MARINE BIODIVERSITY MONITORING

Monitoring protocol for marine benthos: Intertidal and subtidal macrofauna

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Introduction

The world depends on self-sustaining biological systems that include many kinds of organisms. This requires the preservation of the variety of life, i.e. biological diversity, or biodiversity. Such efforts require inventory knowledge and an understanding of natural and artificial changes in biodiversity. Our knowledge of biological diversity is still very poor, with no more than 1/10 of the worlds species presently known (Langreth 1995). Similarly, we are only now beginning to detect and quantify changes to understand the nature, extent and ecological implications of changes in biodiversity. Thus efforts are needed in assessing taxonomic diversity and the processes that affect diversity.

Methods for the actual study of biodiversity in particular habitats are not well known and often differ so as to complicate comparisons of results. A Biodiversity Working Group has been established within the Ecological Monitoring and Assessment Network of Environment Canada to develop standardized biodiversity monitoring protocols for the marine environment.

Marine organisms can be conveniently categorized as benthic, nektonic, or planktonic according to the areas they customarily inhabit. Those that habitually live in or near the sea bed at any time during their life history constitute the benthos. Benthic organisms represent a major component within the marine environment. The benthos is normally divided into three functional groups, the infauna, epibenthos and hyper-benthos, those organisms living within the substratum, on the surface of the substratum and just above it respectively. This division also reflects differences in sampling techniques for the three groups. Sampling differences also result in the division of the benthos into two major habitat groups, the soft-bottom benthos and hard-bottom benthos. The benthos may also be subdivided into animal and plant components although the infauna, as its name suggests contains no plant species (some species of diatoms burrow temporarily into the sediment but are really epibenthic). In temperate regions the diversity, or

species richness, of the benthos in soft substrates on the continental shelf and slope may rival that in shallow tropical seas (Brusca and Brusca 1990). In comparison to the soft-bottom benthos, that on hard bottoms generally has both a higher abundance per unit area and a greater species diversity. One reason for this is that many benthic organisms support a second, diverse, community of epibiotic (living on the surface of other organisms) animals and plants. Even in temperate waters, intertidal and subtidal, hard-bottom benthic communities frequently colonise close to 100% of the area of the available substratum. Thus the benthos should play a major part in the strategy of biodiversity conservation. Studying the benthos is also useful in understanding changes in biological diversity. The use of benthos in aquatic ecological research, and particularly in evaluating marine pollution, is especially effective in assessing long term changes and detecting input from diffuse sources. The benthos reflects the effects of pollutants or organic enrichment by responding through detectable changes in population dynamics on a time scale of weeks to years. This is in contrast to plankton which shows a more immediate change to point sources with no long term consequences to the populations (Gray et al 1992). Impact from diffuse sources is also unlikely to be detected through analysis of plankton. Thus benthic assemblages are used because this biota consists of largely sessile organisms that must tolerate the pollution or die. Other advantages include the fact that many benthic organisms are resident year round, are naturally abundant and diverse and most are not fished or intentionally managed by man. Benthic monitoring is also a relatively sensitive, effective and reliable technique that can detect subtle changes that serve as an early indicator before more drastic environmental changes occur. Most other monitoring methods (e.g. video monitoring for bacterial mats and sediment parameters) generally detect the later, more drastic changes.

It is the intent of this report to produce recommendations or guidelines for sampling, sample processing and data analysis of marine benthos to establish a degree of uniformity in the procedures that will make data from different investigations more readily comparable. At the same time it is recognized that decisions on the methodology, equipment and analysis will depend on the particular aims of a study, on the nature of the habitat involved, on the staff and facilities available, and on historical or personal preferences. However, in the context of collaborative or regional and national biodiversity sampling programs the use of standard or common methodology is important if results from different institutions are to be linked and compared for an evaluation of large scale ecosystems. Presently consideration will be limited to the interand

subtidal zoobenthos macrofauna, comprising the burrowing fauna (infauna) and surface living fauna (epifauna) retained in a 0.5 mm mesh (McIntyre et al 1984), and excluding the smaller interstitial meiofauna (small metazoans), microfauna (protozoans and organisms of bacteria size) and the phytobenthos which all require special techniques.

Species are recognized as the essential baseline for understanding diversity. Thus the sampling and identification methods and procedures required to obtain reliable measures of species richness and diversity are emphasized here. However, many of the techniques are also suitable for simultaneous assessments of taxonomic and community diversity.

It must be recognised that sampling any of the benthos is an inefficient process, and

that because of this our knowledge of species diversity in this group of organisms is very poor. The degree of difficulty in sampling any of the benthos increases with depth. Thus the intertidal, which is directly accessible at low tide, is relatively well understood. The immediate subtidal, down to 30m or so can be sampled and observed by SCUBA equipped biologists but sampling efficiency declines rapidly with depth as working time, manual dexterity and visibility all decrease. From 30 to 100m or so the bottom can be observed using video cameras on ROV's, tethered underwater vehicles controlled from a ship, however, most ROV's are incapable of sampling. Submersibles can be used for observation and limited sampling at virtually any depth, but only at very high expense and on an infrequent basis.

