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**GUIDELINES FOR THE STUDY OF  
THE EPIBENTHOS OF SUBTIDAL ENVIRONMENTS**

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Our cover photo was taken by N. Penny Holliday aboard the RRS “Discovery” in rough seas in the Rockall Trough.

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This series presents detailed descriptions of methods and procedures relating to chemical and biological measurements in the marine environment. Most techniques described have been selected for documentation based on performance in ICES or other intercalibration or intercomparison exercises: they have been carefully evaluated and shown to yield good results when correctly applied. They have also been subject to review by relevant ICES working groups, but this is not to be construed as constituting official recommendation by the Council.

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## 1 Introduction

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These Guidelines for the Study of the Epibenthos of Subtidal Environments document a range of sampling gears and procedures for epibenthos studies that meet a variety of needs. The importance of adopting consistent sampling and analytical practices is highlighted. Emphasis is placed on ship-based techniques for surveys of coastal and offshore shelf environments, but diver-assisted surveys are also considered.

The account extends earlier work by the ICES Benthos Ecology Working Group on methods for studying the benthic communities of hard substrata (Connor, 1995; see Annex 1). It also complements the *ICES Techniques in Marine Environmental Sciences* (TIMES) guidelines for the study of the soft-bottom macrofauna (Rumohr, in prep.), the phytobenthos (Kautsky, in prep.), and other publications dealing with benthic sampling methods, notably Eleftheriou and McIntyre (2005).

Coverage of sampling gears is not exhaustive, and others may be added in future editions. The target audience includes marine scientists new to epibenthic studies as well as established practitioners who require further detail on sampling practices in order to meet various objectives of contemporary interest.

### 1.1 Definition, role, and importance of the epibenthos

The epibenthos comprises the animals and plants that inhabit the seabed surface for most or all of the adult stage of their life cycles. They range from sessile forms, such as seaweeds, sponges, and colonial hydroids, to errant forms, such as crabs, shrimps, and fish. The size range of epibenthic organisms is considerably greater than their endobenthic (within-sediment) counterparts, and setting ecologically plausible boundaries, e.g. according to sieve mesh size, can be difficult. Thus, many species inhabiting rocky or coarse ground habitats are colonial, crustose, or cushion-forming, making it impractical to use colony size as a consistent means to distinguish between species groups or to enumerate individuals. However, qualitative information on the larger sedentary or sessile component of the epibenthos is likely to be the most reliable. It is also essential to take account of operationally determined factors, especially gear design, efficiency, and selectivity, when interpreting ecological data at different locations or between studies. These factors are further addressed in the following sections.

Aspects of the role of the epibenthos in marine ecosystems, which makes it an important target for scientific study, include its significant contribution to benthic production, e.g. subtidal mussel beds and foliose algae in the photic zone. Habitat-forming algae and sessile colonial animals, e.g. kelp forests, coral reefs, and sponge reefs, may provide attachment points and shelter for eggs and juveniles of fish and shellfish of commercial interest. Many epibenthic species are prey for fish, birds, seals, and cetaceans. The epibenthos can also play a significant role in the bioaccumulation and subsequent transfer of contaminants through the food chain (e.g. Kennish, 1997; Gunnarsson *et al.*, 2000).

As a group, the epibenthos accounts for a significant proportion of marine biodiversity (Thorson, 1957). The vulnerability to human activities of the more conspicuous elements of coastal and offshore assemblages has attracted much attention recently in relation to the conservation of species and habitats (e.g. the deep-water coral *Lophelia*: Fosså *et al.*, 2002; Jones *et al.*, 2006; see also European Communities, 1992; [www.ospar.org](http://www.ospar.org)). There is also much interest in the functional role of the epibenthos

in marine ecosystems, e.g. in foodweb studies (Jennings *et al.*, 2002) and in evaluations of “essential fish habitat” (Cappo *et al.*, 1998; Benaka, 1999; Rosenberg *et al.*, 2000). Other studies have addressed the longer term effects of climatic and fishing-induced changes (e.g. Aronson and Blake, 2001; Hobday *et al.*, 2006; Callaway *et al.*, 2007), while several epibenthic species are commercially fished.

## 1.2 Objectives of epibenthos studies

The following objectives highlight the main areas of contemporary interest.

- assessment of the contribution of the epibenthos to marine biodiversity and ecosystem functioning;
- conservation of species or assemblages;
- association of biological and seabed features in the context of large-scale mapping of benthic “biotopes”;
- environmental quality assessment (e.g. through the derivation of Ecological Quality Objectives);
- responses to climatic changes;
- fundamental research, including experimental, behavioural, and biomedical studies of individual species.

## 1.3 Design and conduct of epibenthos surveys

The design of epibenthos surveys will be determined by the study objectives and the nature of the habitat(s) to be sampled. It is not feasible to cover all eventualities in these guidelines, especially if there is a research focus to studies. However, the design of monitoring programmes raises a number of common issues, including the importance of accurate georeferencing, which are outlined in Annex 2.

A useful operational distinction may be made between low- and high-relief terrain, which generally serves to differentiate between sedimentary, i.e. “level-bottom”, and rocky habitats. The former opens up wider possibilities for remote sampling, including the use of towed gear, which may be more efficiently and safely deployed than over rocky areas. The results from many of these devices depend as much on their design and mode of deployment as on the natural disposition of the epibenthic assemblages. This is particularly true for trawls and dredges. Caution should therefore be exercised in interpreting the data, and evaluations of gear efficiency should be encouraged (e.g. Rees *et al.*, 1999; Reiss *et al.*, 2006). This does not mean that operational constraints invalidate trend assessments, but it does highlight the need to adopt consistent sampling practices within and between studies. It is essential that these practices are fully documented in survey reports so that the scope for comparisons between studies can be determined (see e.g. Rumohr, 2008).

Sections 2–4 describe a range of devices for the collection or *in situ* observation of the epibenthos and for characterization of the associated physical habitat. These include both established practices and prototype equipment with potential for further development and/or wider application. A distinction is made between destructive and non-destructive (i.e. observational) sampling practices.

Under the category of destructive sampling, the two most widely used types of equipment are trawls and dredges. Descriptions are given of the design and operation of a selection of these. Although conventional grab samplers are not generally suitable for epibenthic surveys, they, along with suction samplers, may be suitable for more narrowly defined objectives, e.g. quantification of the smaller sessile com-

ponent, especially of coarser substrata supporting species-rich assemblages. Accounts are therefore provided of the Hamon grab (Oele, 1978) and other large prototype and operational devices that may be effective in collecting epibenthic organisms (see Section 2.1.4). Finally, an appraisal of the merits of all gear types against various operational criteria is given in the Conclusions (Section 8).

A number of the selected sampling devices are versatile across depths, whereas others are specifically designed for deep-sea surveys (see Gage and Bett, 2005). These guidelines generally cover deployment/sampling practices for shallower shelf sediments.

## 2 Destructive sampling methods

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### 2.1 Towed gear

Many towed gears for the remote destructive sampling of the epibenthos yield qualitative or, at best, “semi-quantitative” data (for observations on trawl-sampling efficiency see e.g. Eleftheriou and Moore, 2005; Reiss *et al.*, 2006; Rumohr, in prep.). They are usually deployed at the seabed while the vessel is proceeding under power at a constant speed, while drifting or, in some cases, using winch power while anchored. Standardization of sampling may include towing over fixed distances or over specified time intervals; a measure of sampler efficiency may also be obtained from an odometer wheel or transponder, which reflects the distance over which actual seabed contact is maintained (see below). The more sophisticated devices, usually those designed to skim a known surface area of sediment to a standard depth, or to entrain a known volume of water just above the seabed, have opening/closing mechanisms.

Mechanical devices for measuring tow distances, such as an odometer wheel, may work well over even surfaces. However, they may be less reliable over mixed substrata and may fail as a result of fouling with filamentous epigrowths (e.g. colonial hydroids). This risk may be reduced through the use of paired odometer wheels (e.g. Lavaleye *et al.*, 2002; see also Section 2.1.3.1, Aquareve epibenthic sled). A prototype of an electronic device for real-time evaluation of bottom contact during trawl sampling was tested during a collaborative North Sea epifauna survey (R. Callaway, pers. comm.) and is now marketed ([www.remontec.com](http://www.remontec.com)) as a microprocessor-controlled system using a sonar communication link to send and receive data. Visualization, and hence control of performance, may also be achieved by attaching a video camera to e.g. a trawl beam or epibenthic sledge.

The efficiency of the gears often depends on the different tidal states and wind conditions at the time of sampling. For offshore surveys, it is rarely practical to coordinate effort in such a way as to ensure close comparability on all sampling occasions. Thus, sample size and quality may vary, irrespective of whether tows are conducted over fixed times or fixed distances. It is therefore essential that information on tidal state and weather conditions is recorded because these may contribute to the differences between stations identified during data analysis.

For most of the more elementary designs, which are intended to sample at the seabed as well as just above it, bottom sediments will often be penetrated during deployment to an unknown extent, e.g. across mixed substrata. An obvious consequence is that samples from such devices will combine elements of both the epibenthos and endobenthos, and will require separation during processing.

Initial processing of the contents of samples from most towed gears can be conducted at sea, and sorted material can be preserved for later laboratory attention or discarded, as necessary. For quality control, representative specimens of all identified species should be retained (see Section 5.2.2.5).

#### 2.1.1 Trawls

Trawl studies have traditionally taken two forms. One is an examination of the incidental “bycatch” of epifaunal species collected by commercial-sized trawls, typically during fish-stock assessment surveys. The other is the independent sampling of the epifauna using smaller equipment deployed at relatively low speed and over shorter time intervals. The latter may incidentally include commercial fish species, but usually with low catch efficiency. The former have yielded useful data on the distribu-

tional properties of species over wide sea areas (e.g. Dyer *et al.*, 1983). However, because of the large mesh sizes typically associated with commercial-sized towing gear, the epifaunal (or “megafaunal”) bycatch may bear limited resemblance to the *in situ* status of natural assemblages.

The reduced dimensions, smaller mesh sizes, and shorter deployments that characterize “experimental” trawls typically result in the more efficient sampling of the sessile and more sedentary components of the epibenthos. The data provided by trawl sampling across a level, sandy seabed should be reasonably accurate, more so than that obtained by sampling across coarser or mixed substrata. The outcomes of trawl surveys are therefore closely linked to gear design and sampling practices, i.e. they are operationally determined. As with epibenthic bycatches from commercial-sized trawls, caution must therefore be exercised when interpreting survey data.

#### 2.1.1.1 Beam trawl

A 2 m width beam trawl is commonly used in scientific studies. For example, it has been successfully deployed in small- and large-scale studies of epifaunal biodiversity in the North Sea area (Frauenheim *et al.*, 1989; Jennings *et al.*, 1999; Rees *et al.*, 1999, 2001; Zühlke *et al.*, 2001; Callaway *et al.*, 2002b; Reiss and Kröncke, 2004; Smith *et al.*, 2006). Other studies have used larger versions. For example, Serrano *et al.* (2006) sampled the epifauna of the Cantabrian Sea with a 3.5 m beam trawl, whereas Creutzberg *et al.* (1987) and Duineveld and van Noort (1990) used a 5.5 m beam trawl for epifaunal sampling in the southern North Sea. Further details are given in Section 6.

Riley *et al.* (1986) described a 2 m beam trawl with a wooden crossbeam that has been widely employed for young fish and epibenthic surveys. Up to three tickler chains are typically attached. More recent modifications to increase the success rate across coarser deposits and in rough weather have included the use of a steel crossbeam, the widening of the shoes, and the addition of a chain mat to the underbelly to exclude boulders (Jennings *et al.*, 1999; Figure 2.1.1).

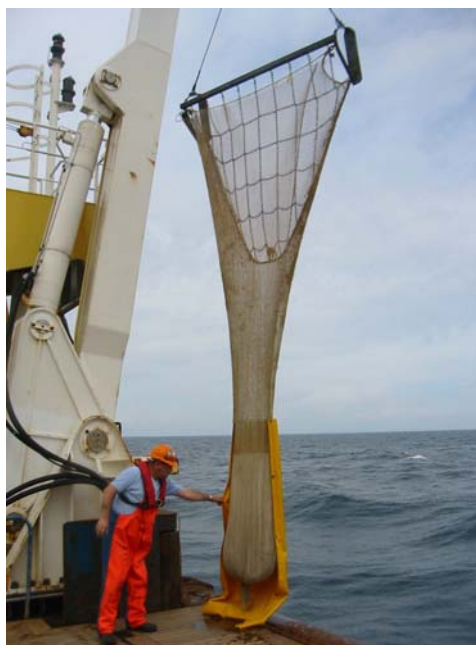


Figure 2.1.1. A 2 m beam trawl with a chain mat and codend chafer (see Jennings *et al.*, 1999) being recovered over the stern of a research vessel (source: Cefas, UK).

The use of a standard sampler design and standard deployment practices is essential if studies are to yield internally consistent results. As 2 m beam trawls have been widely used in epibenthic surveys, more detailed guidance on their use is provided below (see also Section 5 for details on sample processing):

- Trawls should generally be towed at a maximum speed of 1.5 knots (i.e.  $\sim 0.75 \text{ m s}^{-1}$ ) over the ground for distances of  $\sim 500 \text{ m}$ , usually taking 5–10 min. The use of a track-point system (above) will allow the actual path of the tow to be established but, as a minimum, positions at the time of first bottom contact and at the start of hauling should be recorded, allowing an approximate calculation of the area sampled. Towing over relatively short distances/time intervals should ensure that most samples are of a manageable size for processing of the entire contents, thereby minimizing the need for subsampling.
- Beam trawls are towed on a pair of bridles attached to a single towrope or line. A weight placed in the codend of the trawl can be used to sink the net during deployment and hence reduce the risk of fouling across the beam. The length of the warp should be approximately 3–4 times the maximum expected water depth. Evidence that the device has maintained good bottom contact during towing should be sought from an examination of the warp under tension, which may be quantified using an attached odometer wheel or sonar device (see Section 2.1).
- The use of a 2 m beam trawl is limited to relatively uniform, low-relief terrain. In the event of serious damage to the net or frame, the possibility of trawling at nearby locations should be considered before a station is finally abandoned. On retrieval, trawls should be routinely inspected for any damage. Repairs should be conducted immediately, or the gear substituted, and the events noted in the field log. Nets should be washed down after retrieval to ensure that there is no cross-contamination of sampled material between stations.
- It is important to recognize that the data typically generated from 2 m beam trawl surveys will, at best, be semi-quantitative (see e.g. Rumohr, in prep.).

Further work is required in order to improve the quality and comparability of epibenthic data generated from trawl surveys. Agreement on trawl-sampling procedures for recent collaborative North Sea-wide surveys (Jennings *et al.*, 1999; Zühlke *et al.*, 2001; Callaway *et al.*, 2002b; Reiss *et al.*, 2006; see also [www.mafcons.org](http://www.mafcons.org)) represents a significant advance. Specifications for gear design and sampling practices included:

- **Net.** 20 mm mesh size (stretched: 10 mm from knot to knot) for the belly and 4 mm mesh size (knotless) for the codend.
- **Towing.** 5 min at 1–1.5 knots, recorded at the start and end of bottom contact, which typically gave a towing distance of 300 m.
- **Sample processing.** Conducted over a 5 mm mesh sieve; animals passing through this but retained on a 2 mm mesh sieve were also qualitatively assessed.

#### 2.1.1.2 Agassiz trawl

An Agassiz trawl has the advantage that it will sample with equal efficiency regardless of which way it lands on the seabed. In the example shown in Figure 2.1.2, the

frame consists of square steel tubing, with a net opening 300 cm wide and 80 cm high, and is equipped with a chain on both sides. Eleftheriou and Moore (2005) note that an Agassiz trawl is less efficient than a beam trawl at catching fish. Examples of its use in offshore surveys include Basford *et al.* (1989), who deployed a 2 m wide Agassiz trawl with a final mesh opening of 20 mm. Lavaleye *et al.* (2002) report on the deep-water deployment of a 3.5 m wide Agassiz trawl fitted with a mechanical closing mechanism and a 1 cm mesh net. Distance travelled and seabed contact were determined using two odometer wheels, a video, and a cable tension gauge.



Figure 2.1.2. Agassiz trawl (courtesy KC-Denmark A/S ([www.kc-denmark.dk](http://www.kc-denmark.dk))).

### 2.1.2 Dredges

Several dredge designs have been produced over the years to meet a variety of scientific applications, including the sampling of very coarse substrata. When sampling smaller areas, dredges are generally used rather than trawls. In addition, many of the simpler designs are adaptable: light- or heavyweight versions may be constructed for use in coastal or offshore environments and for deployment from smaller or larger vessels. Dredges are towed along the seabed while the vessel is under power, drifting, or, in some cases, while at anchor using winch power.

For dredges that are unsuitable for the attachment of a video camera or odometer wheel and have no opening/closing mechanisms, inherent uncertainties over the mode of action during deployment will, at best, result in semi-quantitative data.

In general, dredges can operate with high resolution over moderate spatial scales ( $\sim 10$ – $100 \text{ m}^2$ ) and can collect rare or large species that would not normally be sampled by grabs. Most designs are limited to softer sediments and are intrinsically disruptive to the seabed; hence, it would be counterproductive to return to precisely the same location for repetitive sampling. At some locations, heavy-duty devices may result in unacceptable damage to more delicate organisms (both those caught and those left behind). Such devices may also require skilful handling on board ship to minimize safety hazards.

A selection of dredge samplers is described below. Further examples are given in Eleftheriou and Moore (2005).

### 2.1.2.1 Rallier du Baty dredge

The Rallier du Baty dredge (Figure 2.1.3) is designed to operate over substrata ranging from sands to cobbles, and has been widely used in the English Channel and Celtic Sea (see Cabioch, 1968; Prygiel *et al.*, 1988; Sanvicente-Añorve *et al.*, 1996). It consists of a heavy-duty metal ring (55 or 39 cm diameter for large and small models), attached to a central towing arm, which also serves to prevent the entry of very large stones. The finer meshed (typically 0.5 or 1 mm) internal collecting bag is protected by a coarser meshed outer bag, which, in turn, is enclosed by a chafer. The warp is shackled to the metal ring, from which a weak link extends to the forward towing point of the central arm.



Figure 2.1.3. Left: a Rallier du Baty dredge during deployment. Right: front view with sample contents on retrieval, illustrating the central towing arm with four curved hooks attached to the circular leading edge (source: Cefas, UK).

The dredge is deployed over the stern or side of a vessel, with a warp length of 3–5 times the water depth. Contact with the seabed can be judged by the vibration of the warp. The speed and duration of tow will depend on the objectives of the study; for small-scale surveys, deployment might typically involve towing at not more than 1.5 knots for a period of 5–10 min. On retrieval, the net bags are untied and, for convenience, the dredge can then be suspended to facilitate the release of the sample contents onto the deck.

The circular leading edge of this dredge allows some lateral movement as it is towed across the seabed, which has the advantage that the device is less prone to snagging on obstructions, and it can continue to sample in all orientations. However, as with other comparable sampling devices, it may be difficult to determine whether the sample contents are evenly or erratically accumulated over the length of the tow. Samples collected by this method should be treated, at best, as semi-quantitative.

### 2.1.2.2 Anchor dredge

The anchor dredge (Forster, 1953) was designed to be operated from a small vessel in order to sample sandy sediments, although it can produce acceptable samples when used on coarser substrata (see Eleftheriou and Moore, 2005). It consists of a rectangu-

lar metal box, open at both ends, to which fixed or hinged wishbone towing arms are attached. Hinged arms allow collection of a sample in two orientations. A canvas or net collection bag is attached to the rear of the device to retain the sample. In a modified version (see Brown *et al.*, 2002a), the collection bag is replaced by a sealed metal plate, i.e. the dredge consists of a metal box, the open (anterior) end of which is 0.5 m wide and 0.2 m deep.

