weight/m^2, including shells when present.

Video transect method

The video transect method is used as a complement to divers' transects. The principle is the same. Instead of divers, the video camera records the substrate and its contents. Afterwards, a protocol is written based on the video record and the environmental data collected simultaneously (e.g. GIS position, depth, type of substrate, etc.).

For further details, see the section entitled 'Other underwater video techniques'.

Continuous observation methods

In certain programmes, the use of frames has been replaced by observations, made in an uninterrupted way, noting whenever a change in substrate, species occurrence or species coverage occurs. A variation of this method is to make

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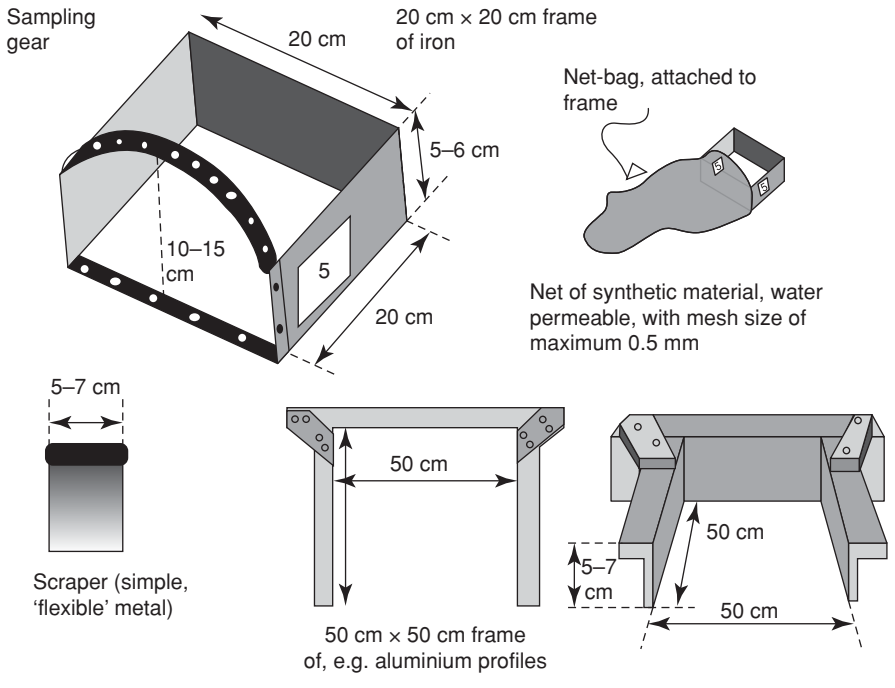

Fig. 9.7 Sampling gear.

free observations at given depth intervals. These approaches (with their positive features and drawbacks) are described below.

Vertical profiling for estimation of species depth distribution

When the scope is to follow species depth distribution and their changes over time, vertical profiles are used. Along the transect line observations may be made in frames at given depths (Fig. 9.8). To obtain a good estimate of the population at a given depth (or region) a number of replicates (at least three) should be taken. This results in good estimates of the species distribution at discrete depths along the transect line. However, to obtain the maximum depth distribution of a single species, additional observations must be performed in between the depths of sampling. If this is not done, this method will record only species depth distribution at discrete intervals, with 1–2 m depth resolution or more, depending on the placement of the frames.

The free estimate approach

When making observations for species distribution along the transect line without using frames (the free estimate approach), the entire area within the corridor is scanned by the divers for species. In practice, 1–2 m ahead and 3–5 m at each side

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Fig. 9.8 Placement of frames along a transect line perpendicular to the depth curves. Observations are made within each frame only. Frames are either placed at fixed depth intervals or distance from shore. (For a colour version of this figure, see Plate 9.2.)

of the transect line are scanned by the observer. A record is made whenever a change in the type and/or composition of the substrate, species occurrence and coverage is observed. For each observation the distance from shore and the depth is noted. Thus, we obtain a continuous description of the plant and animal communities within that corridor and can easily depict any species' depth distribution using different criteria, e.g. individuals in the deepest parts, or when the species covers more than 5% of the substrate, etc. This method gives a true assessment of the communities within the corridor, which, when frames are used, must be estimated with some degree of uncertainty.

As always, the description holds true only for the transect in question, and in order to give areal estimates, the transects have to be accordingly replicated in the given area. In this case each transect is treated as one replicate. This method is fast and as mentioned earlier is observer-independent. As with all methods, the observer has to have a certain skill usually obtained by training and intercalibration.[2]

The free-form estimate method of observing the communities along a corridor usually covers an area 100-fold larger than the placement of frames along the transect line[3]. A standard method for shallow bays prescribes that observations

[2]At a workshop in Helgoland in 2006, the frame approach as well as free estimates of species distribution were carried out along a transect in the tidal zone approximately 50 m long. The free estimate group achieved a detailed description of species distribution and cover within roughly one hour, whereas the frame group completed only three frames (1 m × 1 m) over the same timescale.

[3]In a Norwegian monitoring programme, replicate frames placed at given depths were first selected but after some years they were replaced by free estimates at given depth intervals as the frame observations took too long (Frithjof Moy, pers. comm.).

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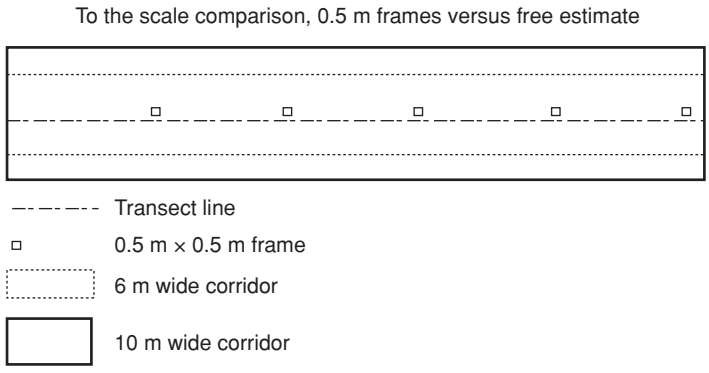

Fig. 9.9 Comparison of area to scale covered by placing a 0.5 × 0.5 m frame at every 10 m distance or performing free estimates of the distribution and cover within a 6 and a 10 m wide corridor along a 50 m long transect line.

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should be made in 0.5 m × 0.5 m frames every 10 m, and if the transect is long, then every 20 m. A comparison of the area covered by this method and the free estimates within 6–10 m wide corridors is illustrated in Fig. 9.9. Along a 50 m long transect the frame method would give observations from a total of 1.5 m^2 (if three parallel samples are taken at each distance, that amounts to an area of 4.5 m^2), where the resolution of change is at best 10 m. The corridor method covers 300–600 m^2 in area, including the exact position of change in substrate, species occurrence and their cover.

A good comparison of the free estimates against sampling using small frames is found in Dethier *et al*. (1993). These authors concluded that around 10–14 small frames are needed to obtain the same precision as the free estimates of the studied area.

Rationale of free estimate approach

The intention of the free estimate approach is to describe exactly the species depth distribution and their coverage along a given transect line. The transect is thus a description of the actual situation in the given corridor, from the deepest point to the surface. To make adequate descriptions of a whole defined area, a number of transects have to be performed, where each is seen as a replicate. If, instead, frames only were placed along the transect, these could be used merely to make an estimate of the species distribution (including statistical uncertainty of the observation), and not to record the true value for the transect, as in the free estimates without frames. Frame observations can be used for larger area estimates, but incorporating an increased uncertainty. To obtain an accuracy similar to the free estimates, more frames have to be sampled. This is a time-consuming process, and free transects have proved to be more efficient to a given precision, as has been demonstrated in several comparative field works and in workshops.

Estimates of abundance based on observations within frames are widely used by ecologists (see Murray *et al*. (2006) for field approach and recent literature). By using replicate frames (or lines), an estimate of the species present with an attached uncertainty can be made. The major drawback is that frames used are seldom larger than 1 m^2, usually only 0.25 m^2 (0.5 m × 0.5 m frame). An alternative would be to look at a sufficiently large area in order to diminish unwanted, spatial variation. Otherwise a large number of replicates have to be made (Dethier *et al*., 1993). Krause-Jensen *et al*. (2000, 2007b) concluded that the larger the frame the better, and found that 5 m × 5 m frames were the minimum required. Some workers extended this into making estimates within a 'free' 6–10 m wide corridor (at least 10 m^2 per observation). The species abundance or/and coverage is estimated in several ways. Besides presence and absence, either a percentage scale is used (classically, the Braun-Blanquet 5-grade scale, modified by Poore (1955) to 6) or the occurrence under the internodes (obtained by a grid subdividing the frame in, e.g. 10 × 10 lines) is counted. The true coverage can be obtained by photogrammetric methods. The method gives accurate measurements of a small area. By repeating the frames an estimate of the community is obtained with an estimated statistical uncertainty attached. Classical statistics are generally easy to apply as each frame is a replicate. The major drawback is the need for a large number of samples to encompass the natural spatial variation of the phytobenthic communities. This is especially the case when the frames are relatively small (e.g. the standard of 0.5 m or 1 m side length). Evaluations in Denmark came to the conclusion that frames of at least 5 m × 5 m (25 m^2) should be used to cope with spatial variation (Krause-Jensen *et al*., 2000).

Photo frames

Instead of estimating species occurrence and cover within frames in the field, the frames can be photographed and processed later in the laboratory. A first, more systematic use of photo frames for the determination of species occurrence and coverage was carried out both in Sweden and Norway (see Lundälv & Christie, 1986). Both used fixed sites marked under water, which were revisited at given time intervals to follow the temporal change. One version of this approach can be seen in the monitoring programme of the Swedish west coast where, however, statistical aspects have guided the set-up. Along a fixed horizontal line 30 m long, five vertical transects are randomly placed at metre distances. Along each transect two parallel pictures are taken at each metre depth. From the surface down to 1 m, pictures are taken every 0.5 m and then every metre down to below 12 m depth where each 2 m depth is documented. Thus, within each station, 2.5 m^2 are observed at discrete depths along the depth gradient. In addition, the most conspicuous species depth distribution along the transect line are noted in order to obtain the true, maximum depth distribution of the species.

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Quantitative samples can be obtained either by photo frames (stereo-photo frames; Lundälv, 1971, 1985; Karlsson *et al*., 1992) or by destructive sampling. These methods give results easily treated by traditional statistical methods. However, a major drawback of destructive sampling means that this method can destroy the habitat. This can be diminished by sampling over a larger area and/or using smaller frames, especially in communities with perennial species but also by using photo frames. In this case, the canopy layer shading the underlying species may be a problem, and, in general, this method can be applied on a fairly steep sloping substrate.

9.5 Other underwater surveying methods

Though diving is the standard method, the study of the subtidal can be carried out in different ways, such as the manta tow and the diver-propulsion vehicle techniques.

The manta tow technique

The classical manta tow consists of a plate being towed at low speed behind a boat, with a diver controlling its height over the seafloor and simultaneously observing and making notes. This method, developed by the military to enable divers to cover large areas without the need for laborious swimming, was the best option before the more sophisticated video-cam reached the market. Relatively large areas can be covered by this method, in which the diver usually manages to give a rough estimate of the conspicuous characteristics of the habitats, such as type of substrate, occurrence of kelp, etc. A fast method, it is used mainly in waters with low turbidity. Its major drawback is that there has to be a SCUBA diver who makes the observations, with very little or no time at all to stop and rest, and with few opportunities to document the observations as there is always something new coming into the observer's vision. In many cases it can also be dangerous for the diver who needs full concentration to keep on track, and therefore, it should only be used in uncomplicated undersea landscapes. The method has been successfully used for surveying the distribution of single, conspicuous species, on sandy substrates, and in tropical regions when mapping the extension of coral reefs, crown-or-torn starfish, etc.

Diver-propulsion vehicle technique

Another version of the manta tow is the diver-propulsion vehicle, also known as an underwater scooter. It enjoys the same advantages as the manta tow but it can, in addition, be stopped by the diver. However, it suffers from similar disadvantages: it is hard to make notes during operation, hence radio communication is recommended. Further disadvantages are their dependence on batteries and their limited range.

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Other underwater video techniques

However, there are alternative methods, including the use of video cameras for remote observations, which can be used to cover large areas. Documenting the seafloor with video is a quick method and may provide an alternative method for SCUBA diving.

Video hanger

The data obtained from a video hanger are visual, and therefore, no signal interpretation is needed. Because the camera can be towed over a relatively large distance, the depth range of the photic zone can be covered within one video session or km-long, horizontal transects. The resolution is high (<0.01 m) especially when the camera is towed slowly (<1 knot). The data from the video profile can be documented and interpreted in the same way as free estimates along transect lines of a known gradient, as described in the more detailed description of the transect method.

Each video observation should be accompanied by its exact position (GPS) and depth information. The equipment can be inexpensive (for around €1000 good functioning systems are available on the Internet). Archived videos provide the verifying data or can be used to extract additional information. However, apart from camera resolution, video quality is affected by the video hanger camera movement and by wave-induced ship movement, which makes some ground-truthing necessary. The low wide-angle view of the camera lens also makes identification of biota more difficult. These problems can be partly solved by technical improvement of the gear, but as a result, the cost increases. A vertical picture gives only a limited view of the surrounding area, and therefore, it is recommended that another camera which has a more horizontal view should be added. This method has been successfully used in the habitat mapping of offshore shallow reefs. It may serve as a survey tool for finding suitable stations for diving transects, as in the start-up of monitoring programmes. An alternative to the video hanger is the use of bottom-towed video cameras mounted on sledges. The advantage of this method is the constant distance to the seafloor, but the picture is usually extremely shaky, making interpretations of the species occurrence rather difficult.

Drop camera technique

An alternative to the video hanger during large-scale habitat mapping is the sampling of central or random points within a grid using a drop camera. However, this technique will only give a limited picture of the spot where it has been deposited. The grid size may be standardised, for example 1 km × 1 km, or of different sizes, depending on the expected heterogeneity of the area. A random proportion of squares within the grid are visited and documented. The observations are made just around the point where the camera has been dropped (1–4 m^2). The results are presented as large-scale maps of species distribution. Here the

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fragmented information obtained from the actual video picture is scaled up to regional or national maps using a modelling approach, where the key environmental factors such as wave exposure, depth and type of substrate are included. Extensive mapping surveys have been performed this way, e.g. along the coast of Finland a grid of 100 m × 100 m was established from which a random choice was made and a certain number of squares were visited by lowering a video camera into the middle of the square. A small fraction of the seafloor then becomes the base for large-scale estimates of the plant and animal communities. There are several programmes (either ongoing or recently completed) dealing with the problems of seafloor mapping (see, e.g. the reports from the EU inter-regional programmes MESH (http://www.searchmesh.net/; accessed 7 November 2012) and BALANCE (http://balance-eu.org/publications/index.html; http://balance-eu.org/xpdf/balance-interim-report-no-30.pdf; http://balance-eu.org/xpdf/balance-interim-report-no-27_draft.pdf; accessed 7 November 2012).

Video transect method

As stated above, the video transect method is used as a complement to divers' transects. The principle is the same. Instead of divers, the video camera records the substrate and its contents. Afterwards, a protocol is written based on the video record and the environmental data collected simultaneously (e.g. GIS position, depth, type of substrate, etc.).

In Sweden, a lightweight system has been developed suitable for small boats, e.g. 5 m, outboard motor is used as a working platform with few operators (two to three persons: one making the observations, one holding the video rack, one steering the boat who could also be the observer). The system is composed of two cameras, one high-resolution camera (3 CCD) gives a vertical picture and one high-resolution (1 CCD) camera is more horizontally placed, which helps the observer to see where the camera gear is heading, and also gives a better overview of the area. Additional light, parallel laser beams, echo-sounder and a depth gauge are placed on the underwater gear. The pictures from both cameras are seen on two monitors at the surface, and recorded separately on two tape recorders together with the GIS position and all environmental parameters measured. The environmental parameters are recorded directly on the audio strip of the videotape. Any measured information can then be seen on the monitor. All video equipment is held in two Pelican boxes, easily transported and mounted on any ship. The system is designed to work from small boats in shallow waters, but can be operated from ships. The equipment has a 75 m long cable (25 + 50 m).

Video output

The result of the video is a 100 to 1000 m long transect of one to a few m wide depending on the distance over the substrate of the video camera. The observations

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are then classified accordingly as described above in the protocol for the divers' transects. For each new observation, the GIS position, depth and other parameters measured are registered in a protocol. New observations are made when either a new species is seen, the coverage of the species changes and/or the type of substrate and its composition changes. Thus, along the transect, several sub-areas are recorded of various lengths. For each starting point the position (GPS) is recorded, together with depth, type of substrate and its coverage and the species and their coverage. In addition, in the Swedish example, the distance of the video camera to the substrate and to the surface is measured by an echo-sounder (0.1 m accuracy) and a depth gauge, respectively, and the declination of the gear and time are recorded. The equipment allows three to five additional sensors to be added.

Remotely operated vehicles (see Chapter 3)

The remotely operated vehicle (ROV) does not depend on the movement of the mother-ship, and as a result, it normally gives a more stable picture. As it is easy to focus on an object of interest, it can provide excellent pictures that are usually easily interpreted. The more advanced ROVs have manipulators and can collect samples. ROVs can be operated even in the deepest sea areas, but, of course, the cost increases dramatically according to the depth of operation. Its major drawbacks are the relatively high cost, the smaller operation range (depending on the length of the umbilical cord) and the need to have a skilled pilot navigating the ROV into position.

Echo-sounding, sidescan sonar (see Chapter 3)

Ship-borne registration tools (e.g. echo-sounder and sonar) are not usually limited by depth and can operate within the phytobenthic zone without difficulty (e.g. video hanger and ROV). Depending on the method, there will be a trade-off between resolution and the area covered. The non-visual methods require longer laboratory time for the interpretation of data.

Echo-sounding and sidescan sonar techniques are capable of covering large areas with a relatively high resolution (>0.1 m). These techniques that use multi-beam have no depth limit, and give good to excellent depth information. The sidescan sonar technique gives good estimates of the substrate type, and can also detect plant cover. One drawback is that the data need interpretation and calibration through ground-truthing. A further disadvantage in using acoustic signals stems from their dependence on the physical characteristics of the water column. For instance, a thermo- or halocline will alter the waves received by the echo-sounder or sonar and, therefore, may generate different pictures of the same seafloor. There is still the need to interpret the data signals, i.e. what they actually show and where are the separating frequencies that can distinguish between the different substrate types or between covered and uncovered seafloor. Also, there is still a need to find

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out which are the best frequencies for discriminating between the phytobenthic communities and the seafloor, and these are probably not the ones currently used for fish detection and depth determination. The sidescan broader view methods have successfully been used in producing marine geological maps of phytobenthic communities, e.g. *Posidonia* meadows in the Mediterranean and seagrass beds in the United States. There are ongoing projects to improve the signal interpretation using echo-sounding (e.g. NIVA-Simrad). But it should be said that there is no reliable standard method today that has proved to hold true for critical testing by scientists.

9.6 Other surveying techniques

Satellite imagery

Of all of the methods under consideration, satellite imagery covers the greatest area though usually with low resolution. Normally, pixel sizes are 50 m × 50 m; however, in special cases, resolution can be increased to 20 m × 20 m. Today it is possible to obtain even higher resolutions. Satellites can actually see objects down to fractions of a metre (SPOT of 2.5 m and Geo-Eye-1 of 0.5 m resolution), but this is seldom used due to limits in interpreting, handling and processing the huge set of data information. Therefore, the scope is often to present maps for planning, the pixel size remaining large. While the amount of field time required is limited, considerable time and expertise is required for processing and interpretation of the data.

Satellite imagery is used to monitor the surface pelagic production. However, the technique may also have some potential for the study of phytobenthic communities and several examples of mapping in shallow and clear waters of coral reef extension and seagrass meadows can be found. However, further development of methods, in particular signal interpretation, is needed to follow more heterogeneous phytobenthic communities. In addition, methods have to be developed to handle the immense amounts of data at reasonably high resolution (1 m × 1 m to 10 m × 10 m grids) where the pixels also are interpreted accurately for phytobenthic species. If it is necessary to have the species information, the resolution of images obtained by satellite is in general too low. A major drawback is that satellite imagery can only see a few metres into the water column in temperate waters. Satellite images cannot cope with the transition from land to sea (they are either set for land or sea reflectance), which might not be a problem in extensive, shallow areas. However, along steep coasts this may mean that only one or very few pixels describe the whole phytobenthic zone before depth exceeds the resolution of the image. Atmospheric interference such as cloud also limits the number of usable images in many regions. Data from satellites can be used to determine turbidity and the location of major land-based outflows, thus providing essential data for the interpretation of results from phytobenthic studies. This is especially useful for the application

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of large-scale models (see, e.g. Kratzer & Tett, 2009). Kutser *et al*. (2006a, 2006b) used satellite measurements for three common Baltic species such as *F. vesiculosus*, *F. lumbricalis* and *Cladophora glomerata* in an endeavour to make maps along the Estonian coast (a project within the EU project BALANCE: Baltic Sea management – nature conservation and sustainable development of the ecosystem through spatial planning). Although the species could be depicted in special cases (such as when growing on contrasting sandy substrates), the method could not be used to determine their depth distribution. These authors stated that:

> Configuration of MERIS (Medium Resolution Imaging Spectrometer) spectral bands allows the recognition of red, green and brown macroalgae based on their spectral signatures provided the algal belts are wider than MERIS spatial resolution.

They also used the method described above in clear waters in Australia, and obtained better results, indicating the problem with the Baltic Seawater colouring and transparency. In the Mediterranean, the *Posidonia* meadows were successfully mapped using satellite images (Fornes *et al*., 2006). It should be noted that all satellite data require ground-truthing.

Aerial photography

Aerial photography can cover quite large areas, and the images thus generated frequently allow observations to be made down to about 3 m depth even in Nordic waters. However, in clear water areas, such as the Mediterranean or tropical areas, aerial photography can be used to map vast areas down to several tens of metres. The resolution is in general high (>0.1 m), at least close to the surface under ideal conditions. The method is nevertheless weather-dependent; clouds, wind and the angle of incidence of the sun upon the water surface all affect the image quality. The images need ground-truthing for the interpretation of the observed structures.

Compared to satellite imagery, aerial photography can cover only limited regions; usually a picture covers about a 500 m $\times$ 500 m area. However, the resolution is much higher than satellite images and objects down to a few decimetres in size can be observed. Since it is a method used to determine the shape of objects, less laboratory time has to be spent in the interpretation of data. Aerial photography has successfully been applied in the mapping of reed belts (Finland), in shallow bays, for instance *Zostera* and *Fucus* communities (Swedish west coast, locally on the east coast), in mapping *Posidonia* meadows (the intertidal Watt area in Northern Germany), mapping coral reef extent, etc.

Laser scanning techniques

A relatively new, potentially highly interesting method using laser techniques for the bathymetry and identification of species is under development. Every species

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has a unique reflection of the laser light, which today is used in the mapping of land-living plant populations. Though the identification of species can be limited by the turbidity of the water, it can collect data down to around 1.5 times the Secchi disk depth, that is, approximately 20 m depth in the Baltic Sea. It can be used to produce charts of high resolution needed for the efficient planning of surveys and modelling of results.