The anchor dredge is deployed over the side or stern of a vessel and, after sufficient warp (typically 3–5 times the maximum water depth) is paid out, the warp is secured. As the name suggests, the dredge is intended to collect a discrete sample from a single point as it penetrates the sediment under the weight of the drifting vessel. However, when used on larger vessels, the dredge may operate (either by default or by design) as a towed body, and the mode of sample collection, i.e. instantaneous or gradual, may be very difficult to ascertain. Also, the gear will tend to be selective for the less motile epifauna. Therefore, the data generated should, at best, be treated as semi-quantitative. The device may be particularly useful in pilot surveys of hitherto unsampled areas, or for the collection in quantity of certain target species for autecological study (e.g. Holme, 1966; Rees and Nicholson, 1989; Jennings *et al.*, 2001a).

#### 2.1.2.3 Newhaven scallop dredge

This dredge was designed to catch scallops in commercial quantities, but it may also be used for the selective sampling of other epibenthic species for scientific assessments (Figure 2.1.4). The dredge may be operated over very coarse terrain, but may suffer damage if towed over bedrock or through large boulder fields. There are several types of scallop dredges in use, of which the Newhaven and French dredges are described by Franklin *et al.* (1980). The leading edge of the Newhaven dredge comprises a bolt-on metal bar to which are attached a row of spring-loaded, downwards-directed teeth. When the dredge encounters large stones, the springs allow the tooth bar to swing back, thus avoiding snagging and reducing the quantity of stones caught. The mouth of the dredge is approximately 0.8 m wide and 11 cm deep during deployment. The lower surface of the collecting bag is made up of heavy-duty metal links (inside diameter ca. 4 cm), whereas the upper surface consists of heavy-gauge nylon mesh. The minimum particle/organism diameter likely to be retained within the dredge is approximately 2 cm.



Figure 2.1.4. Three Newhaven scallop dredges attached to a metal beam with rubber rollers. The undersurface of each dredge has a spring-loaded tooth bar and a mesh of interlaced metal rings to facilitate sampling across coarse substrata (source: Cefas, UK).

Typically, two or three dredges are deployed together (attached to a metal beam) over the stern or side of a vessel and towed for a predetermined time. Care must be taken to ensure that the dredges are deployed the right way up. The epibenthic component of material collected using the Newhaven dredge, and other scallop dredges, will have undergone significant selection in response to the design features, and the eventual data should, at best, be treated as semi-quantitative. Rees (1987) and Kaiser *et al.* (1998) report on surveys of the epifauna in the eastern and western English Channel, respectively, using scallop dredges.

#### 2.1.2.4 Rock dredge

The rock dredge (Nalwalk *et al.*, 1962; Figure 2.1.5) was designed for the collection of samples from deep-water rocky terrain (see also Eleftheriou and Moore, 2005). It consists of a heavy-gauge rectangular metal rim, 0.6 m wide and 0.4 m deep, to which towing arms are attached on either side. To prevent damage to the collecting bag during deployment, the bag can be constructed from interlaced metal rings of the same size as those used for the scallop dredge (above), retaining particles larger than about 2 cm diameter. A finer meshed liner may be fitted within the chain-link bag in order to retain smaller organisms and a “weak link” may be employed to reduce the risk of gear loss during deployment.



Figure 2.1.5. A rock dredge of robust construction consisting of a rectangular mouth attached to towing arms and a collection bag of interlaced metal rings (source: Cefas, UK).

The rock dredge is versatile and will even collect surface scrapings from bedrock. Deployment practices are similar to those described for other dredges. In rocky areas, towing may be achieved using the winch while the ship remains stationary. On retrieval, the dredge is mechanically lifted by a chain attached to the rear of the collecting bag in order to release the sample contents. This device is well suited to surveys in offshore areas that are known or suspected to present significant sampling problems owing to the presence of very coarse substrata. As with many other dredges,

uncertainties over sampling efficiency and selectivity determine that the data generated should, at best, be treated as semi-quantitative.

#### 2.1.2.5 Naturalists' dredge

The Naturalists'—or rectangular—dredge (Figure 2.1.6) was employed as early as the 19th century by natural historians motivated as much by the collecting instinct as by an interest in assigning epibenthic species to the Linnaean classification system. The dredge described by Eleftheriou and Moore (2005) consists of a rectangular metal frame with towing arms, to which a net collecting bag is attached. This dredge is still commonly used to provide a qualitative overview of the biota (e.g. Reise and Bartsch, 1990) but, with caution, may also provide quantitative insights. For example, Collie *et al.* (1997) employed a 1 m wide Naturalists' dredge with a 6.4 mm square mesh liner to sample the benthic fauna of Georges Bank, and identified qualitative and quantitative differences between locations, which were attributable to the degree of disturbance by bottom fishing gears.



Figure 2.1.6. Profile of a Naturalists' dredge, consisting of articulated towing arms extending from the sides of a rectangular leading edge to which a sampling bag is attached (courtesy KC-Denmark A/S ([www.kc-denmark.dk](http://www.kc-denmark.dk))).

#### 2.1.2.6 Kieler Kinderwagen dredge

The Kieler Kinderwagen, or botanical dredge (Schwenke, 1968; Figure 2.1.7), has a 1 m wide rectangular mouth, and functions on both sides like an Agassiz trawl. It is typically equipped with a net bag 3 m in length, with a codend and an inner 0.5 cm mesh net. It is used on sandy and muddy bottoms as well as in the phytal region. If required, its penetration into the sediment (typically 1–3 cm) can be enhanced by additional weights and by single tickler chains on each side of the dredge. Video images revealed efficient sampling over the ground, with good sieving characteristics; irregular bottom contact occurred only in stony areas (H. Rumohr, pers. comm.). An account of its use in central and southern parts of the North Sea is given in Künitzer (1990).



Figure 2.1.7. Kieler Kinderwagen botanical dredge with tickler chains to allow sampling in two orientations (source: H. Rumohr, IfM-GEOMAR, Germany).

#### 2.1.2.7 Triangular dredge

The origin of the triangular dredge (see Schwenke, 1968) can be traced back to the work of naturalists in the 19th century. The modern version shown in Figure 2.1.8 is made of heavy-duty steel, and the triangular frame ( $80 \times 80 \times 80$  cm) supports a 30 mm mesh outer net and a knotless 10 mm square mesh inner net for retaining biological and/or mineralogical samples. It may be used with or without a rubber plate to protect the net. When in use, a buoyancy aid is attached to the upper corner to maintain the correct position at the seabed. Recent examples of the use of a triangular dredge on mixed/rocky terrain include the BIOFAR (Benthic Investigation of the Faroe Islands) and BIOICE (Benthic Investigation of Icelandic Waters) sampling programmes (see Tendal, 1998; Tendal *et al.*, 2005; [www.biofar.fo](http://www.biofar.fo); [ftp.hafro.is/pub/bioice](http://ftp.hafro.is/pub/bioice)).



Figure 2.1.8. Triangular dredge (courtesy KC-Denmark A/S ([www.kc-denmark.dk](http://www.kc-denmark.dk))).

### 2.1.2.8 Deep Digging Dredge (Triple-D)

The Triple-D (Figure 2.1.9) was designed to collect a large sample of the seabed in order to obtain accurate estimates of the densities of the larger and infrequently occurring infaunal and epifaunal species. The prototype (Bergman and van Santbrink, 1994) was equipped with a fixed cutting blade that sliced a strip out of the seabed over a tow length of about 150 m and a width of 0.2 m to a depth of 0.1 m. In a later modification, a hinged cutting blade is operated by compressed air, allowing a haul of a preset length, independent of vessel movement during initial deployment and eventual retrieval. Moreover, the sampling depth increased to 0.14 m. The mesh size in the dredge (0.5 cm square) and the net (0.7 cm square) retains all infaunal and epifaunal specimens with (outer) diameters greater than 1 cm.

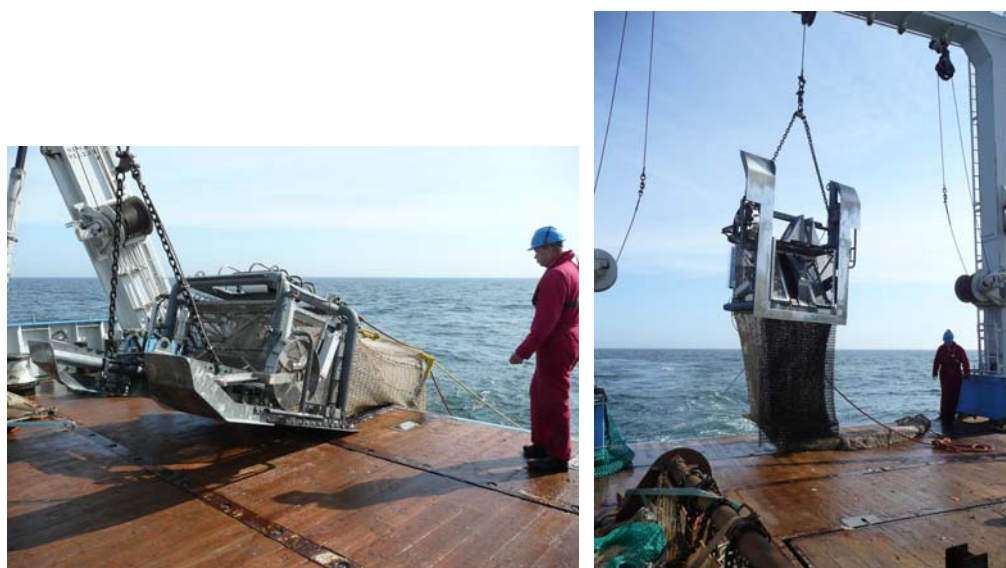


Figure 2.1.9. Deep Digging Dredge (Triple-D: 2008 version: ~1500 kg). Left: oblique view showing the rectangular front end of the sampler, side-mounted odometer wheels, and a 7 × 7 mm mesh collecting net. Right: the underside supports a 20 cm wide retractable cutting blade, which can be closed by two lids and can be preset to penetrate sediments for fixed distances; the triangular leading edge of the gear prevents the lateral escape of epifauna in front of the cutting blade (source: M J. N. Bergman, Royal NIOZ, the Netherlands).

The Triple-D provides reliable density estimates of e.g. (sub)adult molluscs, echinoderms, larger polychaetes, and crustaceans. Although more complex in construction than many other dredges (see above), the capacity for accurate quantification of the targeted fauna is a notable advantage (e.g. Duineveld *et al.*, 2007).

### 2.1.3 Sledges

Sledges typically consist of frame-supported samplers on runners to maintain the height or aspect of the front end for the collection of the epibiota at or just above the seabed. Sampling is therefore more controlled than with simpler dredge designs, although sledges may be less versatile, operating more efficiently over softer, low-relief substrata. As with dredges, several designs have been produced over the years to meet a variety of scientific applications in both shallow water and the deep sea. For sledges that are unsuitable for the attachment of a video camera or odometer wheel and have no opening/closing mechanisms, sampling will typically yield, at best, semi-quantitative data.

In general, sledges can operate with high resolution over moderate spatial scales (~10–100 m<sup>2</sup>) and can collect rare or large species, or the motile hyperbenthos<sup>1</sup>, which would be sampled with low efficiency (or not at all) by grabs. Some are intrinsically disruptive to the seabed, and hence it would be counterproductive to return to precisely the same location for repetitive sampling.

A selection from a range of sledge samplers is described below. Further examples are given in Eleftheriou and Moore (2005) and Gage and Bett (2005).

#### 2.1.3.1 Aquareve epibenthic sled

The Aquareve epibenthic sled was originally designed by Thouzeau and Vine (1991) and used for epifauna studies of scallop grounds on Georges Bank (e.g. Thouzeau *et al.*, 1991). In a modified form (Rowell *et al.*, 1997), it was successfully used to study the effects of otter trawling on the epifauna of a sandy-bottom habitat on the Grand Banks (Prena *et al.*, 1999).

The sled is towed at approximately 1 knot (i.e. ~0.5 m s<sup>-1</sup>). The sampling blade, 0.34 m wide, cuts to a depth of 2–3 cm and therefore can collect some infaunal species. The steel collection box has regularly spaced holes (1 cm in diameter), so most sediment can pass through. Towing distance over the seabed is measured by paired odometer wheels and displayed in the ship's laboratory. At the end of the prescribed tow length (e.g. 50 m), a closing door is activated electronically, and the sled is retrieved. Sampling performance is monitored using an illuminated colour video camera directed backwards towards the mouth of the sled. Underwater and deck control units communicate through a winch-mounted electromechanical cable and slip-ring assembly. Tows of dubious quality (i.e. lifting off the bottom) can be aborted and repeated. The sled is heavy (about 1 t) and, with the specialized cable, block, winch, and electronics, requires a large research vessel to deploy. However, because the area of seabed sampled can be determined with a high degree of accuracy, samples are quantitative (Prena *et al.*, 1999). Captured organisms are subject to damage.

#### 2.1.3.2 Hyperbenthos sledge

Sledge-mounted designs are used for sampling the hyperbenthos (see e.g. Rothlisberg and Percy, 1977; Brattegard and Fosså, 1991; Mees and Jones, 1997; Dauvin *et al.*, 2000; Eleftheriou and Moore, 2005). These typically employ fine-meshed collecting nets (down to 0.5 mm), flowmeters, and opening/closing mechanisms to facilitate quantification, and several of them (e.g. Brunel *et al.*, 1978; Brandt and Barthel, 1995; Dauvin *et al.*, 1995) support additional frame-mounted nets to determine vertical zonation. Hyperbenthic organisms, especially crustaceans, can represent a significant food source for fish and are therefore an important target in resource-assessment surveys. Such surveys must allow for the diurnal changes in swimming activity exhibited by many species (e.g. Kaartvedt, 1986). An example of a hyperbenthic sampler used by Brattegard and Fosså (1991) for surveys of Norwegian waters is illustrated in Figure 2.1.10.

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<sup>1</sup> Hyperbenthos are free-swimming animals inhabiting the water column immediately above the seabed for much of their life cycles.



Figure 2.1.10. Details of a hyperbenthos sledge (see Buhl-Jensen, 1986; Brattegard and Fosså, 1991; Miskov-Nodland *et al.*, 1999) showing (top) the fine-mesh collecting net extending back from the sledge-mounted rectangular sampling box, and (bottom) the front end of the sampler with spring-loaded opening/closing mechanism (source: L. Buhl-Mortensen, IMR, Norway).

#### 2.1.3.3 Ockelmann sledge

The Ockelmann sledge (Figure 2.1.11; Ockelmann, 1964) is a lightweight instrument (10–15 kg) designed to skim the surface of level soft-bottom sediments. It is equipped with adjustable blades on both sides, which determine the sampling depth. The short and fine-meshed (0.5 mm) net bag is sheltered by the broad runners, which also prevent deeper penetration into the muddy sediment. It can be equipped with a light tickler chain between the towing wires, which will gently disturb the upper sediment layer containing any newly settled invertebrate larvae and the temporary meiofauna. This device may be considered to sample the epibenthos, especially the smaller motile component, by default rather than by (original) design, but nevertheless may do so efficiently under the right circumstances. Examples of its use include the collection of algal mats and associated epifauna in shallow Baltic waters (Norkko and Bonsdorff, 1996; Norkko, 1998).

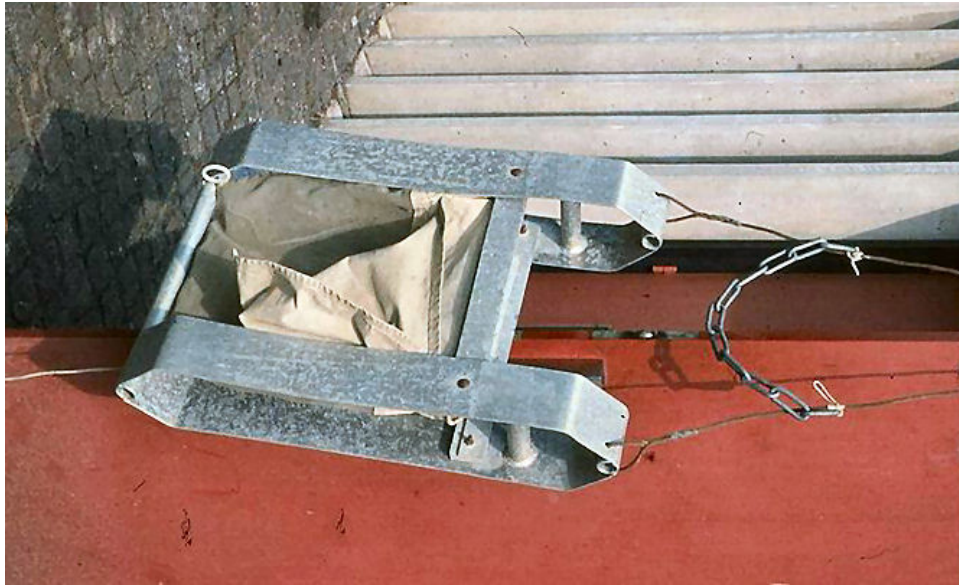


Figure 2.1.11. Ockelmann sledge with a fine-mesh collecting net and runners of lightweight construction (source: H. Rumohr, IfM-GEOMAR, Germany).

#### 2.1.4 Grabs

Because the size range of the organisms encountered is much greater than that of the endobenthos, small (typically 0.1 m<sup>2</sup>) grab samplers are generally unsuitable for sampling the epibenthos. Thus, larger epibenthic organisms tend to be too sparsely distributed relative to sample size, and motile species may easily evade capture. Towed gear, such as trawls and dredges, are more appropriate for sampling these species, although usually at the expense of accurate quantification. Moreover, on mixed substrata or hard ground, grab-sampling devices may operate at low sampling efficiency or not at all. If grabs are to be used, then those sampling larger surface areas (i.e. >0.2 m<sup>2</sup>) are to be recommended.

Details of the design and operation of standard grab-sampling devices for soft sediments may be found in Eleftheriou and Moore (2005) and Rumohr (in prep.). Samplers that operate with some success on coarser substrata may be useful for evaluations of the smaller sessile component, which may be relatively well developed at such locations. Of these, the Hamon grab has proven to be particularly effective, and a description of this device (after Brown *et al.*, 2002a) is summarized below. By their nature, grab samplers operate on a very small spatial scale but provide a higher resolution than many types of towed gear.

##### 2.1.4.1 Hamon grab

The Hamon grab (Oele, 1978; Eleftheriou and Moore, 2005) consists of a rectangular sampling bucket attached to a pivoted arm, supported within a sturdy metal frame (Figure 2.1.12). The arm is free to pivot on contact with the seabed. Tension in the wire at the start of in-hauling then rotates the pivoted arm through an angle of 90°, driving the bucket through the sediment. At the end of its movement, the bucket locates onto an inclined, rubber-covered steel plate, sealing it completely. Weights are usually attached to the supporting frame in order to minimize lateral movement during sample collection. On retrieval, the device is lowered onto a rectangular stand, under which a removable container is placed to receive the sample on release from the bucket.



Figure 2.1.12. A 0.1 m<sup>2</sup> Hamon grab supported on an open plinth to facilitate sample retrieval from the scoop-action bucket. Racks on each side of the grab frame contain lead weights to increase sampler efficiency (source: Cefas, UK).

The Hamon grab is robust, simple to operate, and is particularly effective on coarse sediments. It has been employed in several studies of benthic communities along the French coast (e.g. Desroy *et al.*, 2003) and has been used extensively to assess the biological impacts of marine aggregate extraction (e.g. van Moorsel and Waardenburg, 1991; Kenny and Rees, 1996; Seiderer and Newell, 1999; Boyd and Rees, 2003). The original design was for a grab that samples an area of about 0.25 m<sup>2</sup>. A smaller device, sampling an area of 0.1 m<sup>2</sup>, has since been successfully employed in surveys of coarse substrata around the coast of England and Wales (see Brown *et al.*, 2002a; Boyd *et al.*, 2006a). One drawback of the Hamon grab is that the sediment sample is mixed during closure of the bucket, which prevents the examination or subsampling of an undisturbed surface layer. The attachment of a video camera to the frame has proven useful for the *in situ* evaluation of surface features adjacent to the grab bucket (Brown *et al.*, 2002b).