A new promising technique related to aerial photography is the use of laser scanning from aircraft (e.g. laser range finder LIDAR) where the backscatter gives information as to the depth of the water column and the rugosity of the seafloor. The technique is used for bathymetry and is under development for the interpretation of the benthic communities (see reports by Coggan *et al.*, 2007; Kautsky *et al.*, 2010).

9.7 Conclusion

There is no single comprehensive method incorporating all study techniques, and different approaches have to be applied depending on the scope of the investigation. However, when similar questions are asked it would be of advantage to use the same or at least fully comparable methods, in order to ensure that the collected data are comparable.

Taking into account the several approaches discussed above and national methods referred to at the end of the chapter, there are two main approaches that can be used to broach the problem of describing and using the phytobenthic communities for monitoring purposes. In the first case, the classical approach is to estimate the structure by collecting random samples (frames), to derive a mean value and give an estimate of the statistical error. The other approach is to observe the structure either within fixed frames or in a more extensive area or along corridors following, for instance, a transect line (this may also be aerial photography, video transects, etc.). The temporal change within a fixed frame is only statistically applied to the frame itself. When estimates of the temporal change in a whole area have to be made, there must be a number of frames that co-vary. This co-variation can be statistically evaluated. Therefore, the second approach gives no estimate of the variation (statistical error) unless a number of transects are placed in the area of interest, where each transect is then seen as one replicate. However, this areal approach gives the true value of what it looks like in the frame or along the transect line at the time of observation – it is not an estimate that approximates the truth (with a given statistical error).

If the goal is to establish species distribution along a gradient (e.g. depth) and to monitor change, a cost-efficient approach will be to follow change over fixed transects where observations are made continuously in corridors. This approach has been practised in Sweden since 1974 and has been the standard monitoring method since 1993; it is also recommended in HELCOM, OSPAR (JAMP), is included in the new ISO standard and is practised by several countries.

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Appendix

Short review of methods used for monitoring in Europe and overseas

Baltic Sea region

In Finland, the present monitoring programme is based on the proposal for long-term monitoring as described by Bäck *et al.* (2002). Their proposal was based on the Swedish monitoring programme and in line with the recommendations of HELCOM monitoring programme (COMBINE) and the Nordic Council of Ministers (Bäck *et al*., 1996, 1998).

Field observations are carried out along transects but only in frames at given depth intervals where species composition, algal coverage and canopy height is measured (Bäck *et al*., 2006). In addition, the lower depth limit of the continuous growth of *F. vesiculosus* (defined as 25% cover) and the maximum abundance is noted (Bäck *et al*., 2006). In the design of the monitoring programme, a special interest was paid to areas with high protection values such as NATURA 2000 or HELCOM's BSPA (Baltic Sea Protected Areas) or on aspects that support the implementation of the EU WFD.

In Denmark, the methods chosen for monitoring the phytobenthic communities were thoroughly evaluated through field experiments and an attempt to find parameters that correlate to other environmental parameters such as nutrients, salinity, etc. (Middleboe *et al*., 1997; Middleboe & Krause-Jensen, 1998; Krause-Jensen *et al*., 2000, 2001; Laursen, 2000). In Denmark, it was concluded that the larger the frame size, the better replicates and thus for their investigations a 5 m × 5 m frame size was chosen after evaluating sizes from 0.5 m × 0.5 m frames upwards. They also concluded that opportunistic macroalgae were less reliable for long-term monitoring (Krause-Jensen *et al.*, 2007a), whereas the maximum depth penetration of perennial algae is well correlated to eutrophication (Krause-Jensen *et al.*, 2007b, 2008). Three replicate large fixed frames (5 m × 5 m side length) placed at each depth interval are investigated for species composition and coverage. In addition, perennial species, mainly *Zostera marina*, areal and maximum depth distribution are mapped along transects.

The three Baltic states Estonia, Latvia and Lithuania have coordinated their monitoring programmes by holding common workshops in the field (Martin, 2005). The methods used are directly derived from the HELCOM COMBINE programme and are well in accordance with what is done on the Swedish side of the Baltic Sea (Martin *et al.*, 2003). Observations are made concerning species composition and vertical distribution of phytobenthic communities, biomass and coverage along transects at 1 m depth intervals. Long transects are divided into shorter distances but which cover the depth gradient. In Lithuania, a manta tow is used to estimate the extensive photobenthic *F. lumbricalis* communities that cover large areas lying loosely on sandy substrates (Daunys *et al*., 2007; Bucas, 2009).

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Regular phytobenthos monitoring programmes were initiated in Poland in 2000. Taxonomic composition as well as percentage vegetation coverage were investigated using methods well in accordance with the HELCOM COMBINE recommendations following participation in the ICES BSRP workshops. In addition to estimating species depth distribution and cover of the substrate, quantitative samples are also collected by destructive sampling using a 20 cm × 20 cm frame, the 'Kautsky-frame' (Andrulewicz *et al*., 2004).

For the Baltic Sea area in Germany, two documents are available describing the approach by the German authorities for monitoring and WFD classification (Fürhaupter & Meyer, 2008; Selig *et al*., 2008) based on the work of the group led by Schubert (2004). In the inner waters the depth distribution of plants is monitored by remote technique (video and aquascope) along given transects, where observations are made at given depth intervals in five frames of 1 m × 1 m size. In addition, SCUBA divers are used for ground proofing and verification of the deepest findings. In the outer waters also, transects are monitored where observations are made within frames and given depth intervals. A review of shallow area methods is found in http://www.biologie.uni-rostock.de/oekologie/archives/Endbericht_ELBO.pdf (accessed 7 November 2012).

In the Watt area, it is mainly the large seaweeds that are monitored (*Z. marina and Zostera nana*). A joint study carried out which included Denmark, Germany and The Netherlands (HARBASINS) did not decide on a single approach but chose several methods where remote sensing was used as the base for mapping and transect lines as one way of performing ground-truthing (http://www.waddensea-secretariat.org/workshops/wfd-tmap.html; 4 December 2012).

On the island of Helgoland, repeated sampling of the tidal flat was performed by Bartsch and Tittley (2004). In the subtidal some observations were also carried out (Bartsch & Kuhlenkamp, 2004). The studies used a combination of methods including remote sensing (Hennig *et al.*, 2007) and the placement of frames (0.5 m × 0.5 m). In the subtidal zone, the depth distribution of key species was surveyed using SCUBA divers swimming along given transects (Pehlke & Bartsch, 2008; http://hdl.handle.net/10013/epic.22330; accessed 7 November 2012).

Eastern North Atlantic and North Sea region

On the marine Swedish west coast the national monitoring programme is based on stereo-photography in fixed stations with randomly placed transects every year (Karlsson *et al.*, 1992; https://www.havochvatten.se/en/start.html; accessed 4 December 2012).

Since 1990, Norway, with a coastline of 40,000 km, mostly hard substrate, has followed a coastal monitoring programme (Moy, 1999), which includes transects with free observations at given depth intervals, stereo-photography of fixed frames, kelp monitoring (density and age-group distribution) and a lower depth limit of chosen species in certain fjords. Norway uses an index of perennial to annual to

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describe the water quality, where annual species are considered to increase when the area is eutrophicated. A pollution index has been tried out but it is not consistent between fjords. Red, green, brown ratios have been investigated. Only coarse changes are detectable. An increase in depth and number of fucoids has been seen recently (www.niva.no; accessed 7 November 2012, Langtidsovervåking av miljøkvaliteten i kystområdene av Norge. Kystovervåkingsprogrammet. Årsrapport for 2004.). The newly adopted ISO standards for littoral and sub-littoral hard-bottom communities were written by Norwegian scientist and generally follow their national standards (ISO, 2003). The standards not only include all types of hard substrate in the littoral zone and sub-littoral zone down to 30 m depth, i.e. rock, loose pebbles and stones (mixed), but also include pipelines and other hard underwater constructions. The general method described up to now is the use of gridlines, i.e. a low number (four) of fixed or randomly placed frames of appropriate size and the counting of species within the frame. The overview survey covers simple documentation of bottom conditions and hard-bottom fauna and flora mapping, using divers video observations and/or ROV, description of environmental conditions and trend monitoring.

The United Kingdom has been a world leader with regard to conducting surveys of coastal regions and phytobenthic zone (Kingsford & Battershill, 1998; Davies *et al*., 2001). A number of relevant methods along with good manuals have been produced over the years (see below). However, a national long-term monitoring has not yet been conducted and, regrettably, monitoring of the phytobenthos will now be restricted to temporal studies of fixed frames in the intertidal zone. This decision may limit costs substantially but will only reflect a small part of the rich phytobenthic communities around their islands, losing all possibilities of following the changes in their well-known subtidal regions. Nevertheless, their earlier efforts can still be followed through the material describing relevant methods found on the following links:

http://www.ukmarinesac.org.uk (accessed 7 November 2012)
http://www.marlin.ac.uk (accessed 7 November 2012)
http://www.jncc.gov.uk/csm/ (accessed 18 November 2012)
http://jncc.defra.gov.uk/page-2430 (accessed 7 November 2012)

In France, remote techniques are used to document the seagrass communities. Ground-truthing is carried out by video techniques, dredging and SCUBA diving. Some observations of subtidal cover can be made. Sidescan sonar is currently being tested to achieve maximum depth limit of *Z. marina* and *Zostera noltii*. Biomass of weed is recorded where it is washed up on the shore. Meinesz (2007) used transect methods, deploying video, areal photography and divers (tawn) to map the occurrence of invasive species.

In Spain, Gorostiaga and Diez (1996) followed the fate of pollutants in the Bay of Bilbao for eight years, using transects perpendicular to the seashore. Observations were made in 1 m^2 quadrats at given intervals.

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Portugal collects data of the species that occur in the coastal area and is producing a management plan to protect those species. In the Azores, within the scope of NATURA 2000, sectoral plan, management plans for all Marine Protected Areas (MPAs) were developed, and are gradually being implemented through classification processes in the regional network of protected areas.

Mediterranean Sea region

There are several ongoing programmes going on in the Mediterranean region. For a review, see UNEP - MAP - RAC/SPA (2006), which can be downloaded. In the Mediterranean Sea the *Posidonia* meadows have received special attention (Calvo *et al*., 2003; Romero *et al.*, 2007).

In Spain, within the context of the WFD, a low-budget monitoring of macroalgae in the upper infralittoral down to 1 m depth was suggested, the main goal of which was to implement the European WFD in order to have the ability to class the communities under study in the five ecological status classes of high, good, moderate, low and bad (see, e.g. http://www.unepmap.org/; accessed 4 December 2012). They used five permanent site squares of 5 m × 5 m, which were photo-documented. In addition, one randomly placed quantitative frame (0.2 m × 0.2 m) was used for destructive sampling within the large square (Arévalo *et al.*, 2007; Ballesteros *et al.*, 2007). The sampling was repeated 6 times. The cover value of each taxa in the photo frames was measured and the structure of the vegetation was described. Of the various indices used (Shannon–Weaver, Pielou-evenness, MultiDimensional Scaling (MDS) plot by Bray–Curtis similarity and Ecological Evaluation Index (EEI)), MDS and EEI were better at describing differences. The method is a simplification of the suggested method with a concomitant loss in prediction power.

In Italy, monitoring has mostly been carried out by chemical analysis (Casazza *et al*., 2003). A programme for the phytobenthic communities has been developed and *Posidonia* meadows have been mapped by remote techniques (Calvo *et al*., 2003). Coppejans (1980) described a frame method used in the intertidal where the communities within 0.2 m × 0.2 m frames were chiselled out from the substrate.

There is a tradition of research in the phytobenthic zone in Greece (Haritonidis, 1995). In the context of the WFD, Orfanidis *et al.* (2001, 2003, 2007) suggested several approaches for collecting data for the WFD, which included destructive sampling of frames, coverage and species counting within given frames (10 m × 10 m and smaller).

The Libyan coast was monitoring by using transect and areal photography in combination (Pergent *et al.*, 2002).