#### 2.1.4.2 Videograb

The Videograb (Figure 2.1.13) is a hydraulically actuated bucket grab equipped with video cameras. More sophisticated than a simple bolt-on camera attachment to a conventional grab, it was designed to allow precise selection of station locations, to permit remote control of bucket closure and opening, and to verify proper closure before recovery (Rowell *et al.*, 1997; Gordon *et al.*, 2000). Other examples of video-controlled grabs include the SEABed Observation and Sampling System (SEABOSS) designed by the US Geological Survey for collecting seabed images and sediment samples in coastal regions (Valentine *et al.*, 2005).



**Figure 2.1.13.** Videograb (equipped with DRUMS (Dynamically Responding Underwater Matrix Sonar) being recovered (source: MFO, Canada).

An illuminated colour video camera is mounted obliquely to provide a forwards-looking, wide-angle view while drifting just above the seabed. A downwards-looking, high-resolution video camera is mounted directly above the open bucket and provides imagery of the seabed and closure of the bucket. The bucket is closed hydraulically, and the area sampled is 0.5 m<sup>2</sup>. Sampling depths range from 10–25 cm and, at full penetration, the sediment volume is about 100 l. The device weighs about 1100 kg and is about 2.5 m in height. In most deployments, wire angles are small, and the ship's geographical position (measured at the end of the crane boom) serves as a reasonable proxy for sample location. However, a transponder can be attached for more accurate positioning.

During deployment and retrieval, the winch is controlled by an operator on deck. However, when the seabed comes into sight on the monitor, control of the winch passes to a scientist in the laboratory. The usual procedure is to drift for a few minutes with the Videograb suspended just above the seabed. Once landed on features of interest, the open bucket is poised 20 cm above the bottom. By paying out slack cable, the Videograb is decoupled from the motion of the ship and high-resolution video of the seabed is recorded. Closure of the bucket simultaneously closes a retractable lid on top, which reduces washout during recovery. If sampling is unsuccessful, the operator can lift the Videograb off the seabed, reopen the bucket hydraulically, and select another landing site. A video monitor is also placed on the bridge to assist in ship handling. Video imagery and navigation data are recorded on digital tape. Because of the size and complexity of the Videograb, a large research vessel is required.

The Videograb is an efficient tool for collecting quantitative samples of the epi- and endobenthos with minimal disturbance, and has been used to study the impacts of otter trawling (Kenchington *et al.*, 2001). It works well over soft substrata, although achieving complete closure in gravelly sediments can be problematic. It has been successfully used for targeted sampling to ground-truth/identify video-documented organisms and to collect gorgonian corals attached to small boulders. The video imagery provides information on the undisturbed habitat from which the sample is collected, as well as on sample quality. It leaves a relatively small footprint (a few square metres) and so can be used for time-series studies at a given location. The Videograb can also be adapted to provide information on small-scale structural properties of surficial sediments (Schwinghamer *et al.*, 1996, 1998).

#### **2.1.4.3 Other grabs**

The Baird grab (Baird, 1958) was designed for sampling the epibenthos of coarse substrata. It collects a large surface area (0.5 m<sup>2</sup>) but remains open on retrieval and is therefore of limited use as a fully quantitative sampler. This and a variety of other grabs sampling surface areas greater than conventional (0.1 m<sup>2</sup>) infaunal samplers are described by Eleftheriou and Moore (2005). In general, these are not widely established (or tested) tools.

Another example is the Gordeev grab, which was designed in the 1930s by a Russian engineer to sample the coarse sands and gravel of the Ochotsk Sea. The sampler, weighing 500–1000 kg, sampled an area of 0.25–0.5 m<sup>2</sup>, and had a maximum penetration of 50 cm into these problematic sediments. A sampler still in working condition is stored at the Zoological Museum in St Petersburg, Russia, but published information on its performance and potential for wider use had not been located at the time of publication (H. Rumohr, pers. comm.). Further investigation of the potential use of such samplers (including industrial-scale grabs used in prospecting for seabed resources) in epibenthic studies may be merited.

## **2.2 Suction samplers and other (diver-operated) devices**

### **2.2.1 Suction sampling**

Suction or air-lift sampling involves the release of air at the bottom of a tube, which, as it rises, draws in other material as a result of the Venturi effect. The sample contents are usually collected in a mesh bag. Details of a range of suction samplers, along with procedures for their use in diver surveys, are provided by Rostron (2001) and Munro (2005; see also Eleftheriou and Moore, 2005). Diver- or remotely operated suction samplers have an established role in the collection of the infauna from soft sediments and may also be a cost-effective means for quantifying components of the epibenthos, including the provision of ground-truth information to accompany *in situ* photographic records.

For coarse substrata, suction sampling may be especially useful for quantifying motile and loosely attached sessile epibenthic species. For example, Thomasson and Tunberg (2005) employed a water-jet suction sampler for collecting the motile epifauna associated with vertical rock surfaces in Swedish waters. To provide quantitative data, it is necessary to sample within a quadrat or other area-delimiting device, and as a measure of sampling efficiency, to augment sampling effort with photographic records and/or representative samples scraped manually from the rock (see Section 2.2.2). Cobbles may be sampled in a similar way and, depending on circum-

stances, may be moved to ensure effective sampling of microhabitats beneath and between them.

In deeper water or other areas inaccessible to divers, a remotely operated vehicle (ROV) equipped with a claw-and-suction sampler may be used. A recent example involved the collection of motile fauna associated with deep-sea corals (Buhl-Mortensen and Mortensen, 2004). The ROV was fitted with a suction pump and the water was sieved through plankton meshes within sampling jars mounted in a carousel.

### 2.2.2 Scrape sampling within frames

For quantitative estimation of biomass or biodiversity, scrape samples within frames are commonly collected. The dimensions of the frames depend on the community type. For example, in monitoring programmes in the photic zone of Swedish waters (Kautsky, in prep.; see also [www.helcom.fi](http://www.helcom.fi)), a 0.5 m square frame is used for the canopy layer formed by large plant species such as the bladder wrack (*Fucus vesiculosus*). All specimens with holdfasts located within the frame are collected. For the other strata (the bush and field layer), a 0.2 m square frame is used. Specimens within the frame are scraped into a mesh bag attached to one side of the frame (Kautsky, 1993). A suction sampler may be used as an alternative to scrape sampling into an attached bag (e.g. Gulliksen and Deras, 1975; Dybern *et al.*, 1976; Munro, 2005) and a covered frame can be used to prevent the escape of motile fauna. However, an open frame has the advantage of being easy to handle by a single diver, and experience in Swedish waters demonstrates that most organisms are recovered from within the frame.

Following removal of the canopy layer and motile or loosely attached organisms, estimates of residual presences, such as encrusting bryozoans and red algae, may be made *in situ*. On mixed substrata with large structural heterogeneity (e.g. in the presence of stones and small boulders), the use of frames may be less reliable, because it may be difficult to ensure even contact with the seabed, and there is a risk of the loss of material during sampling. Substrata consisting of pebbles, gravels, and finer fractions do not present such problems.

### 2.2.3 Drop-trap

A drop-trap typically consists of a steep-sided, open, square metal box which, when manually deployed (not necessarily by divers) over soft sediments at depths of <1 m, encloses 0.5–1 m<sup>2</sup> of sediment and the overlying water. Wennhage and Pihl (2007) provide a recent example of the use of this simple device on the Swedish coast. Quoting earlier studies (e.g. Wennhage *et al.*, 1997), they note that it is close to 100% efficient for collecting the dominant species of epibenthic fauna on shallow, soft substrata. Motile species, including fish, are typically extracted from the box using hand-held nets (see also Wilding *et al.* (2001) for guidance on the use of drop-traps for small-fish surveys).

## 2.3 Sediment profile imaging

Sediment profile imaging (SPI) is a standardized technique for imaging and analysis of sediment structure in profile. It was originally developed in the US, where it is known as REMOTS (Remote Ecological Monitoring Of The Seafloor; Rhoads and Germano 1982, 1986). Strictly, this device might be considered a “locally disruptive” rather than a destructive sampler. Although it has only limited direct application to studies of the epibenthos of soft substrata, it may nevertheless provide important in-

formation on microhabitat features, which may in turn help to explain observations on the distribution and densities of the epibenthos sampled by other means.

Functioning like an inverted periscope, the camera consists of a wedge-shaped prism with a front faceplate and a back mirror mounted at a 45° angle to deflect the image of the profile of the sediment–water interface up to the camera. The camera is mounted horizontally on top of the prism and provides images of the sediment column, including the surface, 15 cm wide and up to 23 cm deep. Estimates of a range of physical and biological variables are derived from the images using a video digitizer and an image-analysis system (Rhoads and Germano 1982, 1986; O'Connor *et al.*, 1989; Smith and Rumohr, 2005). Proprietary software allows the measurement and storage of up to 21 variables for each image obtained.

The gear, with a frame and pressure housing, is deployed remotely from ships to sample subtidal sediments, but diver-deployed models are also in use. A summary of its application to habitat quality assessment in Swedish waters is given by Nilsson and Rosenberg (1997), and a compilation of recent studies at various locations is given in Kennedy (2006).

### 3 Non-destructive sampling/sensors

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#### 3.1 Acoustics

Acoustic methodology for seabed characterization is continually evolving, and a summary of some currently used techniques (adapted from Limpenny and Meadows, 2002; see also Foster-Smith *et al.*, 2001; Kenny *et al.*, 2001; Bayle and Kenny, 2005; Smith and Rumohr, 2005; Anderson *et al.*, 2007, 2008) is appropriate in view of their value in the planning and interpretation of epibenthic surveys. Most can be used over large areas and can provide information at high spatial resolution. For example, multibeam bathymetry can resolve features down to the scale of boulders, whereas accompanying backscatter data can be used to infer substratum type. RoxAnn and QTC have been used as exploratory tools in mapping benthic habitats but are yet to be proven (see below). A technique known as synthetic aperture sonar also shows promise but is at a relatively early stage of development (e.g. McHugh, 2000; Hayes and Gough, 2004).

Historically, the most widely used method for geophysical characterization of the seabed habitat is sidescan sonar (SSS; see Section 3.1.3.). Outputs from acoustic surveys can also identify bioherms, such as mussel beds and *Sabellaria* and *Lophelia* reefs (e.g. Magorrian *et al.*, 1995; Bett, 2001; Mortensen *et al.*, 2001; Hendrick and Foster-Smith, 2006; Lindenbaum *et al.*, 2008). It is important to emphasize that accurately georeferenced ground-truthing using conventional sampling devices is an essential accompaniment to such surveys. Reviews of the developing interest in marine habitat mapping and its application to resource and impact evaluations, which commonly combine acoustic, photographic, and conventional seabed sampling practices, include those of Pickrill and Todd (2003), Boyd *et al.* (2006b), Anderson *et al.* (2008), and Rees *et al.* (2008). An appraisal of the resolution of a variety of acoustic (and other) methods relative to the area of coverage is given in Annex 3 (from Kenny *et al.*, 2003). More detailed operational evaluations, including approaches to compensating for scale- and depth-related dependencies, are provided by Anderson *et al.* (2007). Generic guidance on hydrographic surveys, including methods for position-fixing for ship-mounted and towed sensors, is provided by the International Hydrographic Bureau (2005).

##### 3.1.1 Multibeam bathymetry

There are two main types of multibeam (or swathe) sonar systems: true multibeam (or focused multibeam) and interferometric (or bathymetric SSS) systems. True multibeam consists of a transmitter and receiver capable of projecting and detecting multiple beams of sound energy that ensonify the seabed in a fan-shaped swathe. Multiple soundings are thus taken at right angles to the vessel track, as opposed to a single sounding underneath the vessel with a conventional single beam echosounder. This gives a far greater density of soundings, allowing quicker coverage of the survey site.

Interferometric sonar is a variant of SSS technology where electronic techniques are applied to a multiple set of SSS-like transducers arranged to give phase information in a vertical plane. This phase information is used to determine the angle of reception of reflected sound from the seabed and, given the time of flight of the return pulse, a range/angle measurement can be made of the seabed. The main difference is that soundings for a multibeam system are denser directly under the vessel and sparser at full swathe range. The inverse is true for interferometric systems.

Both systems are prone to greater errors on the outer limits of the swathe. Both are also dependent on a very high-quality motion reference unit (MRU) to determine the position and altitude of the transducer. This apparatus significantly increases the cost of the system but is essential to ensure accurate and precise depth measurement. The application of swathe bathymetric techniques demands a lengthy calibration procedure to define any systematic errors in the installation (e.g. heading, latency, and roll). Examples of the contribution of multibeam bathymetric surveys to the mapping of marine habitats and communities include Kostylev *et al.* (2001), James *et al.* (2007), and Lindenbaum *et al.* (2008). An example of survey output is shown in Figure 3.1.1.

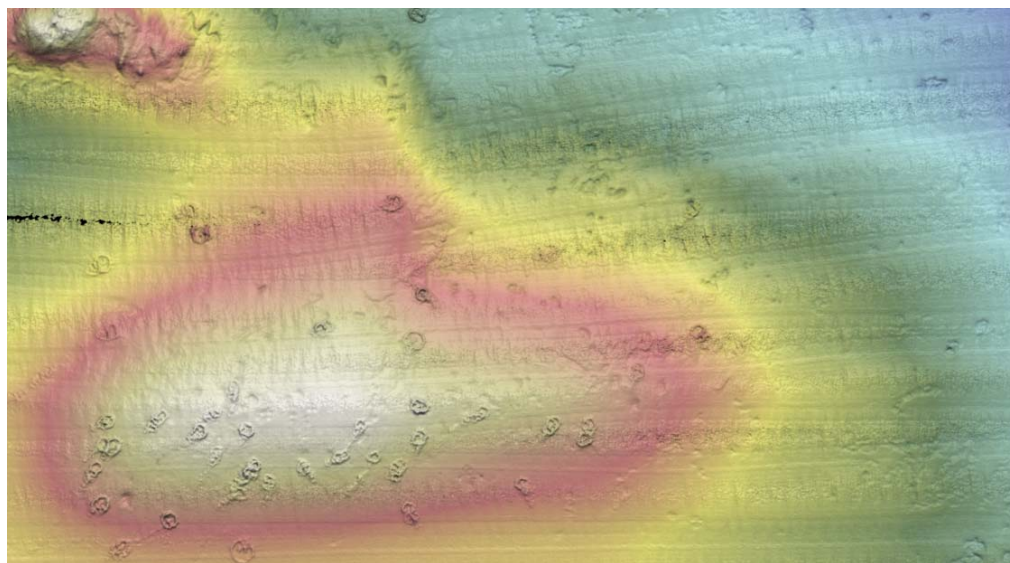


Figure 3.1.1. Output from a multibeam bathymetric survey of a licensed dredged material disposal site off northeastern England. The large oval elevation, consisting of weathered material deposited over many years, is topped with a series of sharply defined local elevations corresponding to recent disposal events (source: Cefas, UK).

### 3.1.2 Acoustic ground discrimination systems

Acoustic ground discrimination systems (AGDSs) are designed to detect differences in the acoustic properties of varying seabed substrata. The technique uses a single-beam echosounder as the host instrument. The sounder provides the acoustic pulse and the returning echo is transferred, by direct electrical connection on the transducer, to the AGDS. The shape and nature of this echo is fundamental to the technique, and all other factors affecting these properties (e.g. pulse length and power fluctuations) must be kept constant.

Significant pitfalls can be encountered using this technique, which have historically given rise to guarded acceptance of the fidelity of certain datasets. The earlier systems are based on simple analogue electronics to detect only one parameter of the first and second returns (RoxAnn: see e.g. Chivers *et al.*, 1990). The first return is the direct bounce off the seabed to the transducer; the second involves a continued pathway bouncing off the sea surface, off the seabed a second time, and back to the transducer. The first return is sensitive to bed roughness whereas the second is sensitive to bed hardness (the harder the seabed the better the reflection). The second return may also be influenced by variation in sea-surface state.

Another more sophisticated approach is the more detailed measurement of the first return only (QTC View: see e.g. Anderson *et al.*, 2002). This has the advantages of using a more reliable acoustic pathway and the ability to operate in a wider range of

environments. Some systems use extremely fast algorithms to extract features of the first return (some use a hundred or so features). They invariably come with bespoke software that operates on the more popular PC operating systems.

Several studies have explored the potential utility of AGDSs in environmental assessment programmes (e.g. Magorrian *et al.*, 1995; Sotheran *et al.*, 1997; Pinn *et al.*, 1998; Hamilton *et al.*, 1999; Pinn and Robertson, 2003; Wilding *et al.*, 2003; Humborstad *et al.*, 2004; Lindenbaum *et al.*, 2008). At present, it is recommended that AGDS methodology be used as an additional tool to complement established methods (e.g. SSS, underwater video and grab/core sampling), rather than in isolation to predict substratum type.

### 3.1.3 Sidescan sonar

SSS data are produced using towed or hull-mounted transducers, which ensonify a swathe of the seabed to either side of the transducers (Figure 3.1.2). The reflected portion of the acoustic signal is received back by the transducers, amplified, and converted into a paper or on-screen image showing levels of strength of return across the ensonified swathe of seabed. Coastal SSS systems are generally designed to work in water depths of up to approximately 300 m, and to be routinely operated at frequencies of 100–500 kHz.

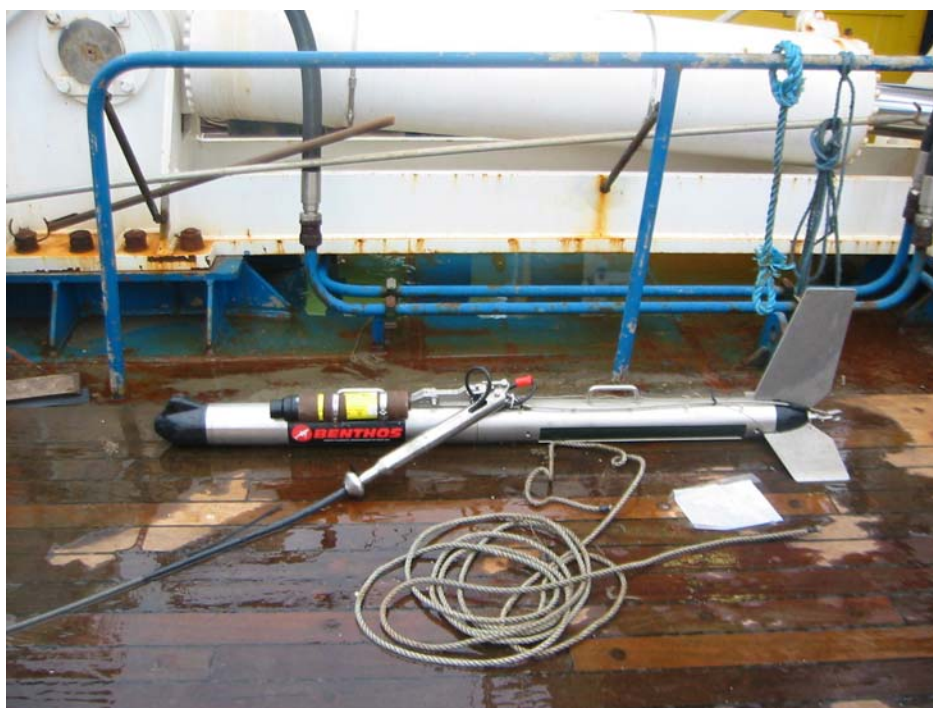
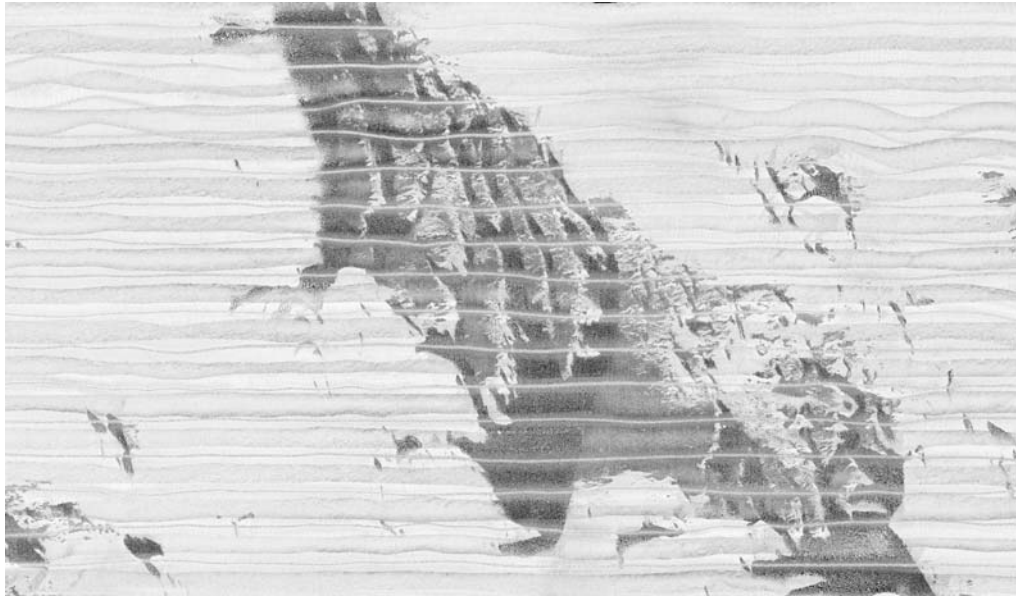


Figure 3.1.2. A typical sidescan sonar fish attached to an umbilical towing cable (source: Cefas, UK).