Other areas

In Canada, monitoring of the phytobenthos has been addressed in several investigations of receiving areas and in the vicinity of, for instance, aquaculture and harvesting of algae. Recently the authorities gave a test monitoring programme within

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the estuaries of Canada (CAMP) a more permanent status (http://www.glf.dfo-mpo.gc.ca/e0006182; accessed 4 December 2012).

In the United States, monitoring of phytobenthos is mainly carried out by NOAA, which has also published several manuals of how to monitor in the phytobenthic communities. Murray *et al.* (2006) concentrate mainly on the intertidal zone using observations carried out by means of frames (http://www.coastalresearchcenter.ucsb.edu/scei/; accessed 18 November 2012). For further references and descriptions of methods see:

http://www.coralreefnetwork.com/quest/methods.htm (accessed 7 November 2012)

For a link to methods used in the Caribbean see:

http://www.unesco.org/csi/act/caricomp/summary14.htm (accessed 4 December 2012)
http://dnr.wa.gov/Publications/aqr_nrsh_szusermanual.pdf (accessed 7 November 2012)

In Australia, there has been an emphasis on standardising several methods used to monitor coral reefs in the Pacific region, which include different transect methods (chain, intercept, parallel, etc.) frames and photo/video recordings. Australia has a long tradition of monitoring the coastal area (English *et al*., 1994) and recent descriptions can be found in Crawford (2007) and Waycott *et al*. (2009) and in http://www.aims.gov.au/docs/research/monitoring/reef/reef-monitoring.html (accessed 5 December 2012).

In Japan, monitoring programmes were launched following oil spills, etc. Kawai *et al*. (2007) followed an oil spill by revisiting transects parallel to the shore and fixed squares.

References

Andrulewicz, E., Kruk-Dowgiallo, L. & Osowiecki, A. (2004) Phytobenthos and macrozoobenthos of the Slupsk Bank stony reefs, Baltic Sea. *Hydrobiologia*, **514**, 163–170.

Arévalo, R., Pinedo, S. & Ballesteros, E. (2007) Changes in the composition and structure of Mediterranean rocky-shore communities following a gradient of nutrient enrichment: descriptive study and test of proposed methods to assess water quality regarding macroalgae. *Marine Pollution Bulletin*, **55**, 104–113.

Bäck, S., Ekebom, J., Johansson, C., Kangas, P., Kautsky, H., Krause-Jensen, D., Mäkinen, A. & Nielsen, K. (1998) *Operative Methods for Mapping and Monitoring Phytobenthic Zone Biodiversity in the Baltic Sea*, TemaNord 1996, No. 5678. Nordic Council of Ministers, Copenhagen, pp 1–72.

Bäck, S., Ekebom, J. & Kangas, P. (2002) A proposal for long-term baseline phytobenthos monitoring programme for the Finnish coastal waters: monitoring submerged rocky shore vegetation. *Environmental Monitoring and Assessment*, **79**, 13–27.

https://telegram.me/Geologybooks

Bäck, S., Ekebom, J., Kangas, P., Kautsky, H., Mäkinen, A. & Rönnberg, O. (1996) *Mapping and Monitoring of Phytobenthic Biodiversity in the Northern Baltic Sea-Background, Methods and Recommendations*, TemaNord 1996, No. 559. Nordic Council of Ministers, Copenhagen, pp 1–92.

Bäck, S., Kauppila, J., Kangas, P., Ruuskanen, A., Westberg, V., Perus, J. & Räike, A. (2006) A biological monitoring programme for the coastal waters of Finland according to the EU Water Framework Directive. *Environmental Research, Engineering and Management*, **4**, 6–11.

Ballesteros, E., Torras, X., Pinedo, S., García, M., Mangialajo, L. & de Torres, M. (2007) A new methodology based on littoral community cartography dominated by macroalgae for the implementation of the European Water Framework Directive. *Marine Pollution Bulletin*, **55**, 172–180.

Bartsch, I. & Kuhlenkamp, R. (2004) WRRL-Klassifizierungssystem WK Helgoland: Historisches Leitbild der Makrophytenvegetation Helgolands Klassifizierungsvorschlag der Helgoländer Makrophytenvegetation Monitoring als Grundlage der Umsetzung der europäischen Wasserrahmenrichtlinie. Bericht im Auftrag des Landesamtes für Natur- und Umwelt des Landes Schleswig, Holstein, Flintbek. http://hdl.handle.net/10013/epic.22330 (accessed 6 November 2012).

Bartsch, I. & Tittley, I. (2004). The rocky intertidal biotopes of Helgoland: present and past. *Helgoland Marine Research*, **58**, 289–302.

Beuchel, F., Gulliksen, B., & Carroll, M.L. (2006) Long-term patterns of rocky bottom macrobenthic community structure in an Arctic fjord (Kongsfjorden, Svalbard) in relation to climate variability (1980–2003). *Journal of Marine Systems*, **63**, 35–48.

Borum, J., Duarte, C.M., Krause-Jensen, D. & Greve, T.M. (eds) (2004) European seagrasses: an introduction to monitoring and management. The M&MS Project, http://www.seagrasses.org/handbook/european_seagrasses_low.pdf, pp 1–95 (accessed 6 November 2012).

Bucas, M. (2009) Distribution patterns and ecological role of the red alga *Furcellaria lumbricalis* (Hudson) J.V. Lamouroux off the exposed Baltic Sea coast of Lithuania. Biomedical sciences, ecology and environmental sciences areas (03B), hydrobiology, marine biology, aquatic ecology, limnology (B260). PhD Thesis. Klaipeda University.

Calvo, S., Ciraolo, G. & La Loggia, G. (2003) Monitoring *Posidonia oceanica* meadows in a Mediterranean coastal lagoon (Stagnone, Italy) by means of neural network and ISODATA classification methods. *International Journal of Remote Sensing*, **24**, 2703–2716.

Casazza, G., Silvestri, C. & Spada, E. (2003) The use of bio-indicators for quality assessments of the marine environment: examples from the Mediterranean Sea. *Journal of Coastal Conservation*, **8**, 147–156.

Coggan, R., Populus, J., White, J., Sheehan, K., Fitzpatrick, F. & Peil, S. (eds) (2007) Review of standards and protocols for seabed habitat mapping, 192 pp. www.searchmesh.net/ (accessed 6 November 2012).

Coppejans, E. (1980) Phytosociological studies on Mediterranean algal vegetation: rocky surfaces of the photophilic infralittoral zone. In: *The Shore Environment: 2. Ecosystems*, Systematics Association Special Volume: 17(b) (eds J.H. Price, D.E. Irvine & W.F. Farham). Academic Press, London, pp 371–393.

https://telegram.me/Geologybooks

Crawford, C. (2007) *Manual for the Assessment of the Health of Georges Bay: Community Monitoring*. Tasmanian Aquaculture and Fisheries Institute, Hobart. http://eprints.utas.edu.au/6821/ (accessed 6 November 2012).

Daunys, D., Olenin, S., Paškauskas, R., Zemlys, P., Olenina, I. & Bučas, M. (2007) Typology and classification of ecological status of Lithuanian coastal and transitional waters: an update of existing system. Procurement of Services for the Institutional Building for the Nemunas River Basin Management, Technical Report for Transition Facility Project No. 2004/016-925-04-06, pp. 1–66.

Davies, J., Baxter, J., Bradley, M., Connor, D., Khan, J., Murray, E., Sanderson, W., Turnbull, C. & Vincent, M. (2001) *Marine Monitoring Handbook*, 405 pp. http://www.ukmarinesac.org.uk (accessed 4 December 2012).

De Jonge, V.N., Elliotte, M. & Brauer, V.S. (2006) Marine monitoring: its shortcomings and mismatch with the EU Water Framework Directive's objectives. In: Estuarine and Coastal Sciences Association Conference, Scarborough 08/2003. *Marine Pollution Bulletin*, **53**, 5–19.

Dethier, M.N., Graham, E.S., Cohen, S. & Tear, L.M. (1993) Visual versus random-point percent cover estimations: 'objective' is not always better. *Marine Ecology Progress Series*, **96**, 93–100.

English, S.A., Wilkinson, C. & Baker, V. (eds) (1994) *Survey Manual for Tropical Marine Resources, ASEAN-Australian Marine Science Project: Living Coastal Resources*, Australian Institute of Marine Science, Townsville, 368 pp.

Fornes, A., Basterretxea, G., Orfila, A., Jordi, A., Alvarez, A. & Tintore, J. (2006) Mapping *Posidonia oceanica* from IKONOS. *Photogrammetry and Remote Sensing*, **60**, 315–322.

Fürhaupter, K. & Meyer, T. (2008) Handlungsanweisung zum Monitoring in den äußeren Küstengewässern der Ostsee nach den Vorgaben der EU-Wasserrahmenrichtline. Qualitätskomponente Makrophyten BALCOSIS Verfahren Bearbeitungsstand, 30 Juni.

Gorostiaga, J.M. & Diez, I. (1996) Changes in the sublittoral benthic marine macroalgae in the polluted area of Abra de Bilbao and proximal coast (Northern Spain). *Marine Ecology Progress Series*, **130**, 157–167.

Haritonidis, S. (1995) Marine research in Greece and the additional Greek marine research centres: progress and present situation. *Helgoländer Meeresunters*, **49**, 417–422.

Hennig, B.D., Cogan, B.C. & Bartsch, I. (2007) Hyperspectral remote sensing and analysis of intertidal zones: a contribution to monitor coastal biodiversity. *Geospatial Crossroads GI Forum* (eds A. Car, G. Griesebner & J. Strobl). Proceedings of the First Geoinformatics Forum Salzburg 2007. Wichman Verlag, Heidelberg, pp 62–73.

HELCOM (1999) Annex for the HELCOM COMBINE Programme, 26 March 1999. http://www.helcom.fi/stc/files/CombineManual/PartC/AnnexC9.pdf (accessed 6 November 2012).

Hiscock, K. (1998) Biological monitoring of Marine Special Areas of Conservation: a review of methods for detecting change. Joint Nature Conservation Committee (JNCC) Report No. 284), pp. 1–96.

ICES Benthos Ecology Working Group (BEWG) (2008) Report CM 2008/MHC: 10.

ICES (2009) Report of the Joint ICES/OSPAR Study Group on Integrated Monitoring of Contaminants and Biological Effects (SGIMC). 12–15 January 2009. Copenhagen, Denmark. ICES CM 2008/ACOM: 30, 64 pp.

https://telegram.me/Geologybooks

Isaeus, M. (2004) Factors structuring *Fucus* communities at open and complex coastlines in the Baltic Sea. Dissertation, Stockholm University, 40 pp.

ISO (2003) Water quality – guidance on marine biological surveys of littoral and sublittoral hard bottom. ISO Proposal ISO/TC 147/ SC 5, N395, pp 1–22.

Jansson, A.-M. & Kautsky, N. (1977) Quantitative survey of hard bottom communities in a Baltic archipelago. In: *Biology of Benthic Organisms* (eds B.F. Keegan, P.O. Ceidigh & P.J.S. Boaden), Pergamon Press, New York, pp 359–366.

Jansson, B.O., Aneer, G. & Nellbring, S. (1985) Spatial and temporal distribution of demersal fish fauna in a Baltic archipelago as estimated by SCUBA-census. *Marine Ecology Progress Series*, **23**, 31–43.

Joint Nature Conservation Committee (2001) UK special protection areas and wetlands of international importance (Ramsar sites). Status Tracking Report as at 31 March 2001. Joint Nature Conservation Committee, Peterborough.

Karlsson, J., Nilsson, P. & Wallentinus, I. (1992) *Monitoring of the Phytal System on the Swedish West Coast – A Pilot Study.* Mimeograph, pp. 1–15. University of Göteborg, Gothenburg. www.havochvatten.se (accessed 4 December 2012).

Kautsky, H. (1989) Quantitative distribution of plant and animal communities of the phytobenthic zone in the Baltic Sea. Askö Contribution No. 35, Stockholm University, Stockholm, 80 pp.

Kautsky, H. (1992) Quantitative change of phytobenthic plant and animal communities due to bleached pulp mill effluents in the Baltic Sea. In: *Environmental Fate and Effects of Bleached Pulp Mill Effluents*. Proceedings of Swedish EPA Conference, Saltsjöbaden 19–21 November 1991, Vol. Report 4031 (ed. A. Södergren), SEPA, Solna, pp 270–282.

Kautsky, H. (1993) Methods for monitoring of phytobenthic plant and animal communities in the Baltic Sea. *Proceedings, Ecological Conference*, 10–13 December 1992, Sopot, Poland, pp 21–59.

Kautsky, H. (1995) Quantitative distribution of sublittoral plant and animal communities in the Baltic Sea gradient. In: *Biology and Ecology of Shallow Coastal Waters* (eds A. Eleftheriou, A.A. Ansell & C.J. Smith), 28th EMBS, 23–28 September 1993. Olsen & Olsen, Crete, Fredensborg, pp 23–31.

Kautsky, H. (2013) Sampling marine phytobenthic communities. *ICES Techniques in Marine Environmental Sciences*, in press.

Kautsky, H., Kautsky, U. & Nellbring, S. (1988) Distribution of flora and fauna in an area receiving pulp mill effluents in the Baltic Sea. *Ophelia*, **28**, 139–155.

Kautsky, H., Martin, G., Mäkinen, A., Borgiel, M., VahteriI, P. & Rissanen, J. (1999) Structure of phytobenthic and associated animal communities in the Gulf of Riga. *Hydrobiologia*, **393**, 191–200.

Kautsky, H., Tulldahl, M., Steinvall, O., Strömbeck, N. & Wikström, S. (2010) New technologies for benthic habitat mapping FOI, Technical Report FOI-R-3013-SE, Swedish Defence Research Agency, Linköping, pp 1–56. http://emma.slu.se/tasks/sites/emma/assets/File/NewTechnologiesFOI-R–3013.pdf (accessed 6 November 2012).

Kautsky, N., Kautsky, H., Kautsky, U. & Waern, M. (1986) Decreased depth penetration of *Fucus vesiculosus* L. since the 1940's indicates eutrophication of the Baltic Sea. *Marine Ecology Progress Series*, **28**, 1–8.

Kawai, H., Kamiya, M., Komatsu, T., Nakaoka, M. & Yamamoto, T. (2007) Ten years' monitoring of intertidal macroalgal vegetation of hyogo prefecture, Northwestern Coast of Honshu, Japan to assess the impact of the Nakhodka oil spill. *Algae*, **22**, 37–44.

https://telegram.me/Geologybooks

Kingsford, M. & Battershill, C. (eds) (1998) *Studying Temperate Marine Environments. A Handbook for Ecologists*, Canterbury University Press, Canterbury, 335 pp.

Krause-Jensen, D., Carstensen, J. & Dah, K. (2007a) Total and opportunistic algal cover in relation to environmental variables. *Marine Pollution Bulletin*, **55**, 114–125.

Krause-Jensen, D., Laursen, J.S., Dahl, K., Hansen, J. & Larsen, S.E. (2000) Test af metoder til marine vegetationsundersøgelser (test of methods for investigations of marine vegetation). Faglig rapport fra DMU 323. Afdeling for Sø- og Fjordøkologi, Afdeling for Havmiljø og Afdeling for Vandløbsøkologi. http://www2.dmu.dk/1_viden/2_Publikationer/3_fagrapporter/rapporter/fr323.pdf (accessed 6 November 2012).

Krause-Jensen, D., Middelboe, A.L., Carstensen, J. & Dahl, K. (2007b) Spatial patterns of macroalgal abundance in relation to eutrophication. *Marine biology*, **152**, 25–36.

Krause-Jensen, D., Sagert, S., Schubert, H. & Boström, C. (2008) Empirical relationships linking distribution and abundance of marine vegetation to eutrophication. *Ecological Indicators*, **8**, 515–529.

Krause-Jensen, K., Sund Laursen, J., Middelboe, A.L., Stjernholm, M. & Manscher, O. (2001) Bundvegetation. NOVA 2003. Teknisk anvisning for marin overvågning. Overvågning 2001:12 Miljø- og Energiministeriet & Danmarks Miljøundersøgelser Köpenhamns Amt 2003 Overvågning.

Kratzer, S. & Tett, P. (2009) Using bio-optics to investigate the extent of coastal waters: a Swedish case study. *Hydrobiologia*, **629**, 169–186.

Kutser, T., Vahtmäe, E. & Martin, G. (2006a) Assessing suitability of multispectral satellites for mapping benthic macroalgal cover in turbid coastal waters by means of model simulations. *Estuarine, Coastal and Shelf Science*, **67**, 521–529.

Kutser, T., Vahtmäe, E. & Metsamaa, L. (2006b) Spectral library of macro algae and benthic substrates in Estonian coastal waters. *Proceedings of Estonian Academy of Science*, **55**, 329–340.

Laursen, J.S., Krause-Jensen, D. & Larsen, S.E. (2000) Interkalibrering af metode til undersøgelser af havbundvegetation i marine områder. Afdeling for Sø-og Fjordøkologi og afdelning for Vandløbsekologi 34 Faglig rapport fra DMU 329. http://www2.dmu.dk/1_viden/2_Publikationer/3_fagrapporter/rapporter/fr329.pdf (accessed 4 December 2012).

Lewis, J.R. (1964) *Ecology of Rocky Shores*, English University Press, London, 323 pp.

Lundälv, T. (1971) Quantitative studies on rocky-bottom biocenoses by underwater photogrammetry. A methodological study. *Thalassia Juguslavica*, **7**, 201–208.

Lundälv, T. (1985) Detection of long-term trends in rocky sublittoral communities: representativeness of fixed sites. In: *The Ecology of Rocky Coasts* (eds P.A. Moore & R. Seed), Hodder and Stoughton, London, Sydney, Auckland, Toronto, pp 329–345.

Lundälv, T. & Christie, H. (1986) Comparative trends and ecological pattern of rocky subtidal communities in the Swedish and Norwegian Skagerrak area. *Hydrobiologia*, **142**, 71–80.

Lüning, K. (1985) *Meeresbotanik*, Georg Thieme Verlag, Stuttgart, New York, 375 pp.

Martin, G. (2005) G E F Baltic Sea Regional Project Phase I: 2003–2005. *BSRP Training Workshop on Phytobenthos Monitoring Methods*, 23–27 May. Kõiguste Field Station, Laimjala. http://www.ices.dk/projects/balticsea/CD/PHYTOBENTHOS%20MONITORING/Martin_BSRP%20training%20workshop%20on%20Phytobenthos%20methods%2023-27%20.pdf (accessed 6 November 2012).

https://telegram.me/Geologybooks

Martin, G., Kotta, J., Torn, K. & Orav-Kotta, H. (2003) Estonian marine phytobenthos monitoring programme: preliminary results and future perspectives. Proceedings of Estonian Academy of Science. *Biology Ecology*, **52**, 112–124.

Meinesz, A. (2007) Methods for identifying and tracking seaweed invasions. *Botanica Marina*, **50**, 373–384.

Middleboe, A.L. & Krause-Jensen, D. (1998) *Vurdering af Metoder til Undersögelse af Marin Vegetation.* Mimeograph, DMU, Afd for fjord og sö-ökologi, pp 1–7.

Middleboe, A.L., Krause-Jensen, D., Nielsen, K. & Sand-Jensen, K. (1997) Interkalibrering af bundvegetationsundersögelser. Faglig rapport fra DMU, nr. 220, Miljö og Energiminiseriet, Danmarks Miljöundersökelser, pp 1–36.

Moy, F.E., Aure, J., Dahl, E., Green, N., Magnusson, J., Pedersen, A., Rygg, B. & Walday, M. (1999) 2 Nordic Marine Sciences Meeting I: Norwegian coastal monitoring programme of Southern Norway – the first ten years (Abstract). Norske Havforskeres Forening.

Murray, S.N., Ambrose, R.F. & Dethier, M.N. (2006) *Monitoring Rocky Shores*, University of California, Berkeley, CA, 220 pp.

Orfanidis, S., Panayotidis, P. & Stamatis, N. (2001) Ecological evaluation of transitional and coastal waters: a marine benthic macrophytes-based model. *Mediterranean Marine Science*, **2/2**, 56–54.

Orfanidis, S., Panayotidis, P. & Stamatis, N. (2003) An insight into the ecological evaluation index (EEI). *Ecological Indicators*, **3**, 27–33.

Orfanidis, S., Papathanasiou, V., Sabetta, L., Pinna, M., Gigi, V., Gounaris, S., Tsiagga, E., Nakou, K. & Theodosiou, T. (2007) Benthic macrophyte communities as bioindicators of transitional and coastal waters: relevant approaches and tools. *Transitional Waters Bulletin (TWB)*, **3**, 45–49.

Pehlke, C. & Bartsch, I. (2008) Changes in depth distribution and biomass of sublittoral seaweeds at Helgoland (North Sea) between 1970 and 2005. *Climate Research*, **37**, 135–147.

Pergent, G., Djellouli, A., Hamza, A., Ettayeb, K., El Mansouri, A., Talha, F., Hamza, M., Pergent-Martini, C. & Platini, F. (2002) Characterization of the benthic vegetation in the Farwà Lagoon (Libya). *Journal of Coastal Conservation*, **8**, 119–126.

Poore, M.E.D. (1955) The use of phyto-sociological methods in ecological investigations. *Journal of Ecology*, **43**, 226–269, 606–651.

Rees, H.L. (ed.) (2009) Guidelines for the study of the epibenthos of subtidal environments. *ICES Techniques in Marine Environmental Sciences*, No. 42, 90 pp.

Romero, J., Martínez-Crego, B., Alcoverro, T. & Pérez, M. (2007) A multivariate index based on the seagrass *Posidonia oceanica* (POMI) to assess ecological status of coastal waters under the water framework directive (WFD). *Marine Pollution Bulletin*, **55**, 196–204.

Sandnes, O. & Gulliksen, B. (1980) Monitoring and manipulation of a sublittoral hard bottom biocoenosis in Balsfjord, northern Norway. *Helgoland Marine Research*, **33**, 467–472.

Schiewer, U. (ed.) (2008) *Ecology of Baltic Coastal Waters*, Springer-Verlag, Berlin, 428 pp.

Schramm, W. & Nienhuis, P.H. (1996) *Marine Benthic Vegetation. Recent Changes and the Effect of Eutrophication*, Springer-Verlag, Berlin, 364 pp.

https://telegram.me/Geologybooks

Schubert, H. (2004) Entwicklung von leitbildorientierten Bewertungsgrundlagen für innere Küstengewässer der deutschen Ostseeküste nach der EU-WRRL. Forschungsbericht zum BMBF Projekt ELBO, Universität Rostock, Institut für Aquatische Ökologie, pp 1–167. http://www.biologie.uni-rostock.de/oekologie/archives/Endbericht_ELBO.pdf (accessed 6 November 2012).

Schwenke, H. (1974) Die Benthosvegetation. In: *Meereskunde der Ostsee* (eds L. Magaard & G. Reinheimer), Springer-Verlag, Berlin, pp 131–146.

Selig, U., Eggert, A., Schories, D., Schubert, M., Blümel, C. & Schubert, H. (2007) Ecological classification of macroalgae and angiosperm communities of inner coastal waters in the southern Baltic Sea. *Ecological Indicators*, **7**, 665–678.