Output from an SSS survey provides information on the texture of the substrata within the survey area. From this, it is possible to predict the particulate nature of the sediments and assign sediment descriptions to regions of the seabed (e.g. gravel, mud). SSS also allows sediment transport features, such as sand waves and ripples, lineated gravel features, and scour marks, to be identified. Geological features, such as outcrops of bedrock and aggregate deposits associated with submerged river valleys, may also be mapped using this technique. Examples of SSS output are shown in Figure 3.1.3.



**Figure 3.1.3.** Output from a typical sidescan sonar survey showing coarse sediment patches (“sorted bedforms”) as dark tones and fine sand as light tones. The survey was conducted in the vicinity of the Dogger Bank, North Sea (source: Cefas, UK).

As with the output from other acoustic techniques, information on the nature of the substratum may be matched with existing data on (epi)benthic communities or used to guide new biological sampling effort, depending on the study objectives (e.g. Service and Magorrian, 1997; Brown *et al.*, 2004; Humborstad *et al.*, 2004; Smith *et al.*, 2007; Lindenbaum *et al.*, 2008; Yeung and McConnaughey, 2008). Inferences concerning seabed sediment transport pathways and allied hydrodynamic influences may also have interpretational value in accounting for the structure of epibenthic communities.

### **3.2 Video and photography**

Video and/or stills photography is a valuable, non-destructive method of assessing all types of seabed habitats (see Rumohr, 1995; Smith and Rumohr, 2005). It can be particularly useful over hard and consolidated ground, where the efficiency of other physical sampling methods is low, and two examples of still images of offshore coarse substrata are given in Figure 3.2.1.



**Figure 3.2.1. Top: a diverse epifauna, including sponges, sea anemones, and the bryozoan *Pentapora foliacea*, inhabiting a stable sandy-gravel substratum in the eastern English Channel. Bottom: mature colonies of the bryozoan *Pentapora foliacea*, together with numerous small sea anemones, on higher-relief terrain off the southwest English coast (source: Cefas, UK).**

The quality of photographs and video images depends largely on water clarity. This can vary considerably, even at the same location, depending on the state of the tide and season of sampling. The chances of encountering good visibility can be increased by deploying the equipment at slack-water periods. The quality and versatility of the equipment are, of course, also critical in determining the success of surveys; options may include low-light intensity cameras or remote-controlled transmitted light sources. For towed gear, speeds of less than 1 knot over the seabed are generally required to obtain clear images. The data from such surveys can be treated at a number of levels, which will be partly determined by the quality of the images obtained. Still

photographs taken at regular intervals along a transect can be treated as point quadrats, the epibenthos being identified to the appropriate taxonomic level and quantified. Data obtained by freezing the video image at regular intervals can be treated in a similar manner. Further details are given in Section 5.2.1.

Unless video/photographic surveys are confined to the identification of conspicuous (usually larger) epibenthic species, it is usually essential to obtain ground-truth physical and biological samples at appropriate intervals using trawl, dredge, or grab, with the material being preserved for later laboratory analyses. The resulting data are used to assist in the identification or verification of many of the organisms appearing in the video/still images. Ground-truthing can also be done using a video-assisted grab (Section 2.1.4.2). For example, this approach proved to be effective in deep-water coral studies (Buhl-Mortensen and Mortensen, 2004). Accurate georeferencing of camera deployments at individual stations or along transects, as well as of accompanying ground-truth samples, is essential.

For conspicuous habitat-forming epibiota, such as coral reefs and kelp forests, inhabiting shallow areas with high water clarity, aerial photographic surveys (or satellite imagery) may be cost-effective, especially for quantifying the spatial extent of cover over relatively large geographical areas, and changes over time (see e.g. [www.unesco.org/csi/pub/source/rs12.htm](http://www.unesco.org/csi/pub/source/rs12.htm)).

A summary of good practice in the use of imaging methods is given in Annex 4.

### 3.3 Direct visual observations

In favourable conditions, divers may be used to fulfil a wide range of survey objectives, mainly in inshore waters (typically to depths of 30 m), and may be the only cost-effective option for systematic quantitative surveys of mixed or rocky terrain in the photic zone (see Annex 1). Detailed coverage of approaches, including survey design, recording techniques, quality control, and safe working practices, is well documented elsewhere (e.g. Davies *et al.*, 2001; Munro, 2005; see also Sanderson *et al.*, 2008). The following example summarizes a methodology for visual profiling along gradients using line transects. Further information is provided by Kautsky (in prep; see also [www.helcom.fi](http://www.helcom.fi)).

For a complete census, continuous observations are made within a corridor 6–10 m wide along a line marked at 1 m intervals covering the area of interest. Working from one end to the other, the divers systematically record occurrences and densities (or percentage cover) within the corridor on writing plates, along with information on distance from the shore, water depth, substratum type, siltation, etc. Specimens can also be collected for later identification/verification, as necessary. A widely used variation of this approach is the line intercept transect, where divers only record information along the line itself, either at fixed (e.g. 1 m) intervals or along the entire line. This method generates sample information and must be repeated to permit population/community estimation. A third variation of the line transect is the placement of frames of standard unit area at intervals along the profile line (e.g. every 5 m), which again generates sample information and requires repeated effort along the transect to permit reliable community estimation. The data from frames have the advantage of being easy to evaluate statistically; the number of replicates (or frame size) required is typically relatively large (Kautsky, in prep.; see also Murray, 2001).

## 4 Non-destructive sampling/platforms

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The most familiar and technologically simple platform for the collection of video and still images is the scuba diver equipped with hand-held devices (see e.g. Munro, 2005). For remotely deployed gear, cameras have been mounted on a variety of platforms to facilitate *in situ* studies of epibenthic communities. These may be categorized as

- devices that are capable of moving or being directed under their own power, such as remotely operated vehicles (ROVs; see Section 4.4);
- devices that are lowered to a point above the seabed (e.g. remotely operated hoisted platforms) or are towed along the seabed, such as photographic sledges.

In recent years, the most commonly used method for epibenthic surveys of the seabed over relatively large areas has been the camera sledge (see Section 4.1), which is robust and simple to operate. It typically includes a vertically mounted stills camera and a forwards-or sideways-directed television camera linked by an electrical umbilical cable to a recording unit on the survey vessel. Still photographs may either be remotely triggered at locations of interest (as identified on the video camera) or taken at regular intervals. Using a fixed frame of view (determined according to distance above the seabed), the area covered by each image can be calculated and, coupled with information on the distance travelled, allows quantitative transect-type studies to be carried out.

General guidelines for the deployment of towed or piloted underwater camera systems (after Rees and Service, 1993) include the following.

- Underwater photographic systems should normally consist of at least one video camera and a high-quality stills camera.
- Where towed sledges are used, the field of view of each camera should be known from previous calibration while immersed in water.
- The distance travelled by the sledge should be known, from the use of the ship's electronic navigator (using appropriate offsets), transponders, or a meter wheel attached to the sledge.
- Towing should be at constant speed.
- For objective quantification of assemblage structure or population sizes along transects, still photographs should be taken at fixed intervals on either a distance or a time basis. These can be backed up by opportunistic shots of features of special interest identified on the video monitor.
- When using ROVs, the distance travelled, heading, height above seabed, and field of view must be recorded.

### 4.1 Camera sledge

The sledge design illustrated in Figure 4.1.1 is typical of a class of towed gear that has been widely used for subtidal surveys of the epibenthos and small-scale habitat features. It has proven to be robust over rough terrain and effective across soft sediments (see Shand and Priestley, 1999; Smith and Rumohr, 2005). It is generally towed at slow speeds (ca.  $0.5\text{--}1\text{ m s}^{-1}$ , or while drifting) from the stern of research vessels. In Figure 4.1.1, the sledge has a downwards-pointing 35 mm stills camera at the front and a forwards-looking video camera towards the rear. Service and Golding (2001) describe procedures for surveys using a towed sledge, and summarize a methodol-

ogy for generating species abundance data from video and still images (see also Section 5.2.1; Magorrian and Service, 1998; Smith and Rumohr, 2005).



Figure 4.1.1. Conventional towed camera sled for collecting still and video images of the seabed (source: Cefas, UK).

## 4.2 Drop-frame

The drop-frame and comparable devices provide photographic data over relatively small spatial scales but at high resolution (see e.g. Davies *et al.*, 2001; Smith and Rumohr, 2005). Three examples are given below.

### 4.2.1 Diver-operated, frame-mounted cameras

Diver-operated, frame-mounted cameras can provide high-resolution information on epibenthic communities. Especially when combined with fixed station locations, they can also provide a valuable long-term monitoring tool, as demonstrated by Lundalv (1976; 1985) for the epibenthos inhabiting rock surfaces in Swedish waters (see also Bullimore, 2001; Munro, 2005).

### 4.2.2 Campod

Campod (Figure 4.2.1) is a lightweight tripod supporting video and stills cameras (Milligan *et al.*, 1998; Gordon *et al.*, 2000). It was designed with an open profile and wide stance to minimize disturbance to the seabed. It is deployed while the research vessel is stationary or slowly drifting, and collects high-resolution imagery from a relatively small area of the seabed.



**Figure 4.2.1.** Campod being deployed near the Hibernia oil platform on the Grand Banks (source: MFO, Canada).

An illuminated colour video camera is mounted obliquely to provide a forwards-looking, wide-angle view while drifting over the seabed. A downwards-looking, high-resolution video camera is mounted on the central axis of the frame. The stills camera and two high-speed flashes are also mounted on the frame. The length of the legs is adjustable, and the photographic image covers about 0.2 m<sup>2</sup> when Campod is on the seabed. Because of its relatively light weight (340 kg), substantial wire angles can develop on deeper deployments so a transponder is routinely attached to provide accurate positioning on the seabed.

During deployment and retrieval, an operator on deck controls the winch. However, when the seabed is sighted on the monitor, control of the winch passes to a scientist in the laboratory. The usual procedure is to drift with Campod suspended just above the seabed, then to land on features of interest to obtain higher resolution video imagery and take still photos. In favourable conditions, it is possible to drift considerable distances (typically 500–1000 m). A video monitor is also placed on the ship's bridge to assist with ship handling. Video imagery and navigation data are recorded on digital tape. Because of the size (2 m in height) and complexity of Campod, a large research vessel is required.

Campod is an efficient tool for obtaining high-resolution video and photographic imagery of habitat type and epibenthic organisms. Applications have included studies of the impacts of drilling waste (Muschenheim and Milligan, 1996), hydraulic

clam dredging and otter trawling (Gilkinson *et al.*, 2003), mapping of benthic communities (Kostylev *et al.*, 2001), and the study of deep-water corals (MacIsaac *et al.*, 2001). Except for the small footprint when it lands, Campod is non-destructive so that time-series observations can be made at a given location. It has also been used to carry other equipment, such as optical backscatter sensors, a silhouette camera, and a water sampler (Milligan *et al.*, 1998). Campod can be used over any kind of seabed regardless of relief, including steep walls of submarine canyons.

Video transects positioned to cover different habitats are useful for mapping large areas (on scales of 1–10 000 km) and large organisms (>5 cm). Typically, a transect can be 500–2000 m. It is important to have good positioning systems for georeferencing of video observations, especially for deep-water surveys. Campod has proven to be very useful for this (Mortensen and Buhl-Mortensen, 2004).

#### 4.2.3 Circular drop-camera frame

The circular drop-camera frame (Figure 4.2.2) is a robust platform for investigating locations of special interest, such as may be identified from acoustic surveys of the seabed. It can also be used during pilot surveys of new areas, especially where there is uncertainty about the nature of bottom substrata, e.g. the occurrence of rock outcrops. The video and stills cameras, lights, and flash unit are housed within the protective metal frame, and are orientated to collect images of the seabed directly below. Holt and Sanderson (2001) describe procedures for conducting video surveys of the epibiota using a comparable drop-down device (see also Brown and Coggan, 2007).



Figure 4.2.2. A drop-camera frame for collecting still and video images of the seabed (source: Cefas, UK).

### 4.3 Towed bodies

For benthic studies, towed bodies are typically deployed a few metres above the seabed, with altitude controlled by shipboard winch or by fins on the platform. Towing speed is usually about  $1 \text{ m s}^{-1}$ .

The most commonly used sensor is a colour video camera. This is generally angled to look ahead and scan a path of seabed to a width of about 1 m (depending on lens and altitude). It is preferable to have the video signal transmitted to the ship by conductor cable so that the imagery can be viewed in real time. A stills camera can also be mounted on a towed body to take pictures at regular intervals or on command from the surface. It is also possible to mount a sidescan sonar (SSS) fish for high-resolution SSS surveys.

Towed bodies can collect images over large areas in a relatively short time and therefore are appropriate tools for conducting general reconnaissance surveys. Imagery can discern major habitat features, such as substratum type and bedforms, and associated epibenthic species. No data processing is necessary in the field.

Towed bodies of this type can only be used to collect seabed images and cannot be used to collect physical samples. They also provide data of low resolution: organisms less than approximately 10 cm in diameter are difficult or impossible to discern. However, they are non-destructive so the same area of seabed can be surveyed repeatedly. Their use is limited to waters with low turbidity and to seabeds of relatively low slope and relief in order to avoid the risk of damaging/losing the gear.

As an example, the Towcam (Figure 4.3.1) is a towed vehicle which collects low-resolution colour video imagery of the seabed over a large area (i.e. transects many kilometres in length). It is towed at a constant altitude above the seabed (generally  $\sim 2 \text{ m}$ , which gives a field of view about 1 m wide) at a speed of  $\sim 1 \text{ m s}^{-1}$  (Gordon *et al.*, 2000).



Figure 4.3.1. Towcam fish (1999 version) being deployed off the stern (source: MFO, Canada).

The aluminium towfish is fitted with a colour video camera, a pair of halogen lights, and an acoustic altimeter. The effective maximum operating depth is about 200 m. All electrical power is supplied from the surface so there is no limit on tow duration. The towfish weighs 110 kg and is about 2 m long (including bridle). A transponder is attached to determine the exact location of imagery over the seabed. Altitude above the seabed is controlled by adjusting the amount of cable paid out, and video images are displayed in the laboratory and on the bridge to assist in ship handling. Video imagery and navigation data are recorded digitally.

Towcam has proved to be an efficient tool for conducting general reconnaissance surveys of substratum type and bedforms, fish, and large epibenthic organisms (greater than approximately 10 cm). Towcam is non-destructive and has the potential to carry other sensors, such as SSS and a digital stills camera. It can be used over any kind of seabed provided that the relief is relatively low and the water is not turbid.

#### **4.4 Remotely operated vehicles and manned submersibles**

Cameras can also be mounted on ROVs. These are self-propelled vehicles controlled by commands from the surface that are relayed down an umbilical cable, which also carries the video and other telemetry signals. The advantage of ROVs over towed vehicles is their greater manoeuvrability, allowing objects to be viewed from a variety of angles; they can also be stopped or moved back onto an object for further study. However, small ROVs are restricted by their limited capability to operate in current speeds in excess of 1.5 knots (i.e.  $\sim 0.75 \text{ m s}^{-1}$ ). The area covered by ROVs is generally restricted by the length of umbilical cable and water depth. They may be especially useful for examining the epibenthic communities of offshore hard substrata (e.g. Hardin *et al.*, 1994) or for non-destructive ground-truthing of high-relief features identified from acoustic surveys (see Annex 1). Characteristics of some of the larger (and more sophisticated) ROVs for deep-sea scientific applications are summarized in Smith and Rumohr (2005).

The use of an ROV is normally an expensive alternative to systems such as drop-frames (see Section 4.2) and is mainly useful for video recording at smaller spatial scales (100–1000 m). ROVs are particularly useful when more detailed information on abundance, size, and morphology of large organisms is needed. The ROV can be equipped with additional sampling gears (e.g. claw-and-suction samplers: see Buhl-Mortensen and Mortensen, 2004), depth sensor, compass, and two parallel laser beams. The laser beams can provide a scale for measuring the size of seabed structures and organisms. In areas with relatively high current speeds, the effect of drag on the cable may cause problems. The ROV should have a navigation system to facilitate accurate quantification of observations. The employment of ROVs (along with autonomous underwater vehicles (AUVs), manned submersible, acoustic and other survey methods) has been a feature of recent studies of the Mid-Atlantic Ridge under the Mar-Eco project ([www.mar-eco.no](http://www.mar-eco.no); see also Bergstad and Godø, 2003; Bergstad and Gebruk, 2008; Gebruk *et al.*, 2008).

A hybrid device in this class is the remotely operated towed vehicle (ROTV), whose depth and altitude are controlled by rotors. Such devices allow faster towing speeds and the possibility of midwater observations. However, the cost of the elaborate control systems required for these devices will tend to limit their use by smaller organizations.

Equipped with a variety of sensors and sampling capabilities, manned submersibles have found scientific applications mainly in the deep sea, with familiar and at times

spectacular outcomes arising from benthic studies. By their nature and size, they generally have a limited role in epibenthic surveys in shallower waters; a brief review of the capabilities of some of the larger devices is provided by Smith and Rumohr (2005). Masuda and Stone (2003) provide a recent example of the use of a manned submersible to assess deep-water scallop populations in fished and unfished areas.

## 5 Sample processing and data analysis

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### 5.1 Field processing

The following account is adapted from Cooper and Boyd (2002) and Rumohr (in prep.), and relates to the processing of epibenthic samples collected using towed gear.

On retrieval and transfer of the sample to a container, an estimate of the total volume of the catch should be made, along with a summary and photographic record of the contents, noting especially the presence of stones, rock, etc. It is essential that all organisms are removed from the net or bag, which should be thoroughly washed down on deck after every deployment. To avoid the risk of cross-contamination of samples, final reassurance may be achieved by a short tow at the sea surface with an open codend.

For most routine surveys employing towed gear, it is recommended that samples are processed over a frame-supported 5 mm mesh sieve, or a sieve of at least the same minimum mesh size as that of the sampling device. Any material passing through a 5 mm mesh sieve may be examined qualitatively (see Section 2.1) or discarded, depending on the objectives of the study. Callaway *et al.* (2002a) discussed the merits of 5 or 10 mm mesh sizes for processing 2 m beam trawl samples, concluding that the former was preferable for wide-scale surveys of the North Sea.

If feasible, the sample contents should be identified and counted at sea. An appropriate range of taxonomic keys should be available, and waterproofed copies are strongly advised. A full census should be made of all the less common species. Densities of very abundant solitary species may be estimated from subsamples, e.g. by subdividing the catch on the sieve mesh or by examining a known volume or weight of material, counts from which can then be related to the total sample size. Typically, the subsampled count should represent at least 10% of the total numbers present in a haul. It is essential that records of any subsampling activity are made.