Selig, U., Marquardt, R. & Porsche, C. (2008) Vorläufige Handlungsanweisung zur Erfassung der Angiospermen – und Makroalgenbeständen in den inneren Küstengewässern der Deutschen Ostseeküste. Bewertung entsprechend den Vorgaben der EU-Wasserrahmenrichtlinie Qualitätskomponente Makrophyten. Mimeograph, Universität Rostock, FB Biowissenschaften, AG Ökologie Bearbeitungsstand: 2. Fassung, pp. 1–23.

Stephenson, T.A. & Stephenson, A. (1972) *Life Between Tidemarks on Rocky Shores*, W.H. Freeman and Co., San Francisco, CA, 425 pp.

Underwood, A.J. (1997) *Experiments in Ecology. Their Logical Design and Interpretation Using Analysis of Variance*, Cambridge University Press, Cambridge, 504 pp.

UNEP - MAP - RAC/SPA (2006) *Proceedings of the Second Mediterranean Symposium on Marine Vegetation*, Athens, 12–13 December 2003, RAC-SPA edit, Tunis, 255 pp.

Waycott, M., Duarte, C.M., Carruthers, T.J.B., Orth, R.J., Dennison, W.C., Olyarnik, S., Calladine, A., Fourqurean, J.W., Heck, K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Short, F.T. & Williams, S.L. (2009) Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proceedings of the National Academy of Sciences*, **106**, 12377–12381.

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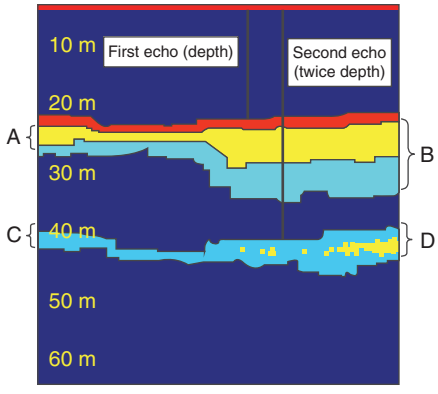

Plate 2.1 A typical screen display of an echo-sounder (signal strength decreasing from red to blue) as though a vessel had passed over soft, smooth ground onto hard, rough ground. 'A' is the small tail of the first echo (smooth surface); 'B' is a more extended tail (rough surface); 'C' is a weak second echo (soft material) and 'D' is a stronger second echo (hard material). Overall this output describes a transition from a smooth soft seabed to one that is relatively rough and hard.

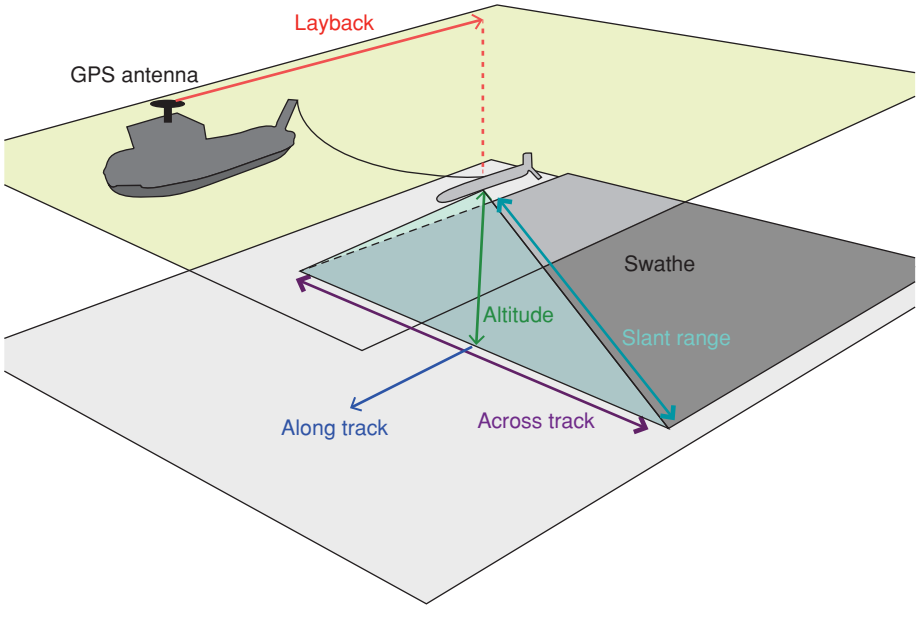

Plate 2.2 Standard deployment and geometry for a typical sidescan sonar system.

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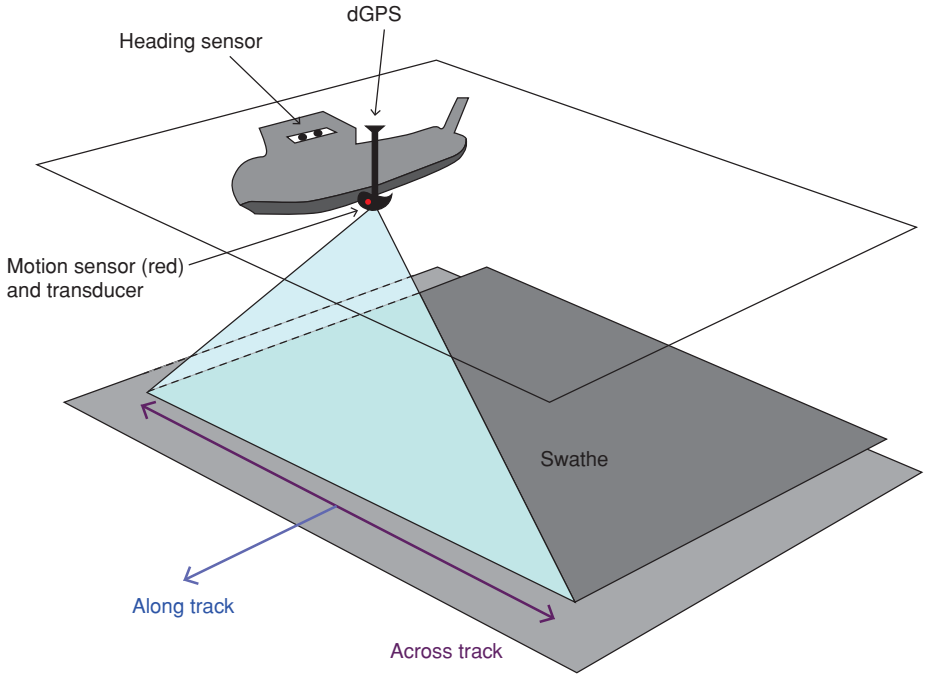
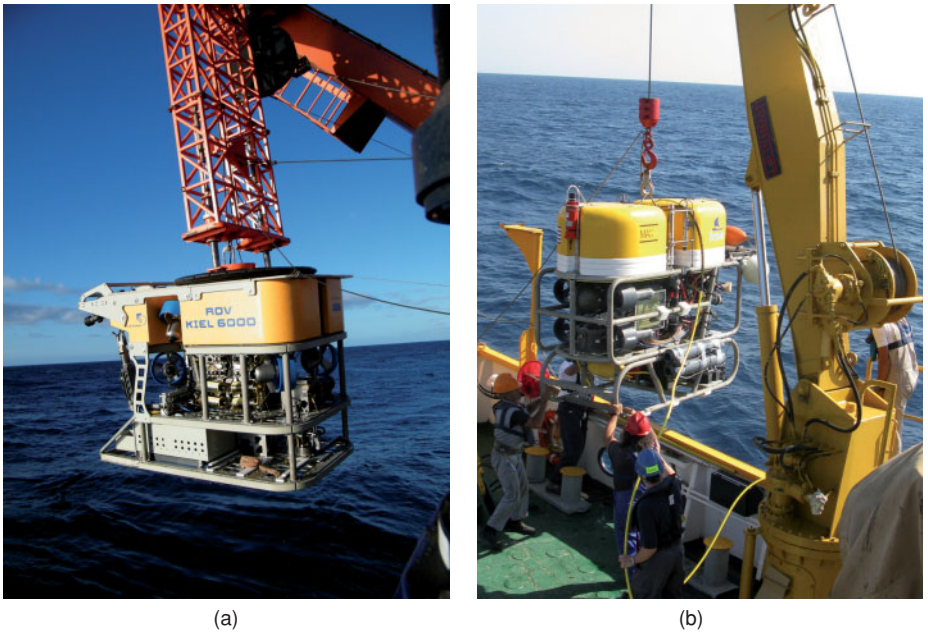


Plate 2.3 A typical set-up for a pole-mounted swathe bathymetric system. Offsets are measured for heading/dGPS sensors and transducers. Motion sensors are precisely positioned within the transducer V-plate.



(a) (b)

Plate 3.1 Science Remotely Operated Vehicles (ROVs) (a) Kiel 6000 (credit: GEOMAR) (b) HCMR 2000 m Max Rover.

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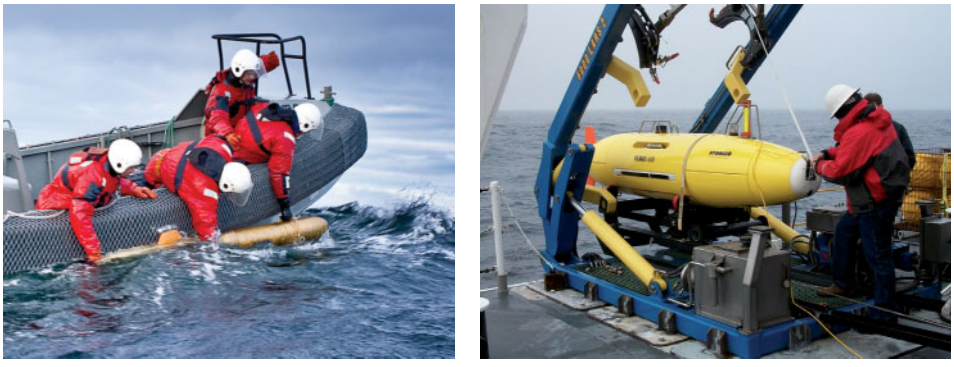

(a) (b)

Plate 3.2 Autonomous Underwater Vehicles (AUV) (a) Gavia coastal AUV system with primarily imaging sensors (courtesy of Teledyne Gavia) (b) Remus 6000 large oceanographic AUV system with near-bottom imaging capabilities (credit: Hydroid).

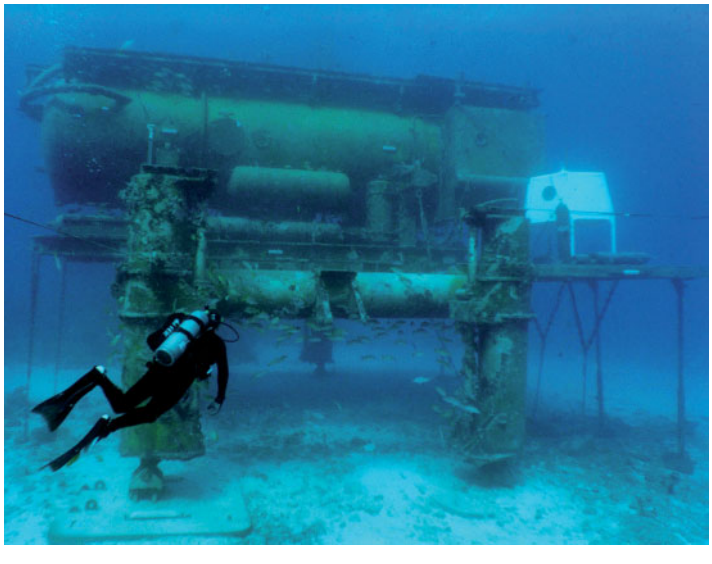

Plate 4.1 The undersea laboratory *Aquarius*, a diving habitat off the coast of Florida. © University of North Carolina at Wilmington. Reproduced with permission. Aquarius is owned by NOAA and is operated by the University of North Carolina at Wilmington.

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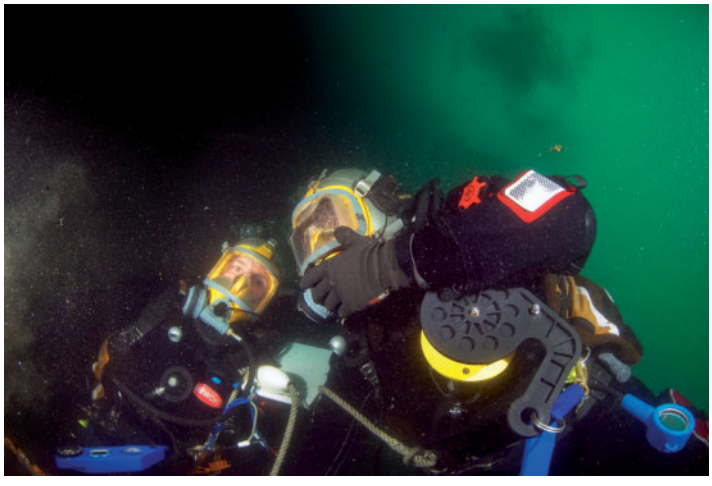

Plate 4.2 Plymouth University Diving & Marine Centre professional diving students using positive pressure full-face masks. Diver on right can be seen operating press-to-talk through-water *Buddy Phone* voice communications. © Colin Munro.

Plate 4.3 A photo-monitoring framer being used to photograph *Alcyonium glomeratum* soft coral colonies. Orange floats are to compensate for weight of the framer. © Skomer Marine Nature Reserve, Countryside Council for Wales.

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Plate 4.4 Apparent growth of an erect sponge due to peripheral distortion and steepened perspective. © Colin Munro.

Plate 4.5 Distortion inherent in a wide-angle lens causing changes across a photo-monitoring image. © Colin Munro.

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Plate 4.6 Video system being used to record behaviour in the sea hare *Aplysia punctata*. © Colin Munro.

Plate 4.7 Six inch diameter plastic fishing floats attached to a concrete paving slab by floating polypropylene line, used to mark a monitoring station. © Colin Munro.

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Plate 4.8 A diver-operated air drill being used to mark a sub-littoral monitoring station in Milford Haven. © Colin Munro.

Plate 4.9 A diver collecting a core sample in soft sediment. Coring can create large plumes of suspended sediment, temporarily reducing visibility. © Colin Munro.

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Plate 4.10 Divers using a suction sampler. © Dale Rostron, Subsea Surveys.

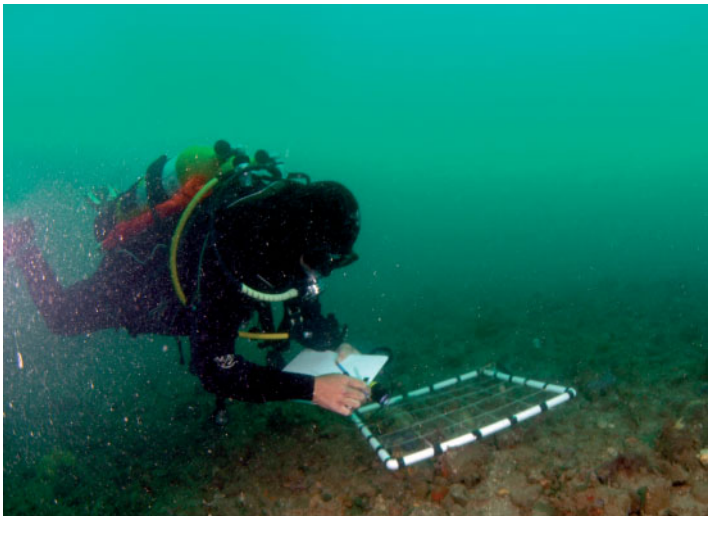

Plate 4.11 A diver counting individuals within a strung quadrat. © Colin Munro.

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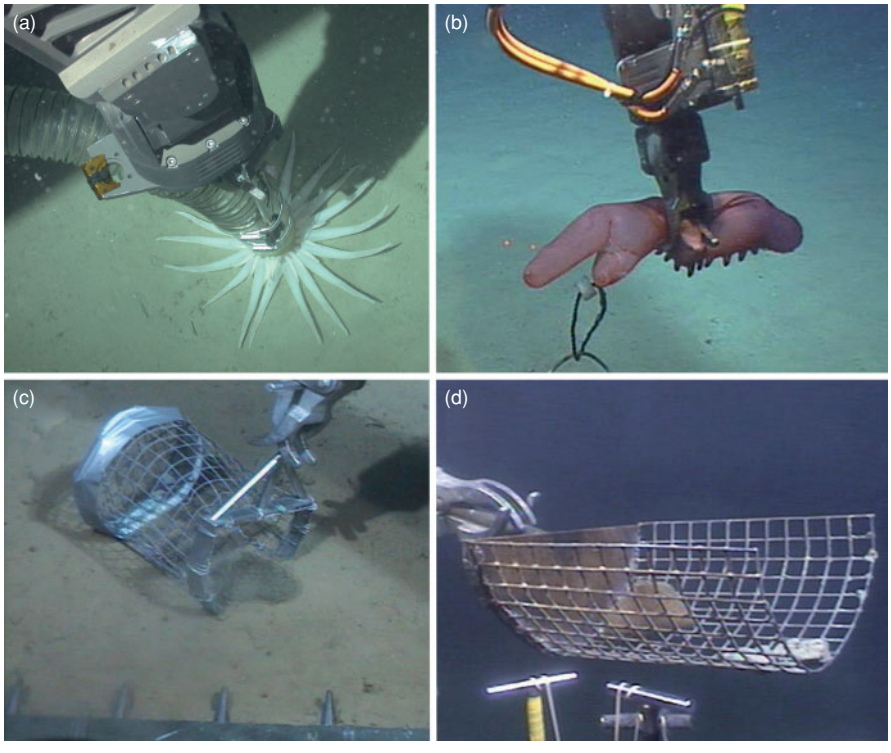

Plate 7.1 Collecting organisms from the deep-sea benthic using a Remotely Operated Vehicle (ROV). (a) Suction sampling an anemone. (b) Direct collection of holothurians using the manipulator arm. (c, d) Collecting echinoids using the baskets and scoops, respectively. (Images © the National Oceanography Centre, Southampton (a, b), and © Ken Smith, MBARI, the Deep Submergence Group, WHOI, USA (c, d).)

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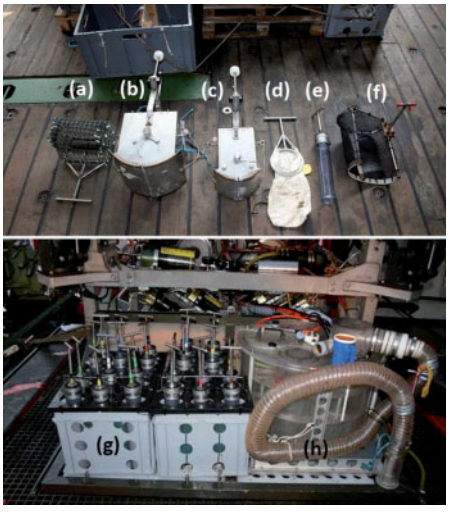

Plate 7.2 Examples of Remotely Operated Vehicle (ROV)-operated tools used in deep-sea research for collecting organisms and sediment. (a) Epifauna basket, (b) and (c) sediment grabs, (d) sample net, (e) sediment push core, (f) epifauna scoop, (g) 24 push cores mounted on the tool tray and (h) suction sampler. (Images © the National Oceanography Centre, Southampton.)

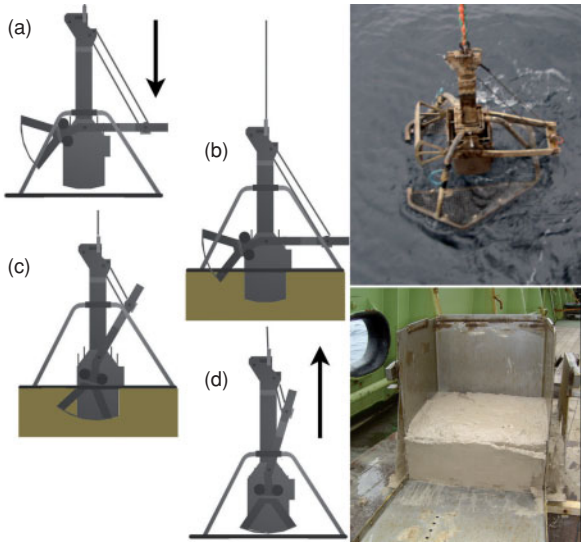

Plate 7.3 The USNEL Mk II box core operations. (a) The spade is in the cocked horizontal position during descent. (b) The weighted central column drives the box into the sediment while the outer frame rests on the seafloor. (c) The locking pin deactivates and hauling begins to rotate the spade under the box. (d) The corer is hauled to the surface with the block of sediment sealed inside the box. To the right is an image of a box corer being deployed and an example of a sediment sample taken from 4000 m deep. (Images © the University of Aberdeen, UK.)

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Plate 7.4 The Bowers and Connelly Maxicore being deployed and a close-up of two successful cores from the deep sea. (Images © the University of Aberdeen, UK.)

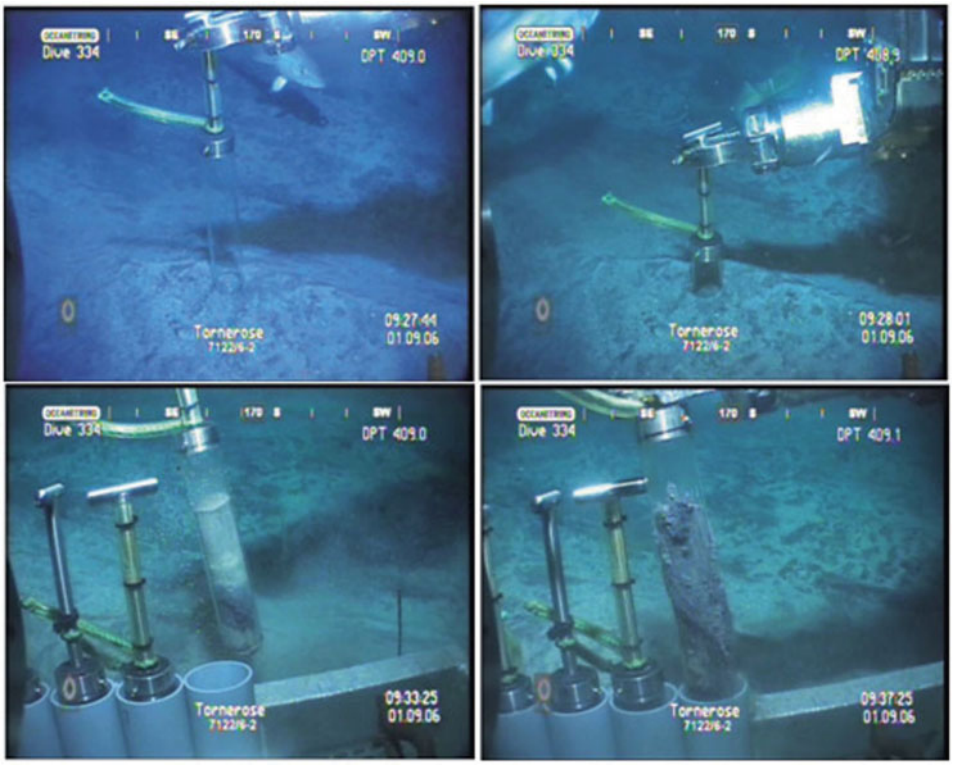

Plate 7.5 Remotely Operated Vehicle (ROV) push cores in use in the Barents Sea. (Images © SERPENT.)