Algal and colonial animal species are generally recorded on a scale of relative abundance, such as the SACFOR scale (Super-abundant, Abundant, Common, Frequent, Occasional, Rare: Hiscock, 1996; see also [www.jncc.gov.uk](http://www.jncc.gov.uk)). Encrusting organisms, such as barnacles and serpulids, may also be evaluated in this way when present in very large numbers; alternatively, densities may be estimated from subsamples. The presence of any infaunal organisms arising from the fouling of soft sediment should also be noted, together with the occurrences of pelagic species. However, these additional records should be excluded from data compilations prior to statistical analyses.

At least one individual of each species encountered should be retained for inclusion in a reference collection, along with other specimens that cannot be reliably identified. Samples should be fixed in 4% formaldehyde solution in seawater. For sponges, it is preferable to preserve specimens directly in alcohol in order to protect the spicules, which are required for later identification. Each specimen container should be clearly labelled with the cruise and sample number, date, location, and gear type. Prior to preservation, there may be advantages in recording the weight or volume of a specimen, and in obtaining a photographic record in live condition, as this may aid later identification.

It is essential to compile electronic records of the results from on-board sample processing as soon as possible after completion, especially on extended sea trips. This

should avoid later difficulties in interpreting handwritten notes, especially where large numbers of taxa are encountered, where subsampling procedures have been

adopted for certain common taxa, or when a number of scientists are communicating information on species occurrences and counts to a single data recorder.

Estimates of the biomass (as wet blotted weight) of individual species or major groups can be made using motion-compensated, sea-going balances (see e.g. Jennings *et al.*, 2001b; Smith *et al.*, 2006). The procedure followed should be the same as that used for later laboratory analysis (see Section 5.2.2). Drawing from earlier experiences with infaunal Analytical Quality Control (AQC) exercises (see Section 7), the scope for interoperator and interlaboratory error in biomass determinations is likely to be high. Field and laboratory intercomparison exercises are therefore strongly recommended.

## **5.2 Laboratory processing**

### **5.2.1 Still/video images**

#### **5.2.1.1 Photographs**

Ongoing technological advances are such that post-processing image-analysis software can now achieve a higher resolution than that initially obtained at sea. As a result, it is possible routinely to resolve epibenthic species in photographs down to a scale of several millimetres. This is invaluable when the objects of interest are small taxa or juvenile stages, and represents a more versatile and efficient technique than previous methods used to enhance resolution, including stereomicroscopy and projection of magnified images onto a screen. It is difficult to be prescriptive about approaches to image analysis because of the range of options available, but it is essential to work to Standard Operating Procedures (SOPs), with regular updating as necessary. A summary of good practice in the use of imaging techniques is given in Annex 4. Approaches to processing still images are also summarized in Bullimore (2001), Service and Golding (2001), and Smith and Rumohr (2005).

#### **5.2.1.2 Video images**

For the purposes of identifying and enumerating the epibenthos over large spatial scales, a wide variety of video camera and platform systems are available. In general, post-processing of video images permits enumeration only of larger specimens of the epibenthos because of the distance and angle of the camera system from the seabed, combined with towing speeds. Most video surveys are conducted along transects of different distances. Often, these surveys can be viewed simultaneously with the recording of the video (i.e. in real time). However, quantitative analyses of the images, in order to extract the desired information, are generally performed later, (i.e. post-processing).

When larger, more dispersed taxa are being enumerated, the transects may be viewed in their entirety and total counts taken as the recording is played back. This is not possible for large taxa occurring at high densities (e.g. sand dollars), particularly when replicate transects are available. In other cases, if towing speed is relatively uniform, the transect may be divided into equidistant intervals based on time and counts made within each interval. An example of this approach is given by Mortensen and Buhl-Mortensen (2004). Sequence length will depend on habitat patchiness and community type(s). Good results have been made with sections of 10–50 m. When analysing sequences, the position is noted at the beginning. Coverage of substratum,

number of organisms identified, and other relevant information is recorded. The sequence is ended by again noting the position. This method makes it possible to estimate the densities of organisms and to relate them to a particular depth and substratum type.

Regardless of the method, there are limitations to resolution of the epibenthos in images that will be a function of the actual survey parameters (e.g. altitude of video camera above the seabed, towing speed, and variation in these two parameters), the type of video (e.g. digital signal for maximum quality), and the quality and types of monitors and recorders used for display. Post-processing of videos includes counts over continuously run segments of the recordings, as well as at pauses (either random or targeted) in the video. For the latter, static video images can be captured and imported into various PC-run, image-analysis systems for a variety of purposes. In all cases, the quality (i.e. resolution) of paused or captured video images is less than that afforded by high-quality photographs taken of the same area of seabed.

A summary of good practice in the use of imaging techniques is given in Annex 4 (see also Magorrian and Service, 1998; Smith and Rumohr, 2005).

### **5.2.2 Biological samples**

The following account (adapted from Cooper and Boyd, 2002) covers the laboratory processing of entire epibenthic samples, as well as of subsets of specimens retained from field samples, either for later validation of identifications or for resolution of taxonomic difficulties. In practice, most samples should be amenable to processing at sea, which has the advantage of reducing problems associated with the handling of large amounts of preserved material in the laboratory.

#### **5.2.2.1 Sorting of epibenthic samples**

The formalin fixative must be removed before sample processing. Because it is toxic and carcinogenic, this must be carried out under fume extraction while wearing disposable gloves and protective clothing. Specimens may then be transferred to labelled Petri dishes or sorting trays (depending on sample or specimen sizes) for identification and enumeration, typically with the aid of low- and high-power binocular microscopes.

If estimates of biomass are required, then it is advisable (in the short period before measurements are made) to maintain specimens in water rather than in an alcohol-based preservative. Once the entire sample has been processed, the sieve residue can be returned to the original container, formalin or alcohol added, and the sample stored until the quality control measures have been satisfactorily completed.

#### **5.2.2.2 Identification and enumeration**

All specimens of solitary taxa should be enumerated and identified to the lowest possible taxonomic level, usually species, using standard taxonomic keys. It is essential that competent personnel are employed in order to ensure accurate and consistent identification of specimens. The skills of personnel involved in species identification should be regularly assessed and updated through attendance at training workshops and participation in exercises designed to test proficiency (see Section 7).

Where appropriate, records should include reference to the occurrence of juveniles or adults of particular species; alternatively, measurements of specimen sizes may be made. Common species that are readily identifiable can be enumerated using digital counters. Colonial species (e.g. hydroids and bryozoans) may be recorded on a pres-

ence–absence basis or quantified according to the number of colonies or degree of substratum cover (see Section 5.1).

Nomenclature should conform to established inventories; for European waters, these are Howson and Picton (1997) and Costello *et al.* (2001, 2004). Both of these also serve as useful reference sources for taxonomic keys, and all references employed during identification should be documented. In cases where specimens cannot be assigned to species level owing to damage, the lowest definitive taxonomic level should be recorded. In some cases, it may be reasonable to denote uncertainty by a question mark before the second epithet for a species binomen (e.g. *Sabellaria ?spinulosa*) and before the generic name at genus level (e.g. *?Sabellaria*). In others, it may be necessary to express greater uncertainty, e.g. by using *sp.*, *spp.*, *sp. A*, *sp. B*, etc. as designators, rather than tentative specific names. These may be further qualified: e.g. “damaged”, “indeterminate”, or “juvenile” convey information to others on the reasons for uncertainty and the likelihood (if any) of further progress being made on expert re-examination. Occasionally, because of taxonomic uncertainties in the literature, dual assignments may be necessary (e.g. *Genus sp. x/sp. y*). Identified specimens of each species should be transferred to numbered containers of appropriate size (one per species) containing preservative.

#### 5.2.2.3 Biomass determination

For routine purposes, biomass estimates may be determined at sea as wet blotted weight (see Section 5.1). However, follow-up laboratory estimates may be necessary for a variety of reasons, e.g. for intercalibration against sea-going estimates for selected species to evaluate accuracy and precision, to derive length–flesh weight relationships for shelled organisms, and to weigh small specimens beyond the resolving power of sea-going balances. Specimens are initially placed upon absorbent paper and, once blotted dry, should be transferred to a weighing balance as soon as possible. The wet blotted weight should be recorded once equilibrium has been attained, or after a fixed time interval.

Specimens that tend to retain fluid in the body cavity following preservation (e.g. *Echinus*) should be punctured and drained before blotting. Where possible, faunal or plant fragments should be assigned to the appropriate species and included as part of the biomass estimates for those species; otherwise, they should be weighed separately and then allocated across appropriate taxonomic groups. According to the degree of accuracy required, relationships may be established between whole-organism weights and measurements of the size of component parts (e.g. body or appendage widths), from which biomass estimates may be derived.

Ideally, estimates of biomass should be provided for each identified species and reported, together with the estimated total biomass for each sample. Procedures are in principle the same as those for the infauna, including the employment of wet/dry/ash-free dry weight conversion factors (see e.g. Rumohr, in prep.). However, it may be necessary (and desirable) for individual laboratories to determine anew such relationships for several species. Full documentation, both of the methods employed (through Standard Operating Procedures (SOPs), see Section 7) and of the results obtained, will be essential to allow interoperator and interlaboratory evaluations of their dependability and hence the need for any future improvements.

#### 5.2.2.4 Preservation and storage

After identification, enumeration, and estimation of biomass have been completed, specimens from each sample should be transferred to a single container, and a pre-

servative solution of 70% ethanol/industrial methylated spirits (IMS) applied. Sample containers should be fully labelled in accordance with SOP (see Section 7) and stored at least until all quality assurance needs have been fully addressed.

#### **5.2.2.5 Reference collections**

A separate reference collection should be catalogued and maintained in a curatorial manner for all epibenthic surveys. This involves the separate preservation of at least one individual of each species encountered. Specimens should be preserved in 70% ethanol/IMS and labelled with at least the following information: species name, station number, sample number (where replicates are taken), date of sampling, and name of the identifier. This collection can be used to validate identifications between samples and surveys.

#### **5.2.2.6 Sample tracking**

Collected samples constitute a valuable resource, both financially and in terms of the data they provide. Sample tracking, i.e. information concerning the location and status of samples at all stages following collection, is an essential part of any quality assurance programme (see Section 7).

### **5.3 Data analysis**

A typical dataset arising from a spatial survey of epibenthic communities will exceed 100 species. Numerous techniques can be employed to elucidate structure in the data, and a summary of those commonly applied to benthic datasets is given in Annex 5. Each of these techniques can be used to partly fulfil the objectives of data analysis. However, it is recommended that parallel application of a range of techniques will help both to differentiate patterns and to confirm real trends in the data. For a comprehensive review of statistical methods, reference should be made to general texts (e.g. Sokal and Rohlf, 1987; Green, 1979; Clarke and Warwick, 1994; Underwood, 1997; Magurran, 2004; Underwood and Chapman, 2005).

## 6 Characterizing the epibenthos: case studies

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This section summarizes approaches to characterizing the epibenthos and their habitats by reference to several case studies, and considers the effects of sampling efficiency, spatial scale, habitat complexity, and study effort. Particular attention is given to level-bottom surveys using towed gears. Multivariate statistical techniques are now routinely employed as aids to classify field survey data and to identify links between biotic and environmental variability. A review of methods is given in Annex 5. Mention is made of their use in case studies but the outcomes are not described in detail.

The term assemblage (rather than community) is used to describe a group of co-occurring epibenthic species in samples from trawls or dredges, thereby avoiding assumptions about the existence of interactions among them.<sup>2</sup>

### 6.1 Characterizing the habitat

The greater habitat complexity of high-relief (rocky) terrain accounts for the wider variety of communities found there, compared with elsewhere (see Annex 1). This is evident in the European Nature Information System (EUNIS) marine habitat classification (European Environment Agency, 2004) and also in the UK marine habitat/biotope classification of Connor *et al.* (2004). Variation in environmental influences (e.g. the degree of exposure to tidal currents and wave action) and biotic interactions add further layers to the classification. However, one clear advantage is that rocky substrata are generally invariable over modest time-scales, and the biological component of interest is exclusively surficial in nature.

For soft sediments, divisions used in the hierarchical classification of biotopes by Connor *et al.* (2004) are illustrated in Table 6.1. At this level, distinctions are expressed in terms of sediment types, depth zones, salinity, macrophyte dominance, and biogenic structures. These parallel the descriptors used by Jones (1950), Pérès (1961), and Glémarec (1973) to discriminate between benthic community types and habitats. Descriptions of individual biotopes (i.e. combining habitat and biological features: see also Olenin and Ducrotoy, 2006) within these categories are not exclusive to the epi- or endobenthos. However, Connor *et al.* (2004) recognized that the ability to integrate such information was constrained by both the nature and quantity of currently available data. Thus, in contrast to the highly resolved but small-scale habitat and faunal information generated from individual grab or core samples, data for the epibenthos from trawl or dredge tows are typically more poorly resolved and, in heterogeneous areas, may integrate across a range of habitat types (see below).

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<sup>2</sup> MacGinitie (1939), quoted in Thorson (1957), defined a biotic community as: "...an assemblage of animals or plants living in a common locality under similar conditions of environment and with some apparent association of activities and habits". For the epibiota of a sheltered rock face, there is a high probability that species occurrences are a product of close association, and hence constitute a community by this definition. For the epifauna of a level sandy bottom, the probability of significant interaction among sparsely represented species may be low, and hence only the first part of the definition safely applies.

Table 6.1. Sublittoral sediment types for classifying biotopes (from Connor *et al.* 2004).

SUBLITTORAL COARSE SEDIMENT	SUBLITTORAL SAND	SUBLITTORAL MUD	SUBLITTORAL MIXED SEDIMENT	SUBLITTORAL MACROPHYTE- DOMINATED SEDIMENT	SUBLITTORAL BIOGENIC REEFS
Sublittoral coarse sediment in variable salinity (estuaries)	Sublittoral sand in low or reduced salinity (lagoons)	Sublittoral mud in low or reduced salinity (lagoons)	Sublittoral mixed sediment in low or reduced salinity (lagoons)	Maerl beds	Sublittoral polychaete reefs
Infralittoral coarse sediment	Sublittoral sand in variable salinity (estuaries)	Sublittoral mud in variable salinity (estuaries)	Sublittoral mixed sediment in variable salinity (estuaries)	Kelp and seaweed communities on sublittoral sediment	Sublittoral mussel beds
Circalittoral coarse sediment	Infralittoral fine sand	Infralittoral sandy mud	Infralittoral mixed sediment	Sublittoral seagrass beds	Coral reefs
Offshore circalittoral coarse sediment	Infralittoral muddy sand	Infralittoral fine mud	Circalittoral mixed sediment	Angiosperm communities in brackish conditions	
	Circalittoral fine sand	Circalittoral sandy mud	Offshore circalittoral mixed sediment		
	Circalittoral muddy sand	Circalittoral fine mud			
	Offshore circalittoral sand	Offshore circalittoral mud			

Coarse or mixed sediments are rarely uniform in character over larger areas and may typically include gradients or small-scale patchiness in the proportions of different particle sizes, e.g. in areas supporting sand and gravel admixtures (Schneider *et al.*, 1987). The epibenthos will be responsive to such variability, but the presence of discrete assemblage types may be masked in samples collected by trawls and dredges. In energetic environments, appreciable changes in substratum type, and hence in faunal assemblages, may also occur over time, e.g. as a result of sand transport across coarse deposits or bedrock (Holme and Wilson, 1985). The difficulty of routinely accounting for variability in substratum type along trawl tows has been highlighted in several studies and may be resolved by the parallel use of acoustic and photographic imagery (see Sections 2–4).

## 6.2 Characterizing the epibenthos

Important considerations include:

- the diffuse boundaries that may exist between components of the benthic ecosystem. For example, Jones (1950) considered that there was no clear-cut division between the infauna and epifauna, especially of coarse deposits, but accepted that, in general, they were sufficiently distinct for the terms to have descriptive validity. Thorson (1957) highlighted contrasts in the environment inhabited by the infauna and sessile epifauna in support of the ecological distinction. Both recognized that demersal fish and motile invertebrates were components of benthic communities, although not necessarily bound closely to them. Biotope descriptions may combine information on the infauna and epifauna and, in principle, overcome such concerns.

- the adoption of sampling approaches that accommodate the larger size range and comparative rarity of many epibenthic species. Destructive sampling using towed gear has become established practice for many off-shore level-bottom environments, but usually at the expense of resolving power (cf. infaunal grab/core samplers).

A review of recent publications on trawl surveys of the epibenthos conducted over relatively large geographical scales (see Annex 6) identified variability in gear design, deployment practices, and methods of sample and data processing. Some involved the analysis of the epibenthic bycatch arising from established fish-stock assessment surveys and made best use of opportunities to generate data relevant to those surveys, as well as having wider ecological interest. Others were conducted independently of such effort. All were “fit for purpose” and insightful, particularly when covering newly sampled areas, but they are clearly not all suited to combined analyses of the data (at least not without considerable caution). Some studies routinely excluded occurrences of infaunal or pelagic species, while others did not.

For epibenthic bycatch surveys, there may be some scope for harmonizing approaches to sample processing across studies, thereby widening the utility of the data. For separate (or parallel) epibenthic sampling, the use of a 2 m beam trawl together with standard procedures for deployment and sample processing in collaborative North Sea programmes sets an encouraging precedent for future work in the region and perhaps more widely. All sampling programmes employed multivariate techniques to aid in identifying assemblage types, which were then examined for any correlations with a range of potentially explanatory variables. In some cases, the apparent “looseness” of the association with substratum type may reflect the difficulty of accurately summarizing physical variability along tows.

Although it does not overcome sampling dependencies, adopting a consistent approach to characterizing the epibenthos might facilitate comparisons with similar studies elsewhere and with studies addressing other ecosystem elements. The partitioning of sampling effort for the epifauna, infauna, and demersal fish is generally a practical necessity in routine studies. However, there is increasing demand for combined characterizations to meet the needs of benthic ecosystem assessments, including reports on the status of biotopes and “essential fish habitat” (e.g. Kaiser *et al.*, 1999; Callaway *et al.*, 2002b; Connor *et al.*, 2004; Reiss and Rees, 2007).

Epibenthic assemblages may also be characterized using functional properties. Recent work has included an examination of trophic structure and other life-history/biological traits (Jennings *et al.*, 2001b, 2002; Lavaleye *et al.*, 2002; Bremner *et al.*, 2003, 2006), in addition to conventional determinations of assemblage biomass. Again, such work has special value for assessments of ecosystem quality, trophic interactions, and inputs to ecosystem models.

### 6.3 Discussion

On larger scales, the distribution of epibenthic assemblages identified from trawl surveys frequently matches those of the macroinfauna and demersal fish, indicating similar responses to environmental changes (e.g. Callaway *et al.*, 2002b; Yeung and McConnaughey, 2006; Reiss and Rees, 2007). On smaller scales, patterns may diverge mainly as a result of contrasts in resolving power that result from differences in the size of sampling units, and hence the ground covered by, e.g. a grab vs. a trawl. For soft sediments, the relative rarity and larger size of several adult epibenthic species necessitates sampling over a considerably greater unit area than is feasible by a grab.

On coarser ground, characterized by the relatively common occurrence of sessile epibiota and smaller sedentary associates (in addition to larger and rarer taxa), the use of grabs, underwater photography, or diver observation can reveal the existence of appreciable small-scale variability in epibenthic assemblage structure comparable with that of the infauna (e.g. Holme and Wilson, 1985; Rees *et al.*, 2008).

Describing epibenthic assemblages in terms of the main characterizing taxa and their relationship to substratum type, depth, and/or relevant hydrographic factors seems to be generally followed. However, descriptions can be inconsistent between studies owing to differences in sampling and analytical practices. The framework for biotope descriptions in Connor *et al.* (2004) appears to be useful. With further refinement, it may be expected to lead to the addition of new biotopes as the effort committed to offshore surveys and their spatial resolution increases.<sup>3</sup> However, difficulties associated with “unsighted” sampling using towed gear across multiple habitats/biotopes, especially in heterogeneous areas, must be recognized. Agreement on a set of generic rules for describing epibenthic assemblage types would be an advantage both in allowing new survey work to be interpreted in a wider ecological context and for ease of communication.