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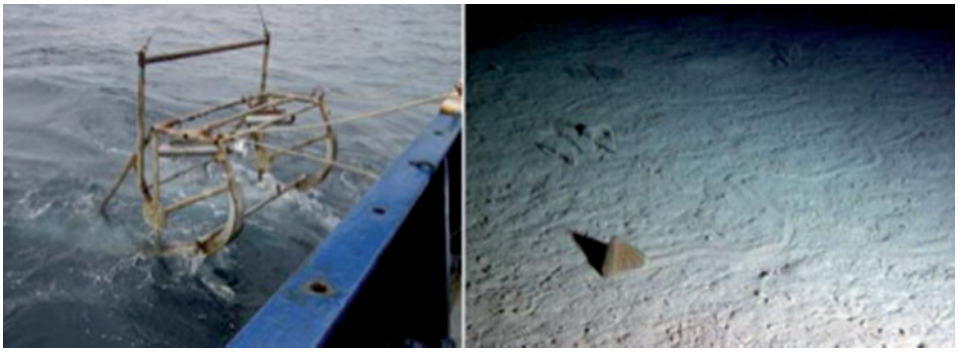

Plate 7.6 An example of a towed camera sledge; the Scripps camera sledge (left) and an image from the deep-sea floor (right). (Images © Ken L. Smith Jr., MBARI, USA.)

Plate 7.7 The Deep Tow Imaging System (DTIS) (left); an example of an image taken of fragile coral mounds taken by DTIS (right). (Images © the National Institute of Water and Atmospheric Research, New Zealand.)

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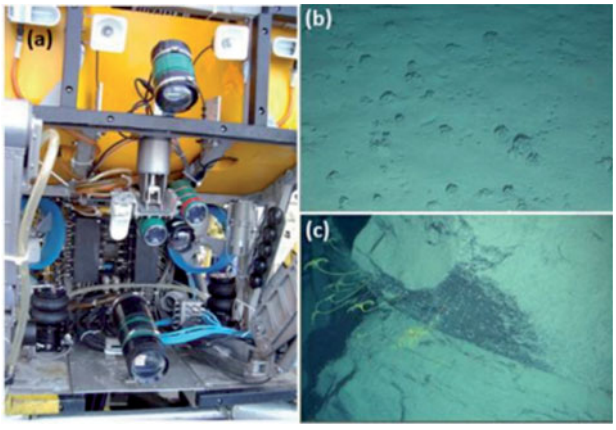

Plate 7.8 (a) An example of a work-class oceaneering Remotely Operated Vehicle (ROV) set-up for imaging. From top: with lights, strobe (for still camera), pan-and-tilt unit (with low-light video, standard-definition video and high-definition video) and still camera. (b) Examples of horizontal transect showing xenophyophore populations. (c) Examples of vertical transect up a canyon wall showing stalked crinoids. (Images © SERPENT (a) and National Oceanography Centre (b,c).)

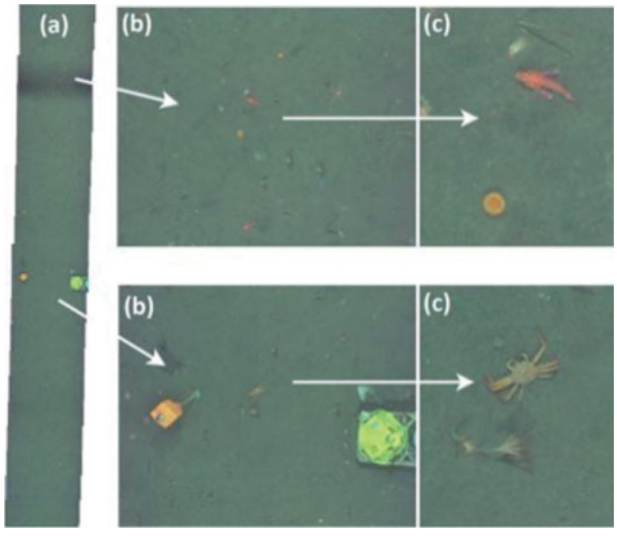

Plate 7.9 Examples of photo mosaicking from Autonomous Underwater Vehicles (AUVs). Large areas of seafloor can be visualised in a mosaic (a), which are constructed from single still images (b), which can be magnified to identify specific organisms or features (c). (Images © MBARI, USA.)

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Plate 7.10 Examples of images from long-term time-lapse imaging landers: NOCS' Bathysnap (left) and the Scripps camera tripod (right). (Images © DEEPSEAS Group, National Oceanography Centre, Southampton, UK and Ken L. Smith Jr., MBARI, USA.)

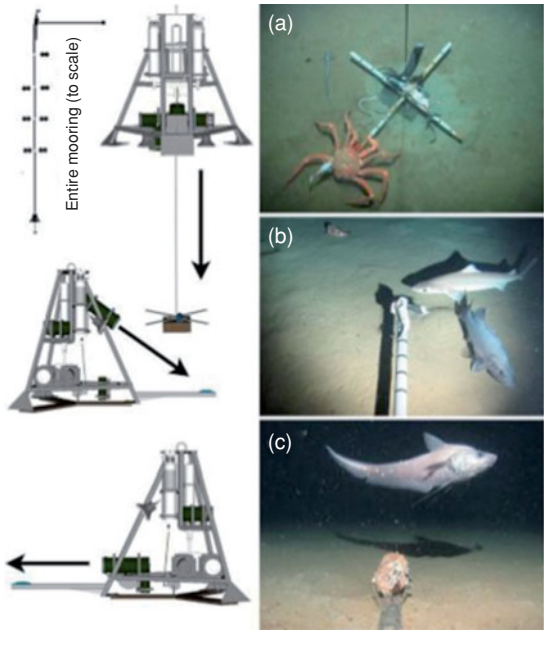

Plate 7.11 Examples of different baited camera configurations of the ROBIO lander. (a) Vertical imaging from 2 m above the seafloor (field of view = 2.1 m × 1.6 m). (b) Oblique 30° imaging at 1 m above bottom (field of view = 1.4 m across centre). (c) Horizontal imaging at 0.3 m above bottom (field of view = 0.55 m along bottom). (Images © the University of Aberdeen, UK.)

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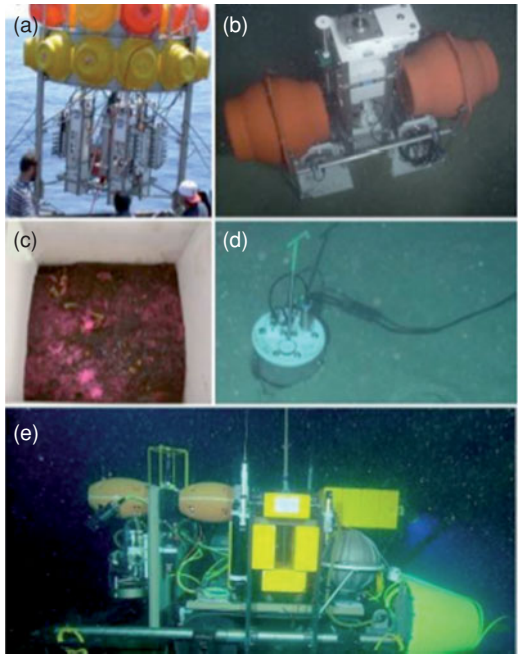

Plate 7.12 Examples of deep-sea chamber incubating systems. (a) Modular chamber lander carrying three chambers. (b) A single autonomous chamber delivered by Remotely Operated Vehicle (ROV). (c) Sediment recovered from the chamber with ^{13}C-labelled algae and luminophore deposits visible of the sediment surface. (d) Simple ROV operated chamber system. (e) Two chambers mounted on the front of a deep-sea crawler vehicle. (Images © the University of Aberdeen, UK and Ken L. Smith Jr, MBARI, USA.)

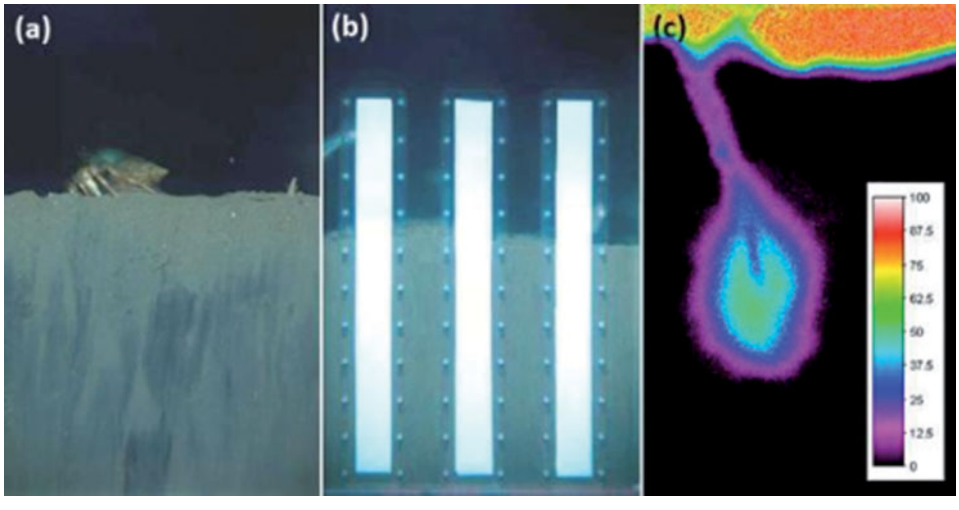

Plate 7.13 Examples of imaging the sediment profile. (a) A typical Sediment Profile Imaging (SPI) image showing a crab on the sediment surface, (b) a SPI image with three integrated gel strips and (c) a 2D oxygen image of a brittlestar burrow from a Planar Optode. (Images © the University of Aberdeen, UK (a,b) and the Scottish Association for Marine Science, UK (c).)

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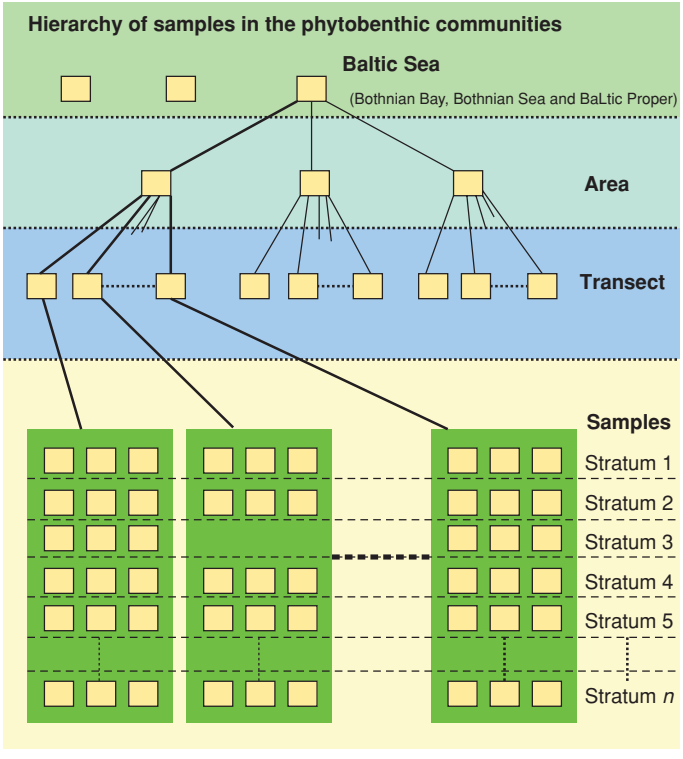

Plate 9.1 Hierarchical tree of observations (quantitative samples). Starting from the base with strata defined by species depth distribution, repeated by transects within the same area to repeated areas in a region to whole water bodies.

Plate 9.2 Placement of frames along a transect line perpendicular to the depth curves. Observations are made within each frame only. Frames are either placed at fixed depth intervals or distance from shore.

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