For more accurate characterization of poorly studied offshore environments, the large geographical scales involved point to the benefits of high-resolution habitat mapping linked to biological ground-truthing. This should at least permit the broad dispositions of assemblage types to be predicted, and features of special interest to be identified, at manageable cost. Examples of such effort are given in Section 3.

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<sup>3</sup> Thorson (1957) notes that an ecologist “... may divide the material procured into smaller or greater units, but it is up to him [sic] to see that his division will help to simplify conditions for work, and not to complicate them”; see also the cautionary remarks of Connor *et al.* (2004).

## 7 Quality assurance of epibenthos studies

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A quality assurance (QA) strategy should be produced at the outset of an investigation and should encompass the objectives and design of programmes, as well as practical matters relating to their execution.<sup>4</sup> Thus, the adoption of consistent and reliable practices, both at the field sampling and laboratory analytical stages, will provide confidence in the validity of the output. However, they cannot make up for a sampling design that no longer serves its intended purpose. QA must therefore include regular re-evaluation of reported outcomes in relation to the original objectives of a study.

For benthic ecological studies, all-encompassing QA/AQC systems are still evolving, but the trend is towards increased involvement by individuals and institutes, especially those engaged in collaborative work requiring the synthesis of data from several sources. In the UK, an example of this trend is the establishment of a National Marine Biological AQC Scheme, which includes an epibenthic component ([www.nmbaqcs.org](http://www.nmbaqcs.org)). Although originally designed to service the needs of the UK National Marine Monitoring Programme, it has recently combined with a self-funding EU initiative (Biological Effects Quality Assurance in Monitoring Programmes (BEQUALM); see [www.bequalm.org](http://www.bequalm.org)) and currently offers Europe-wide participation. As part of the BEQUALM project, a training CD-ROM on benthic sampling methods was produced (accessible at [www.asa-multimedia.de/benthos](http://www.asa-multimedia.de/benthos); see also Rumohr, in prep.). Other European examples of QA/AQC schemes include the quality assurance panel of the German Marine Monitoring Programme (GMMP) of the North and Baltic seas. Approaches to the QA of biological measurements in the Baltic Sea are contained in the HELCOM COMBINE manual (accessible at [www.helcom.fi](http://www.helcom.fi)). Efforts to enhance the quality of biological data generated in support of habitat mapping programmes have been made under the EU MESH project (Mapping European Seabed Habitats; see e.g. Curtis and Coggan, 2006). Finally, the Identification Qualification (IdQ) scheme involves the certification of individual competence in species identification and is operated by the UK Natural History Museum ([www.nhm.ac.uk](http://www.nhm.ac.uk)).

Guidelines for the establishment of quality systems are given in Rees (2004), with the emphasis on marine biological studies. The degree of sophistication will clearly depend upon laboratory size, and it would be inappropriate to attempt to cover the needs of all recipients in the present document. However, one of the most important practical tools in a QA system is the Standard Operating Procedure (SOP). General guidance on the production of an SOP is given in Annex 7.

The commitment of adequate resources to the systematic recording and management of all information relevant to the interpretation of epibenthic surveys is essential. Thus, information on sampling and analytical practices, and other potential error sources (e.g. weather conditions at the time of sampling), is required in addition to biological records. Taxonomic naming conventions, coding systems, and accompanying information should conform to standards for the construction of a permanent electronic archive. These should meet agreed local/national needs and also ensure compatibility at an international level (e.g. with the Integrated Taxonomic Information System (ITIS): [www.itis.gov](http://www.itis.gov); the European Register of Marine Species, see

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<sup>4</sup> Quality assurance (QA) is the total management scheme required to ensure the consistent delivery of quality controlled information fit for a defined purpose. The scheme must take into account as many steps of the analytical chain as possible in order to determine the contribution of each step to the total variation. Analytical quality control (AQC) encompasses procedures that maintain the measurements within an acceptable level of accuracy and precision.

Costello *et al.*, 2001, 2004; and the Ocean Biogeographic Information System (OBIS): [www.iobis.org](http://www.iobis.org)), depending on the aims and scope of surveys. Vanden Berghe *et al.* (2007) report on a recent data management initiative encompassing collaborative macrofauna surveys of the North Sea under ICES auspices (see also Vanden Berghe *et al.*, in press).

## 8 Conclusions

An assessment of the performance of the gears outlined in Sections 2–4 against various operational criteria is given in Table 8.1. As expected, there is trade-off between resolution, spatial coverage, and the costs associated with sample/data processing. The most versatile gears tend to be the non-destructive visual or acoustic methods but, typically, their level of resolution is lower than the conventional (destructive) towed gears or grabs. A combination of these, i.e. seabed-imaging techniques accompanied by more limited biological ground-truth sampling, has proven to be useful in the mapping of habitats and assemblages at reasonable cost over relatively large areas (e.g. Kostylev *et al.*, 2001; Boyd *et al.*, 2006b).

**Table 8.1. Qualitative assessment of gear performance/utility against various operational criteria, conducted by members of the ICES Benthos Ecology Working Group. Key: \* low/small; \*\* medium; \*\*\* high/large.**

DEVICE	RESOLVING POWER (EPIBENTHOS)			VERSATILITY		EXPERTISE		COST	
	AREA COVERED	SPATIAL PATTERN	TEMPORAL TRENDS	HABITAT TYPES	VESSEL SIZE	DEPLOYMENT	SAMPLE/DATA PROCESSING	EQUIPMENT	SAMPLE/DATA PROCESSING
2 m beam trawl	**	**	**	**	**	*	**	**	*
5.5 m beam trawl	***	**	**	**	**	*	*	**	*
Agassiz trawl	**	**	**	**	**	*	**	**	*
R.-du-B. dredge	**	**	*	***	**	*	**	*	**
Anchor dredge	*	*	*	**	**	*	**	*	**
Scallop dredge	**	*	*	**	**	*	*	*	*
Rock dredge	**	*	*	**	**	*	**	*	**
Naturalists' dredge	**	**	*	**	**	*	**	*	**
Kiel. Kind. dredge	**	**	**	**	**	*	**	*	**
Triangular dredge	**	*	*	**	**	*	**	*	**
Triple-D dredge	**	***	**	*	***	*	**	**	**
Aquareve sled	**	***	**	**	***	**	**	**	**
Hyperbenthos sledge	**	**	**	**	**	**	***	**	***
Ockelmann sledge	**	**	**	*	*	*	***	*	***
Hamon grab	*	***	***	**	**	*	***	**	***
Videograb	*	***	***	**	***	**	***	***	***
Suction sampler	*	***	***	***	*	***	***	**	***
Quadrat	*	***	***	**	*	*	***	*	***
Drop-trap	*	***	***	*	*	*	***	*	***
SPI	*	***	**	*	**	**	***	***	*
Multibeam	***	*	*	***	**	***	***	***	**
AGDS	***	*	*	***	**	***	***	***	**
SSC	***	*	*	***	**	***	***	***	**
Diver observation	**	**	***	**	*	***	***	**	*
Camera sledge	**	**	*	**	***	**	**	**	*
Campod	**	**	**	***	***	***	**	***	*
Drop-camera	**	**	*	***	**	**	**	**	*
Towcam	***	*	*	***	***	***	**	***	*
ROV	**	**	**	***	***	***	**	***	*

The subtidal epibenthos is a rather loosely defined ecological entity, owing to the variety of the habitats encountered, the variable degree of association of organisms with those habitats, and the wide range in organism size and life-history traits. Thus, the epibenthos may comprise motile or attached species, and colonial or individual forms, and the maximum body size is (in principle) limited only by the dimensions of the sampling gear.

There has been speculation on the functional and evolutionary significance of benthic body-size distributions, especially for the endobenthos (Schwinghamer, 1981; Warwick, 1984; Warwick *et al.*, 1986). Observations have extended to the epibenthos, and the data have considerable interpretative value, including the facility to model trophic and other ecosystem interactions (e.g. Edgar, 1994; Gee and Warwick, 1994; Duplisea and Drgas, 1999; Jennings *et al.*, 2002; Warwick, 2007). Further work is necessary to determine whether epibenthic body size (or mesh selection) might serve as an aid to identifying subgroups of ecological relevance. Although the high morphological diversity of epibenthic organisms clearly presents technical challenges (see Section 1.1), such work may have an additional practical benefit in helping to distinguish between reliably and not so reliably sampled suites of taxa (see e.g. Callaway *et al.*, 2002a).

The nature of the data generated from many of the remote sampling approaches reviewed in this account is strongly gear-dependent. Caution is therefore required in ascribing ecological significance to differences in densities, size, or species composition determined from different studies unless they have been intercalibrated (see e.g. Rumohr and Kujawski, 2000; Callaway *et al.*, 2007). For towed gear, such as a beam trawl, sources of variability include the speed over ground relative to the escape reactions of motile organisms, the capability to dislodge sessile species, the constancy of bottom contact, and changes in the habitat along and between tows. This can make accurate quantification very difficult. For some surveys, there may be a case for identifying subsets of taxa with more dependable catch efficiencies. Paradoxically, the relative inefficiency of some towed gears also explains their versatility as sampling tools for wide-scale descriptive surveys.

In favourable conditions, observations by experienced divers are likely to provide the most accurate information on the *in situ* status of epibenthic communities. However, a central requirement of all surveys is consistency in sampling and analytical practices as a means of quality control. The outcomes, although method-dependent, can then provide a reliable way of identifying trends in response to natural and human influences (Lundalv, 1985; Maurer *et al.*, 1998; Rees *et al.*, 2001; Reiss and Kröncke, 2004; Smith *et al.*, 2006; Yeung and McConnaughey, 2006; Callaway *et al.*, 2007).

Accurate georeferencing of epibenthos samples is essential, not only to facilitate ground-truthing of acoustic survey data (Section 3.1; see also Brown and Coggan, 2007) and the revisiting of stations in monitoring programmes (Annex 2) but also to enhance the value of the historical data archive (Section 7). Approaches to minimizing positional errors for remotely deployed gears include attention to navigational accuracy on ship, calculation of offsets, especially for towed gear, and direct recording of sampler positions during deployment using transponders.

Activities to improve study practices and enhance knowledge of the epibenthos include:

- further innovations in sampler design; publication of gear efficiency studies (e.g. for trawl sampling: Creutzberg *et al.*, 1987; Kaiser *et al.*, 1994; Reiss *et al.*, 2006; see also Eleftheriou and Moore, 2005).

- more effort to standardize sampling/analytical practices for comparable environments (if not already done or if new approaches merit inclusion). International collaborative R&D and monitoring programmes have provided an important incentive (e.g. [www.mafcons.org](http://www.mafcons.org); [www.helcom.fi](http://www.helcom.fi); [www.ospar.org](http://www.ospar.org)) and the preparation and wider dissemination of standard operating procedures, e.g. through ICES, should be encouraged.
- more consistency in descriptions of assemblage types over larger geographical scales, as sampling methods allow.
- more effort to identify and predict relationships between the epibenthos and the physical environment, especially offshore (e.g. Kostylev *et al.*, 2001; Foster-Smith and Sotheran, 2003; Connor *et al.*, 2004; Hewitt *et al.*, 2004; Boyd *et al.*, 2006b), which should be helped by continuing advances in seabed imaging techniques and interpretational tools (Anderson *et al.*, 2008).
- further evaluation of size–weight relationships and benthic production, for environmental quality assessment and to explore functional interactions with other ecosystem components (e.g. Cohen *et al.*, 2000; Jennings *et al.*, 2001a, 2002; Bourget *et al.*, 2003; Hinz *et al.*, 2004; Smith *et al.*, 2006; [www.mafcons.org](http://www.mafcons.org)).
- development of indicators using information on the life-history traits and sensitivities of epibenthic species to natural and human perturbations (see Kaiser, 1996; MacDonald *et al.*, 1996; Rogers *et al.*, 2001; Bremner *et al.*, 2003, 2006; Hiddink *et al.*, 2006; Hiscock and Tyler-Walters, 2006; de Juan *et al.*, 2007).

The availability of a wide range of techniques for studying the epibenthos reflects the diversity of the supporting marine habitats and the need for adaptability and innovation to meet the sampling challenge. This report has highlighted some of the more important sampling and interpretational issues, along with examples of good practice. It is hoped that it will contribute to enhancing the quality of existing survey and analytical effort, and provide a stimulus for further fundamental and applied studies.

## **Annex 1: Methods for studying hard-bottom substrata**

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### **A1.1 Introduction**

This report provides a brief account of the methods available for the study of marine benthic communities that occur on hard substrata, based on Connor (1995) and ICES (1996), with minor updating. Hard substrata are defined as habitats of bedrock, boulder, stable cobble and pebble, artificial substrata, and biogenic reefs (e.g. *Mytilus edulis* and *Modiolus modiolus* beds), on which epibiota communities develop, either in the littoral or sublittoral zone. This definition excludes sediment substrata that support infaunal communities (but may develop epibiota communities if sufficiently stable), including mobile shingle, which may have little in the way of associated biota. Habitats of mixed hard and soft substrata, which are difficult to sample for their infauna, might best be considered here.

In the history of the ICES Benthos Ecology Working Group, most attention has been directed towards studies of soft-bottom substrata, the predominant habitat over most offshore areas and in some coastal areas. In many countries, however, hard substrata form a major component of coastal areas and are consequently subject to many of the pressures of coastal activities, such as coastal defences, urban and industrial development, mineral extraction, coastal quarrying, fishing, chemical contamination, eutrophication, waste dumping, and oil pollution. Rocky habitats often hold much intrinsic appeal, both for their scenery and for the wealth of marine life associated with them.

Although the ecology of littoral rocky habitats is generally well studied, that for sublittoral rock, and particularly for offshore rock, is relatively poorly understood. Much remains to be learned about the basic nature and distribution of rocky communities which, compared with the communities of littoral and sublittoral sediments, undergo very little monitoring.

### **A1.2 The nature of rocky habitats**

The communities of rocky habitats exhibit a very high degree of heterogeneity, according to local conditions, particularly height up the shore (as a result of desiccation) or depth into the sublittoral (as a result of light attenuation, temperature, wave disturbance), exposure to wave action and currents, salinity, temperature, topography, geology, and the effects of suspended sediment or siltation. In addition, biological interactions of predation, competition, and chance recruitment play their role in community structure.

The effect of this is often to yield a great variety of communities in an area, often changing markedly over a few metres, compared with the much smaller range of communities on sediments, which typically cover larger expanses of shore or seabed. Such complexity in rocky habitats presents difficulties in both ecological monitoring and monitoring for human-induced change, and may in part explain why so little is undertaken.

On the shore and in the shallow, kelp-dominated infralittoral zone, one or several species may typically dominate community structure (numerically or spatially); however, in the deeper, animal-dominated circalittoral zone, communities tend to comprise a wide variety of species and present a much patchier community structure; this too can make effective monitoring difficult.

### A1.3 Sampling rocky habitats: general principles

As with sediment studies, the methods adopted need to be appropriate to the end requirements of the study. Consequently, the methods, equipment, and resources required for mapping habitats differ considerably from those for detailed description of habitats or for monitoring studies. The scale of the study, whether local, national, or international, also has a marked effect on the techniques employed and the level of detail appropriate, as does the focus of the study on species distribution and dynamics, community description, productivity, etc. It has not been possible in this brief review to consider all the potential types of hard-substrata sampling to suit every requirement. An attempt is made here to give general guidance about the types of method applicable to baseline resource surveys and monitoring because these are likely to be of most interest to ICES.

The general strategy for sampling should be similar for both littoral and sublittoral rocky habitats, although the specific methods adopted will differ according to the logistics of sampling in each zone.

Because rocky habitats, by definition, support epibiotic communities, they are visible to the eye. Therefore, sampling can typically be undertaken in a non-destructive *in situ* manner, rather than by the infaunal sampling of sediments, which requires removal of samples to the laboratory for analysis. However, destructive sampling may be appropriate for some studies.

Quantitative sampling is difficult because many species are colonial in nature (and thus cannot be counted), hard to count (e.g. stands of filamentous algae), or adhere as a crust over the rock (and so cannot be collected or counted). More effective assessment of quantity for such species is given by estimates of percentage cover. An integration of such percentage cover estimates, with a  $\log_{10}$ -based quantitative abundance scale for species that can be counted, is provided in Hiscock (1990). Fully quantitative sampling, with the removal of the entire sample from the rock to determine biomass, is seldom undertaken (e.g. Christie, 1980, 1985).

In the sublittoral, scuba diving allows detailed recording and sampling to be done, which is particularly important in the description of the community and monitoring. Use of cameras attached to remotely operated vehicles (ROVs) offers advantages over divers in terms of the greater depth ranges that can be covered and the extended time available underwater, and providing a permanent record of the site.

However, for species identification, remote video is able to pick up only the larger species at a site, amounting to only about 50% of the macrobenthic species present (R. H. F. Holt, pers. comm.). It is consequently unsuitable for detailed description of the habitat and for certain types of monitoring.

Monitoring for human-induced change requires previous knowledge of the nature of the community and its natural variability. Such basic information is lacking for the majority of rocky habitats, so the design of monitoring programmes is critical to ensure they effectively answer the aims of the study. Such monitoring should therefore include sufficient study to establish natural variation at the site or parallel monitoring of a reference site, which must be of a comparable nature.

## A1.4 Baseline resource studies

### Mapping of biotopes (i.e. habitats and their associated communities)

Rapid identification of the key biological features that define each community, combined with knowledge of the extent of the physical habitat, can be used to provide maps of the resource without recourse to detailed and time-consuming programmes for sampling of species. Such an approach requires a pre-established biotope classification, such as that developed for the British Isles (Connor *et al.*, 1995, 2004). For littoral habitats, Richards *et al.* (1995) have developed a technique using aerial photographs to define polygons of similar physical habitat, which are then ground-truthed by field surveyors. The data are fed into a geographical information system (GIS) in order to provide maps and allow for spatial analysis of the data. In the sublittoral, a similar approach can be achieved through acoustic survey of the seabed (e.g. by using the RoxAnn or other imaging systems) and ground-truthing with ROV cameras (e.g. Davies and Sotheran, 1995; Sotheran *et al.*, 1997; see also Davies *et al.*, 2001).

### Description of biotopes—main species only

In the sublittoral, ROVs can be used to give a general description of the community, although only the largest conspicuous species can be identified accurately (typically those at least 10 cm in size), accounting for only up to 50% of the macrobenthic species present. ROVs can be used to great depths and have fewer time restrictions compared with divers; they are often used when diving expertise is unavailable.

### Description of biotopes—all conspicuous species

*In situ* recording by experienced field ecologists in order to identify all conspicuous species present in a defined habitat can be achieved through search over a wide area of the habitat to ensure widely dispersed species are recorded. Diving techniques are used for the sublittoral zone. This approach was adopted for the Marine Nature Conservation Review, a major resource survey for the whole coast of Great Britain (Hiscock, 1996; see also Davies *et al.*, 2001). For more restricted surveys specific quadrat or transect approaches may be appropriate in order to provide more quantitative data for some studies.

## A1.5 Monitoring studies

Monitoring requires repeat surveys of the same location at set time intervals. Marking of such sample sites is important to ensure return to the exact location because of the marked spatial variation in community structure over short distances. Epibiota communities lend themselves to photographic monitoring techniques as well as monitoring by *in situ* recording. Stereo photography techniques for sublittoral rocky monitoring have been developed by Lundalv (1971, 1976) and have been used for many years in Sweden. Recent advances in computer-aided image analysis allow direct comparison of photographic images with time (e.g. Fowler and Pilley, 1992, for monitoring growth of individual specimens of slow-growing sponges and seafans). Other publications relevant to monitoring hard-bottom communities include Crapp (1971), Lewis (1976), Jones *et al.* (1980), Hawkins and Hartnoll (1983), Christie (1985), Costelloe *et al.* (1986), Hiscock (1987), Sullivan and Chiappone (1993), Scott (1994), and Davies *et al.* (2001).

### Point source monitoring

Transects away from the source of contamination, with sampling at regular intervals along one or more transects, can be used but are subject to difficulties in interpretation. Heterogeneity of the rocky habitats is such that sample points along the transect are likely to be in different communities and hence not comparable; also samples within quadrats may not be representative of the wider area as a result of spatial variation.

### Long-term change (trend) monitoring—ecological or human-induced

Establishment of the baseline community structure and variability at the site under consideration is important. Sample points need to be spread out over the extent of the habitat being studied to ensure that adequate account of spatial variation is considered, rather than assuming one point to be representative of the habitat as a whole. When measuring human-induced change, a control or reference site is required for each test site. In this case, it is critical that like habitats are selected for comparison.

## A1.6 Parallels with sediment sampling

For consistency of approach it is important to use similar strategies for sampling both rock and sediment habitats. However, the differing nature of the two habitat types leads to marked differences in the techniques used, as does the logistics of sampling in littoral and sublittoral environments. For rocky habitats, there is generally more emphasis on *in situ* recording and photographic techniques. A broad comparison of approaches applicable to rock and sediment habitats is given in Table A1.1. This is not comprehensive as other techniques may be equally valid.

**Table A1.1. Summary of approaches to assessing rock and sediment habitats: baseline resource sampling and monitoring/surveillance.**

Approach	Littoral rock	Littoral sediment	Sublittoral rock	Sublittoral sediment
Mapping (habitat and biotope type)	Aerial photography	Aerial photography	Acoustic survey	Acoustic survey
	OS maps	OS maps	Video (ROV)	Towed video
	Rapid field survey	Field survey with <i>in situ</i> identification	ground-truthing	
Description of biotopes (main species only)	Rapid <i>in situ</i> recording	<i>In situ</i> identification	Rapid <i>in situ</i> recording by diver	Remote sampling (identification on boat)
			Video (ROV)	Diver observations of epibiota
Description of biotopes (all conspicuous species)	<i>In situ</i> recording	Coring (samples worked up in lab.)	<i>In situ</i> recording by diver	Remote sampling (samples worked up in lab.)
Monitoring / Surveillance	Fixed transects or positions	Replicate samples on grid	Fixed transects or positions	Replicate samples on grid
	Quadrats	Regular sampling programme	Quadrats	Regular sampling programme
	Photography		Photography	

## **Annex 2: Stages in the design and conduct of epibenthos surveys**

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This section provides guidance on the development of routine sampling programmes to assess the status of the epibenthos in relation to human activities. Descriptions of the various stages involved are adapted from Rees and Boyd (2002). Useful general sources of information concerning the evolution of sampling designs in benthic studies include Elliott (1971), Cohen (1977), Green (1979), Holme and McIntyre (1984), Andrew and Mapstone (1987), Skalski and Robson (1992), Underwood (1997), and Underwood and Chapman (2005).

### **A2.1 Desk study**

This is an essential precursor to all field sampling effort, and should allow the construction of a realistic hypothesis-testing framework to meet the overall objectives. The desk study should provide an early indication of the most suitable sampling gear(s), and of possible constraints on the amount or type of data in relation to the resources available to carry out the work (see Sections 2–4). This should lead to the drafting or adoption of standard operating procedures (SOPs) for sampling/analytical practices, as part of an overall quality assurance (QA) plan (Rees, 2004).

Prior knowledge of the sampling area may be gained from the published literature, geological maps, and hydrographic charts. Contacts with governmental and research agencies may reveal ongoing research and monitoring initiatives in the area of interest, including the existence of spatial data in geographical information systems (GIS) and archived oceanographic data. Access to the “grey” literature, and consultations with individuals who have local sampling experience, may provide useful background information, which will reduce uncertainties at the planning stage and hence increase the cost-effectiveness of sampling programmes.

### **A2.2 Survey planning**

Information gained during the desk study will aid decisions on the range of sampling equipment needed and, in turn, influence the size and capability of the survey vessels required for field sampling. Critical issues regarding the suitability and seaworthiness of chartered vessels, along with safe working practices for scientists (including divers) at sea, must be considered at this stage by competent and experienced individuals.

The choice of environmental variables for measurement alongside biological sampling will depend upon the survey objectives, and may include combinations of the following:

- substratum type (essential)
- depth below chart datum (essential)
- wind strength/direction (essential for towed gear)
- current speed
- wave action
- salinity
- temperature
- turbidity
- bottom oxygen concentrations
- water-column stratification

- sediment quality, including organic matter content and contaminant concentrations
- manifestations of other sources of human perturbations (e.g. the presence of trawl tracks or of dredged furrows from commercial sand and gravel extraction).

Information on variation in substratum types, scale, and depths of the area to be investigated will be especially important at the planning stage. For example, deepwater locations with hard substrata may create particular difficulties for quantitative sampling. Many deep-water species are rare and/or patchily distributed and will seldom be encountered by random photographing or other methods covering a small area.

Plans for scientific sampling may include the use of epibenthic trawls or dredges with mesh sizes below the minimum legal requirements specified for commercial fishing activity in the study area. In such cases, prior dispensation should be sought from the appropriate regulatory authorities.

When planning for surveys at locations where knowledge of the benthic habitat and the associated epibenthos is limited, but which are suspected to include very coarse or rocky substratum types, then allowance must be made for the possibility of damage or even loss of towed gear in pilot surveys. To allow for such eventualities, survey planning should include the provision of duplicated sampling equipment and/or an adequate supply of spares for on-board repairs.

### **A2.3 Pilot survey**

The need for such a survey will depend upon the availability of existing information for the area of interest. A well-studied location may provide all the information required for selecting suitable sampling tools and designing a baseline survey (see Section A2.4). In its absence, preliminary sampling over a wide area that encompasses the activity or feature of interest may be required, using a range of mechanical sampling devices, along with acoustic and visual methods for ground discrimination (see Sections 2–4). The ability of acoustic methods to provide topographical information on habitat types over large areas may be valuable in a number of respects. For example, it may identify biogenic structures, such as *Sabellaria* or *Lophelia* reefs, aid the selection of representative biological sampling stations, and determine the appropriate length and orientation of transects. Any deficiencies in local knowledge of water movements and their influence on particulate transport may be made good by the deployment of current and turbidity meters.

The adopted sampling design may be random, systematic, or stratified, depending upon the extent of prior knowledge of the area. It may also be selective, e.g. for confirmation of the presence of a particular habitat type. The options are similar to those available for subsequent baseline surveys (see Section A2.4). Thus, in terms of design, the two may differ only in the number of stations visited, if the pilot survey is successful in confirming prior inferences concerning the nature of the benthic environment.

For bottom sediments and the accompanying epibenthos, on-board or direct visual assessments of collected samples or images at a qualitative level will usually suffice at this stage. The purpose will be to

- establish the distribution of habitat types that may influence subsequent sampling design;

- determine the most effective sampling tools to meet the aims of future monitoring;
- provide a preliminary characterization of the nature of epibenthic assemblages.

Observations on assemblage types may influence later decisions on survey approaches, including the size and number of samples to be taken. In many areas, sufficient information may already exist on a larger scale, and pilot sampling may only be necessary to confirm that local conditions conform to the wider pattern. Such an investigation may be conducted immediately prior to a baseline survey in order to refine the sampling design or sampling practices, but it need not involve a separate sampling trip.

Accurate navigation is essential and, with the use of differential global positioning systems (dGPS) and acoustic tracking equipment, it should be possible to determine the location of platforms (both stationary and towed) to within 15 m or less. This will require determination of offsets in order to establish the precise location and orientation of sampling gear during deployment. It is also essential to simultaneously record other data, e.g. depth, ship speed, and heading, as a function of time (GMT), along with the geodetic parameters for position fixing, e.g. datum and projection. Such information will allow the plotting of stations/transects over other georeferenced datasets (e.g. multibeam bathymetry, sidescan sonar, or the output from electronic monitoring systems: see Section 3.1).

#### **A2.4 Baseline survey**

In an area of relative uniformity, this will typically take the form of a systematic grid of stations, although more complex and spatially extensive designs may be necessary according to local circumstances, e.g. to account for human activities such as waste disposal or demersal fishing, or features of conservation interest in the vicinity. A stratified random sampling design may be more appropriate where prior information (e.g. from desk study or pilot survey) reveals well-defined spatial partitioning of habitat types. Ideally, the same sampling methodology will be employed at all stations across level-bottom terrain. However, alternative methods may be necessary in some circumstances, e.g. in the presence of rock outcrops supporting distinctive epibenthic assemblages. Examples of sampling designs for trawl surveys are given in Annex 6.

As the aim is to elucidate and (as far as possible) quantify spatial patterns, a strategy involving the collection of single samples or observations over several stations is preferable to repetitive sampling at fewer stations. The latter approach is more appropriate for ongoing monitoring surveys at representative stations (Section A2.5), but selective sampling at this stage in anticipation of future need is likely to be cost-effective.

#### **A2.5 Ongoing survey**

The main aim of ongoing surveys is to monitor temporal trends. However, a spatial component is also essential. Stations may be located along a transect where effects are predicted to occur, principally along a well-defined gradient away from the human or natural influence of interest, or at representative locations within physically comparable zones. The choice of stations will be facilitated by the outcome of the baseline survey, and sampling to generate the first data points in an ongoing monitoring series may be feasible during this survey. As part of an overall quality-assurance strat-

egy, it will be important to check on their continued validity. This may be achieved by periodically repeating the baseline survey, at intervals appropriate to local circumstances, typically once every 3–5 years.

The number of stations will vary with the complexity of the physical habitat, the dispersive properties of the environment, and the proximity of other human influences. As a minimum, an ongoing sampling design will consist of one treatment station located within the predicted sphere of influence of the activity under investigation, accompanied by two reference stations, one just beyond the predicted sphere of influence and one at some distance away. This approach is comparable with the control/treatment pairing principle of Skalski and McKenzie (1982) and developments (by Underwood, 1992) of the Before/After Control/Impact (BACI) design of Stewart-Oaten *et al.* (1986).

The number of samples or observations to be collected at each station will reflect a balance between the statistical requirements of data analysis, the nature of the epibenthos, and any resource constraints. The choice of sampler(s) combined with habitat variability will strongly influence the capability to accurately quantify epibenthic assemblages (see Sections 2–4), and decisions regarding sample number are therefore likely to be site-specific. In general, a minimum of three replicates or observations should be obtained, either across a fixed point or randomly within a well-defined habitat type.

The timing and frequency of sampling will depend on many factors, including seasonality (see e.g. Reiss and Kröncke, 2004) and the perceived sensitivity of the environment. However, for contributions to monitoring programmes aimed at evaluating sea-wide quality status, surveys of the epibenthos will typically be carried out at the same time each year.

## **A2.6 Summary**

A summary of the overall strategy for the design and conduct of sampling programmes is given in Table A2.1.

**Table A2.1 Summary of strategy for the planning, design, and conduct of sampling programmes incorporating an epibenthic component.**

Desk study	Survey planning	Pilot survey	Baseline survey	Ongoing survey
<b>1 Determine</b> <ul style="list-style-type: none"> <li>testable hypotheses against study objectives</li> <li>survey needs and sampling gear</li> <li>QA strategy</li> </ul>	<b>1 Determine</b> <ul style="list-style-type: none"> <li>survey timing</li> <li>suitability/availability of charter vessel</li> <li>availability of sampling gear</li> </ul>	<b>1 Determine</b> <ul style="list-style-type: none"> <li>local hydrography</li> <li>suitable sampling gear</li> <li>substratum type (qualitative)</li> <li>epibenthos (qualitative)</li> <li>boundaries of survey area</li> </ul>	<b>1 Carry out</b> <ul style="list-style-type: none"> <li>quantitative spatial survey</li> <li>initial sampling at representative stations</li> <li>analysis/AQC of samples</li> </ul>	<b>1 Carry out</b> <ul style="list-style-type: none"> <li>sampling at representative stations over time</li> <li>analysis/AQC of samples</li> <li>hypothesis-testing for natural or human changes</li> </ul>
<b>2 Seek information on</b> <ul style="list-style-type: none"> <li>wave climate</li> <li>tidal/residual currents</li> <li>water-column stratification</li> <li>substratum type</li> <li>epi- and endo-benthic communities</li> <li>valued resources (e.g. fish/shellfish)</li> <li>human activities/impacts</li> </ul>	<b>2 Attend to issues of safety at sea and other operational matters</b>	<b>2 Evaluate findings</b>	<b>2 Analyse data/report and act on findings</b>	<b>2 Report and act on findings</b>
			<b>3 Refine hypothesis-testing framework as necessary</b>	<b>3 Review sampling design/frequency</b>
			<b>4 Repeat at intervals</b>	

### Annex 3: Area mapped per unit effort vs. resolution for different seabed survey techniques

Resolving power is indicated by \* = lower; \*\* = higher. Reproduced from Kenny *et al.* (2003).

SYSTEM	AREA MAPPED (KM <sup>2</sup> H <sup>-1</sup> )	Resolution (horizontal)								REMARKS
		1000 M	100 M	10 M	1 M	0.1 M	0.01 M	0.001 M	< 0.001 M	
Remote sensing, satellite	> 100		**	*						Restricted to operational coverage, mainly shallow seas
Remote sensing, aircraft	> 10	*	*	**	*					Generally restricted to depths <6 m
"Chirp" SSS	10		*	*	**					High-energy broad bandwidth pulse sonar
MBES	5		*	*	**	*				Allows the use of backscatter data to characterize substrata
SSS	3.5		*	*	*	**	*			Swathe width depends on frequency used
Synthetic aperture sonar	3.0					*	**	*		Optimal operation in range of 50–100 kHz
AGDS	1.5		*	*	**	*				Valid for normal (narrow) beam surface coverage only
High-resolution sub-bottom profiler	0.8	*	*	*	**	*				Narrow-beam subsurface coverage
Video camera	0.2				*	**	*			Allows mega-epibenthos identification
Benthic grab/core sampling	0.003					*	**	*		Quantitative data on macro- and meiofauna requires laboratory analysis
Sediment profile camera	< 0.001						*	**		Sediment/water interface inspections
X-ray photography	< 0.001						*	**	*	High-resolution geochemical and physical inspections (water content, density)

SSS = sidescan sonar

MBES = multibeam echo-sounding system

AGDS = acoustic ground discrimination system

## **Annex 4: Good practice in the use of imaging techniques**

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Reproduced from Rees (2004).

### **A4.1 General comments**

No binding procedures have so far been identified for quality control/quality assurance (QA/QC) measures for imaging methods. Retrieval methods can be grouped as follows:

- sea-going and land-based activities
- satellite and airborne imagery
- evaluation and processing of images, videos, and sidescan sonar records
- storage and retrieval of image documents.

Important considerations in relation to diver-based retrieval include parallel checks among several divers; photo and video documentation; double recording of pro-files/transects; need for specific scientific diver training and certification; strict safety rules.

### **A4.2 Best-practice guidelines**

#### **A4.2.1 Sea-going and land-based procedures**

- Sea-going and land-based procedures should follow well-documented standard operating procedures (SOPs). In addition, a precise time log (i.e. time base corrector (TBC) or data input on screen) is a prerequisite for proper evaluation and identification of photographic and video images. Marks on videos and photographs, and in the written log, can help in identifying and tracing back the image documents. However, it should be noted that, for certain purposes, high-quality pictures are needed for publication *without* the on-screen information.
- All technical details of cameras, films, tapes, camera settings, angles, distances, lightings, and parallel measurements must be recorded in writing.
- Films, tapes, and sonar records must be labelled and stored safely (in waterproof conditions).
- Underwater pressure housings should be equipped with hydrophilic drying agents (silica-gel pellets in sacks) to provide proper functioning of cameras.
- Greatest care should be given to O-ring sealings to avoid flooding of pressure housings.
- All safety instructions for diving, safety on board ships, and underwater electricity should be strictly followed.

#### **A4.2.2 Satellite and airborne images**

For satellite and airborne images the same rules apply in principle as for other imaging methods. In particular, it is important to document fully all parameters used in the registration and georectification to a coordinate system.

#### **A4.2.3 Images and recordings**

- Images and recordings should be evaluated following well-documented and repeatable methods conforming to standards of the highest objectivity.

- All steps involved in the processing of the original image (e.g. colour enhancement) must be documented and stated in documents and figure legends. Geodetic parameters must be recorded.
- Images and videos should be stored in suitably labelled magazines so that they can be retrieved by other individuals at a later date.
- Back-ups must be stored in other buildings as video copies or CD-ROMs of photograph collections, or stored on PC drives. Future storage media may include DVD drives.
- Large collections of images should be stored in image databanks in digital form in order to avoid misidentification and loss of images. The use of keywords, providing information about the subject, platform, format, position depth, remarks, etc., is strongly recommended.
- Attention should be given to the possibility that video tapes may lose their magnetic information after >15 years and CD-ROMs after >10 years; thus backups are advised every five years.

This also applies to old film and photographic material that is of documentary and historical value.

## **Annex 5: Approaches to the analysis of data from epibenthos surveys**

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### **A5.1 Objectives of data analysis**

The objectives of the analysis of data arising from surveys of epibenthic communities are comparable with those for the infauna (see Schratzberger and Boyd, 2002, from which much of the following account is derived) and include:

- the identification of spatial patterns in the assemblage(s) and their relationship with environmental information, including the spatial extent of any human influences under investigation (baseline/exploratory data);
- the identification of temporal trends and their relationship with human or natural influences (ongoing monitoring data).

Several statistical techniques described in the following account are included in the PRIMER (Plymouth Routines in Multivariate Ecological Research) software package (Clarke and Gorley, 2001). Details are also provided of CANOCO (CANOnical Community Ordination), including canonical correspondence analysis (Jongman *et al.*, 1987), and of TWINSpan (Two-Way INdicator SPecies ANalysis; Hill, 1979) for multivariate analysis (see Section A5.5), and many others exist. As long as the analyses of data arising from epibenthic surveys are based on sound statistical principles and employ techniques most suited to the individual datasets, the actual software package employed is irrelevant. It should also be noted that both novel statistical approaches for the analysis of biological data and new statistical software packages are continually emerging.

In addition to biological data, analysis will generally include information on sediment type and a range of hydrographic variables. Many of the approaches described below can be applied equally to other determinants, e.g. biomass estimates. However, non-parametric statistical methods may be more appropriate than parametric methods when analysing count data.

### **A5.2 Initial data processing**

Stages in initial data processing are summarized by Clarke and Green (1988). First, the data must be collated and classified using established coding systems (see Section A5.7). A species-sample matrix is then created, which should include both quantitative and presence-absence data. In addition, a matrix of the biomass of individual taxa by sample (typically expressed as ash-free dry weight) and, finally, a matrix of the corresponding environmental data should be prepared.

Once data are collated, any transformations (see Section A5.5) or exclusions of rare species can be made, the rationale for which must be clearly stated. Similarly, presence-absence records will be removed before analysis of quantitative data.

Statistical methods for describing assemblage structure include:

- univariate methods
- distributional techniques
- multivariate methods.

For each of these classes, statistical tests have been developed to determine the significance of differences between replicated samples.

### A5.3 Univariate methods

Diversity measures take into account two factors: (i) species richness (number of species), and (ii) species evenness (the apportioning of individuals among the species). Typically, indices that require no assumptions to be made about the underlying species abundance distributions are used. There are two categories of distribution-free indices (Magurran, 1988):

- 1 ) information theory indices (e.g. Shannon–Wiener index  $H'$ );
- 2 ) dominance indices (e.g. Pielou evenness index  $J'$ ).

More information about the structure of assemblages and their change in response to human or natural events can be obtained by the use of a variety of different univariate measures, including total number of individuals, total number of species, diversity (Shannon–Wiener index  $H'$ ), dominance (Simpson index  $C$ ), species richness (Margalef  $d$ ), and evenness (Pielou  $J'$ ). In general, such measures tend to be highly correlated and therefore there is limited value in calculating a large number of indices, as many will show similar trends in the data. Those indices that depend less on sample size (see Table A5.1) may be more appropriate for data arising from coarse substrata.

**Table A5.1 Summary of the performances and characteristics of diversity statistics (modified from Magurran, 1988).**

Index	Discriminant ability	Sensitivity to sample size	Predominant diversity element
$H'$	Moderate	Moderate	Richness
$C$	Moderate	Low	Dominance
$d$	Good	High	Richness
$J'$	Poor	Moderate	Evenness

There are indices that reflect the species-richness element of diversity and measures that express the degree of evenness in the data. The number of species detected in a sample usually changes much more in relation to sample size or sampling intensity than the distribution of relative abundances (Huston, 1996). Therefore, indices in the first category are generally better at discriminating between samples, but are more affected by sample size than the evenness of diversity measures.

Taxonomic Distinctness indices (Clarke and Warwick, 1998; see also Clarke and Gorley, 2001) express the “relatedness” of the species in each sample. They are generally less dependent on sample size than conventional diversity measures, allowing results to be compared across studies with differing and uncontrolled degrees of sampling effort. These indices are currently being more widely tested, and further refinements may be expected (e.g. Hall and Greenstreet, 1998; Clarke and Warwick, 1999; Rogers *et al.*, 1999; Bates *et al.*, 2005; Ellingsen *et al.*, 2005; Bhat and Magurran, 2006; Callaway *et al.*, 2007).

Biotic indices are derived from the known or perceived sensitivities of species to human influences, especially the effects of contaminants and organic enrichment (e.g. Hily, 1984; Majeed, 1987; Dauer, 1993; Grall and Glémarec, 1997; Weisberg *et al.*, 1997; Borja *et al.*, 2000; see also [www.azti.es](http://www.azti.es)). Although aimed principally at evaluating in-faunal benthic communities, they clearly have the potential for future adaptation to epibenthic studies, and such work is in progress (A. Borja, pers. comm.).

### A5.3.1 Analysis of variance

Testing of the significance of differences in replicated univariate measures between treatments by analysis of variance (ANOVA) is carried out on the assumption that

- the data follow a normal distribution;
- the variance of the sample is independent of the mean;
- the components of the variance are additive.

In general, the variance and mean tend to increase together, and therefore the second condition is never fulfilled. Transformations are an essential procedure before the application of most methods associated with the normal distribution (Elliott, 1971).

Multiple comparisons tests can be performed to identify assemblages that are significantly different at  $p < 0.05$ .

### A5.4 Distributional techniques

Diversity profiles can be visualized by plotting  $k$ -dominance curves (Lambshead *et al.*, 1983). Species are ranked in decreasing order of dominance along the  $x$ -axis and the percentage cumulative abundance ( $k$  dominance) is then plotted against the species rank  $k$  (Platt *et al.*, 1984). The purpose of such curves is to extract information on the dominance pattern within a sample without reducing the information to a single summary statistic, such as a diversity index. Sets of macrobenthic species counts and biomass can be jointly summarized in abundance and biomass  $k$ -dominance curves applying the ABC (abundance biomass comparison) procedure (Warwick, 1986). Grounded in disturbance theory, both procedures have been validated using data from soft-sediment infaunal communities, but may be adapted to account for changes in the epibenthos.

Body size (biomass) spectra have been used to examine the effects of bottom trawling and aggregate extraction on the subtidal epifauna (Jennings *et al.*, 2001a; Smith *et al.*, 2006). Species weights were allocated to  $\log_2$  size classes and normalized (class interval biomass  $\div$  class interval width) so that intersite relationships could be explored.

### A5.5 Multivariate methods

Multivariate techniques identify patterns in the data, which may aid in establishing causal influences. For example, ANOVA can be used to determine which of a range of environmental variables are significantly different between station groups identified from multivariate analysis of the epibenthic data. Further insights into the causative factors may be gained through computing correlations between environmental variables and univariate measures, such as the densities of selected species, diversity indices, or numbers and densities of all species at each station. More sophisticated techniques that employ multivariate approaches to linking biotic and environmental data may also be used (see e.g. Clarke and Warwick, 1994). One useful visual approach is to superimpose environmental data upon the output from station ordination or classification of biological data.

Field *et al.* (1982) describe a strategy for the multivariate analysis of marine biological survey data. Initial considerations include the following.

#### Data transformation

Untransformed data may have the undesirable property of accentuating the influence of very abundant species. Transformations (e.g.  $\log_{10}$ ) will have the effect of increasing equitability of the dataset. The square-root transformation has the advantage that,

when similarity is assessed by the Bray–Curtis measure (see below), the similarity coefficient is invariant to a scale change (i.e. it does not matter whether scores are expressed as  $\text{cm}^{-2}$  or  $\text{m}^{-2}$ ).

#### **Similarity measurement**

The overall similarity between each pair of samples is expressed, taking all the species into consideration. Many options are available, and an example of a commonly used index is the Bray–Curtis similarity measure (Bray and Curtis, 1957), which gives more weight to abundant species than to rare species. Because there will frequently be a requirement for the analyses of presence–absence data from epibenthic surveys, indices such as the Jaccard or Czekanowski coefficients will be appropriate (see Clarke and Gorley, 2001).

#### **A5.5.1 Hierarchical classification**

Hierarchical sorting strategies are used to produce a dendrogram from the similarity matrix. Again, many options are available, but one of the most commonly used methods in marine benthic studies is group-average sorting (Lance and Williams, 1967), which joins two groups of samples together at the average level of similarity between all members of one group and all members of the other.

Analysis of similarity (ANOSIM: Clarke, 1993) can be conducted to test for statistically significant differences in assemblage structure between station groups identified from the dendrogram output. Additionally, it is important to establish which species contribute to observed differences in the data. This can be achieved by ranking species in terms of abundance or by examining the degree to which species contribute to measures of similarity/dissimilarity between individual samples or sample groups (SIMilarity PERcentages (SIMPER): Clarke and Warwick, 1994).

TWINSPAN classifies species and samples to produce an ordered two-way table of their occurrence (Hill, 1979; a Windows version of the program is available at [www.canodraw.com/wintwins.htm](http://www.canodraw.com/wintwins.htm)). The process of classification is hierarchical; samples are successively divided into categories, and species are then divided into categories on the basis of the sample classification. Species demonstrating most difference in occurrence on the two sides (+ve and -ve) of each split are identified and termed Indicator Species. For examples of its application to benthic macrofaunal data see Künitz et al. (1992) and Rachor et al. (2007).

#### **A5.5.2 Non-parametric multidimensional-scaling ordination**

In non-parametric multidimensional-scaling (MDS) ordination, an ordination of the  $n$  samples is produced in a specified number of dimensions. The identity of each species is retained and used integrally with e.g. abundance data to compare assemblages (Austen and Warwick, 1989). The purpose of the MDS is to construct a configuration (“map”) of samples that attempts to satisfy all the conditions imposed by the underlying similarity matrix. The distances between pairs of samples in the resulting plot reflect their relative dissimilarity in species composition.

Initially, the samples are placed in two-dimensional space at entirely arbitrary locations; their relative positions are then gradually refined by an iterative analytical process. The intention is to move samples into positions in which the rank order of their distances from each other becomes ever closer to the rank order in the original similarity matrix. The extent to which the two disagree is reflected in the stress value (Clarke and Warwick, 1994). This coefficient indicates the degree to which the two-dimensional plot provides an acceptable summary of the multidimensional

sample relationships. Stress values of  $<0.05$  indicate an excellent representation with no prospect of misinterpretation, whereas MDS plots with stress values  $>0.3$  should be treated with caution because the points are close to being arbitrarily placed in the two-dimensional ordination space (Clarke and Warwick, 1994).

Analysis of similarity (ANOSIM: Clarke, 1993) can be conducted to test for statistically significant differences in epibenthic assemblage structure between station groups identified from the MDS plots. Additionally, it is important to establish which species contribute to observed differences in the data. This can be achieved by ranking species in terms of abundance or by examining the degree to which species contribute to measures of similarity/dissimilarity between individual samples or sample groups (SIMPER: Clarke and Warwick, 1994).

### **A5.5.3 Canonical correspondence analysis**

Canonical correspondence analysis (CCA) is a canonical (or constrained) ordination technique using multivariate direct gradient analysis to determine relationships between biotic and environmental data (ter Braak 1986, 1987, 1994, 1996). It builds on the method of weighted averaging of indicator species and the ordination method of reciprocal averaging (Hill, 1973, 1974). Ordination axes are constrained to be linear combinations of the environmental variables used in the analysis and provide a simultaneous ordination of species, sites, and environmental variables.

CCA is a reliable method of relating patterns in the biota to environmental factors, even when these are intercorrelated, by using forward selection of the environmental variables (Hill, 1991; Palmer, 1993). Monte Carlo permutation tests (Manly, 1991) are used to test the significance of each environmental variable. The variables can be further evaluated by examining intraset correlations (i.e. correlations between species axes and environmental variables). Detrended correspondence analysis (DCA), an unconstrained ordination technique, can be used to check whether the environmental variables are sufficient to account for the major patterns in the species variance. If the results of CCA and DCA do not correspond the environmental factors are irrelevant in explaining patterns in the biota.

CCA can be performed using the software package CANOCO, which contains both linear and unimodal methods and integrates ordination with regression and permutation methodology (see ter Braak and Smilauer, 1998). The ordination methods include weighted averaging, reciprocal averaging/(multiple) correspondence analysis, DCA, CCA, principal components analysis (PCA), and redundancy analysis (the canonical form of PCA). Permutation methods are included for time-series, line transect, and rectangular grids, and for repeated measurement design, e.g. the Before/After Control/Impact (BACI) design of Stewart-Oaten *et al.* (1986). A recent example of its application to benthic macrofaunal data from the North Sea in relation to the effects of commercial fishing activity can be found in Craeymeersch *et al.* (2007).

## **A5.6 Interpretation of the data**

For the final stage in the interpretation of the results, knowledge of the biology of the various species (e.g. feeding habits, environmental preferences, functional significance) is required to assess whether variables that are empirically related to the species distributions might be causative factors. Thus, it is possible to assess which environmental factors, either natural or resulting from anthropogenic perturbations, are affecting the benthic environment and to what degree. This information may then

be employed in a predictive manner to assess the likely consequences of any changes in these factors in a given area.

### **A5.7 Data management**

Standard procedures should be adopted for the recording and archiving of data, including all relevant information on field and laboratory practices. Species records should conform with established nomenclatural and coding systems (e.g. the Integrated Taxonomic Information System (ITIS): [www.itis.gov](http://www.itis.gov); the European Register of Marine Species: Costello *et al.*, 2001, 2004; Howson and Picton, 1997). It is essential to ensure that adequate resources are provided for database construction and long-term management. Further information is given in Section 7.

## **Annex 6: Approaches to epibenthos surveys using trawls: selected examples**

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### **A6.1 Eastern Bering Sea**

Yeung and McConnaughey (2006) examined the invertebrate bycatch from otter trawls used in annual groundfish surveys across a systematic grid of stations in the eastern Bering Sea. Trawls were fitted with a 3.2 cm mesh codend liner and towed for 30 min at 3 knots, and the contents were identified, quantified, and weighed. About 400 species were encountered across all surveys (1982–2002). A persistent feature was a division between inshore and offshore assemblages associated with a dynamic oceanographic front and a corresponding change in sediment type; the division was also evident in groundfish and infaunal survey data. Periodic reductions in the spatial extent of the inshore assemblage could be linked to higher than average mean bottom temperatures in the preceding summers, and may be explained by the effects on assemblage structure of the offshore migration of certain motile species to cooler waters. The biomass dominants were used in summary descriptions of assemblage types.

### **A6.2 Gulf of Carpentaria**

Long *et al.* (1995) sampled the megabenthos of the Gulf of Carpentaria (Australia) using a 3 m beam trawl with a 30 mm mesh net bag, towed for 15 min at 6 cm h<sup>-1</sup>. Wet-weight biomass was determined for each species, together with densities or colony numbers, and species were allocated to feeding types to aid interpretation. More than 840 species were identified from 107 stations located on a systematic grid. The data (including any infauna) were analysed using multivariate classification of presence–absence data, excluding species occurring at less than two stations. Two main communities were identified, corresponding to predominantly sandy or muddier substrata.

### **A6.3 Cantabrian Sea**

Serrano *et al.* (2006) surveyed the epibenthos at 22 stations in the Cantabrian Sea with a 3.5 m beam trawl and a 9 mm mesh net, towed for 15 min (following ground contact) at 2.5 knots, giving a mean swept-area of ca. 4000 m<sup>2</sup>. Sediment samples were collected with a boxcorer. Stations were allocated to three depth zones, spanning 30–400 m. In all, 241 species were identified, and the data were expressed as numbers per 1000 m<sup>2</sup>. Cluster analysis of log-transformed data identified three main assemblage types. Multivariate analyses also identified gradients in depth/water temperature and sediment characteristics as being significant influences on the distribution of assemblages, but correlations between univariate measures of the epibenthos and environmental factors were weak. Assemblage types were summarized according to the dominant species, depth, sediment type, and organic matter content of sediments.

### **A6.4 North Sea**

Jennings *et al.* (1999) used a heavy-duty 2 m beam trawl with a 4 mm mesh codend liner, towed for 5 min (from ground contact to the start of hauling) at 1 knot at 63 North Sea stations. (The device has since been used in two collaborative surveys of the North Sea epibenthos: see Callaway *et al.*, 2002b, and [www.mafcons.org](http://www.mafcons.org).) Stations were randomly allocated within ICES rectangles. Samples were processed over 10 and 5 mm mesh sieves. Species were identified and counted at sea or in the laboratory; colonial species were recorded on a presence–absence basis. In all, 334 species

were identified. Infaunal and fish species were excluded from multivariate data analysis, which was conducted separately for the attached (qualitative) and free-living (quantitative) component to identify any differences that might be attributable to the level of association with the seabed. Each identified three major groups, the dispositions of which were not identical but were consistent with a north–south division in the vicinity of the Dogger Bank. Influential environmental factors included depth, winter/summer temperature difference for both components and, for attached species only, winter temperature. Assemblages were summarized in terms of the species accounting mainly for the similarities within station groups, and the species accounting for most of the dissimilarity between groups.

### **A6.5 Northern North Sea**

Basford *et al.* (1989) sampled the northern North Sea epifauna with a 2 m Agassiz trawl (final mesh opening of 20 mm), towed for 15–20 min at 1–2 knots. In all, 196 taxa were identified from a systematic grid of 152 stations. From multivariate analyses of the species–abundance data with no exclusions (i.e. the data were not filtered for any infaunal species), they distinguished four major epifaunal groups and identified depth, particle size, sorting, and organic content of sediments as the most important explanatory variables.

### **A6.6 Southern North Sea**

Duineveld and van Noort (1990) sampled the epifauna of the southern North Sea with a 5.5 m beam trawl across a systematic grid of 58 stations, based on ICES rectangles. The trawl was fitted with a 10 mm stretched-mesh codend, and was towed for about 10 min at 3 knots. Fish were measured and weighed at sea, and the invertebrate catch was preserved for later laboratory analysis. Specimens were identified, counted and weighed (then converted to ash-free dry weight (AFDW)); all data were standardized to a surface area of 10 000 m<sup>2</sup>. Infaunal taxa were excluded and the species–abundance data were analysed using multivariate techniques, including and excluding demersal fish. Four main groups were identified, the most conspicuous change in species composition occurring along the northern edge of the Dogger Bank. Patterns were not closely related to sediment type or depth. Further discussion of the data appears in Duineveld *et al.* (1991).

### **A6.7 Southern North Sea, English Channel and western UK seas**

Rees *et al.* (1999) sampled 69 UK coastal and offshore stations using a 2 m beam trawl towed for 5–10 min (from ground contact to the start of hauling), at a speed of ca.

0.5 m s<sup>-1</sup>. Samples retained in a 3 mm mesh codend liner were sieved on a 5 mm mesh screen. The station grid combined stratified and random sampling schemes, and 414 taxa were identified at sea or during later laboratory analysis. From multivariate analyses of presence–absence data, the entire epifaunal component (i.e. after filtering out any infaunal or pelagic organisms) was classified into eight assemblage types, the distributions of which were explained by sediment type, coastal influences, depth, tidal current velocity, and temperature.

### **A6.8 Western UK seas**

Ellis *et al.* (2000) examined the epibenthic bycatch from a groundfish survey of the Irish Sea, St George's Channel, and Bristol Channel (UK), using a 4 m beam trawl with a 40 mm stretched-mesh codend, which was towed for 30 min at each of 101 stations. After removing fish and commercial shellfish, a representative subsample of

known weight from the remaining invertebrate catch was selected for the identification of individual taxa and for their enumeration and weighing, which were then raised to numbers/weight per hour fished. Rocks and broken shells in the subsample were also weighed as an expression of the nature of the seabed sampled. Using multivariate analyses, six assemblages were identified and described according to the taxa mainly responsible for the differences. The distribution of epibenthic assemblages was mainly accounted for by depth, surface water temperature, and substratum type. Distribution patterns were also similar to those for bottom sediments and the associated infauna determined from previous studies.

## **Annex 7: Standard operating procedures: general guidance**

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Standard operating procedures (SOPs) are an integral part of any quality assurance (QA) programme and help to ensure that data collected by a laboratory are scientifically valid, comparable, and adequate to meet the study objectives. The European Communities (1999) defines SOPs as "...documented procedures which describe how to perform tests or activities normally not specified in detail in study plans or test guidelines".

An absolute requirement that all laboratories carry out tasks in exactly the same way would be unrealistic because procedures are often legitimately tailored to local circumstances (e.g. vessel size). However, where approaches differ between laboratories, it is essential to establish that they have no adverse implications for the comparability of data. The following general guidance on the structure and content of an SOP is taken from Rees (2004).

A well-written SOP will help inexperienced members of staff in a laboratory quickly to develop expertise in a sampling or analytical area that is not only consistent with past practice at that laboratory but also compatible with established approaches elsewhere. For those seeking laboratory accreditation, the production of SOPs is an essential part of a wider QA package, but even for others, they provide an important means of fostering good internal practice. However, SOPs in themselves are clearly not guarantors of data quality.

SOPs should describe all steps performed in biological measurement. They should be established to cover the following areas of activity:

- station selection and location, navigational accuracy;
- handling, maintenance, and calibration of field and laboratory equipment;
- handling and use of chemicals (i.e. fixatives, preservatives, reagents) used in marine environmental surveys;
- collection of biological material;
- storage of biological material, including labelling and the checking of preservation status;
- distribution of biological material to external contractors/taxonomic specialists;
- analytical methods for biological material;
- identification of biological material, including taxonomic expertise of the personnel;
- recording of biological and environmental data; data management;
- analysis of biological and environmental data;
- QA of report writing and documentation, including signed protocols in all steps of analysis.

The preparation of SOPs to cover field and laboratory analytical activities is one of the most important practical steps that a laboratory/institute can take in seeking to improve the quality and consistency of its scientific products, and is therefore to be strongly recommended. Once this has been done, interlaboratory comparisons of SOPs may then provide a useful tool in identifying any remaining inconsistencies, and hence in promoting harmonization of methodology at a national and international level (see e.g. Cooper and Rees, 2002). Such periodic comparisons of SOPs are

also strongly recommended. An encouraging example of international effort to standardize approaches to sampling with a 2 m beam trawl is given in Section 2.1.1 (see also Zühlke *et al.*, 2001; Callaway *et al.*, 2002b). More detailed guidance on sample collection and processing is given in Sections 2–5, while advice on good practice in the use of imaging techniques is given in Annex 4. Such information provides a framework for the construction of SOPs to meet future project-specific needs.

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- No. 6 Control procedures: Good laboratory practice and quality assurance
- No. 7 Suspended particulate matter: Collection methods for gravimetric and trace metal analysis
- No. 8 Soft bottom macrofauna: Collection and treatment of samples
- No. 9 Sediments and suspended particulate matter: Total and partial methods of digestion (*videotape available*)
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