Generally speaking, the soft-bottom, infaunal benthos can be sampled relatively well by retrieving quantitative samples of the sediment and sieving them to extract the fauna. Grabs and corers are the devices generally used for this and reviews of these, such as those of Holme (1964) and Holme and McIntyre (1971) show a bewildering variety. The former are almost always lowered from a boat while the latter may be either remotely or SCUBA-diver operated. Both corers and grabs have problems in penetrating sediment to a depth that will include all the biota. The degree of difficulty generally increases with the hardness and increased particle size of the sediment. However, even in soft, virtually homogeneous sediments, biota, stones or debris can prevent efficient sampling. Corers have an advantage over grabs in that they sample an equal volume of sediment regardless of depth of penetration into the sediment, while grabs almost always take a diminishing volume of sediment from deeper layers. Some grabs, such as the Smith-MacIntyre, do dig a relatively parallel-sided hole but can only penetrate relatively shallowly. Corers, however, except for large, unwieldy, box-corers are restricted to fairly small diameters due to the difficulty of retaining the sample. All remotely-operated sediment sampling devices, except when used in very clear shallow waters are "blind", the precise area where the sample will be taken cannot be selected and this effect increases with depth. While this has an advantage in ensuring that samples are taken at random, it prevents sampling of smaller communities or at a precise depth on a slope. There is also no way to prevent such a device hitting the same spot twice. In effect these difficulties mean that only general communities can be sampled at depth and that replication must be high. All remotely operated quantitative devices, too, are relatively small in terms of the area that they sample. Thus, they are inefficient devices for looking at diversity where individuals are widely spaced, a condition very common in this environment.

There are some, non-quantitative or semi-quantitative devices such as anchor-dredges which can sample larger areas, but they are hard to use and tend to damage delicate specimens.

Another difficulty facing those extracting organisms from sediment is the processing of the samples. Inevitably there is a large volume of sediment compared to that of the biota. Biota are usually extracted by passing the sediment through a sieve or series of sieves. This is difficult to do without damage to delicate organisms and is very time consuming. It also limits the minimum size of organism that can be retrieved since sieves finer than 0.5mm retain large volumes of sediment and detritus along with the organisms. Coarser sieves also retain a great deal of non-living material in many circumstances. This must be preserved with the biota and labouriously "picked" at a later date under a stereo-microscope.

In shallow water, some SCUBA diver operated devices such as suction dredges attempt to overcome the difficulty of small sample size, blind sampling and labourious sieving, in that they can be used within a quadrat placed in the precise community of interest, can dig deeply and are partially self-sifting. However, they require skilled operators and complex mechanical back-up and are relatively infrequently used.
Compared to the difficulty of sampling the infauna the epibiota of sedimentary bottoms is even more difficult to study efficiently except in shallow-water by SCUBA divers. Organisms on the sediment surface with a density similar to that of water, tend to get pushed away from descending grabs or corers by the "shock wave" that precedes them. Such organisms are usually sampled by means of an epibenthic sled, but there are difficulties in getting the sled not to dig into and clog in very soft bottoms and with general clogging where the amount of debris and/or detritus is high.
A further degree of difficulty is presented in attempting to efficiently sample the benthos of subtidal hard bottoms. Grabs and corers are useless in these cases and only the advent of SCUBA diving has allowed any reasonable amount of actual sampling to be carried out. Before this, communities could be photographed by remote methods but not efficiently sampled (Gulliksen and Deras 1975, Hiscock 1979, Jan, Dai and Chang 1994, Thomas 1994). Photography is still a widely used method for this group of organisms, but again SCUBA has allowed the use of more detailed and refined methods with adequate replication (Lundalv 1971, Toregard and Lundalv 1974, Rorslett *et al.* 1978, Svane 1988). Even for SCUBA divers, quantitative sampling is still inordinately difficult. The organisms of this group of benthos are mostly either firmly attached to the bottom or are epibiotic on other animals or algae. To obtain samples the diver must scrape all the organisms off the rock within a quadrat and somehow retain them all within a collecting bag or other device (Harmelin 1976). In the presence of current this is difficult. Even in still water, organisms with a density close to that of sea water drift away and are difficult to grasp as the current caused by hand movement deflects specimens unpredictably. Scraping into a suction device is one solution, but this requires a tender moored above. In the case of encrusting animals and plants, which are common in this environment, a portion of the surface rock must be chiselled off to obtain good specimens. Since hard bottom benthos are most prevalent in locations with moderate to high tidal currents, the conditions for sampling are rarely good. In many locations, a sampling "window" of less than one hour per tide, around the time of slack water, is all that is available. Because of these constraints quantitative sampling is most frequently done on a basis of percentage cover of the bottom rather than on a basis of biomass. Percent cover can be done from still or video camera images but is made difficult by the fact that the benthic community structure is highly three-dimensional and because organisms with large portions elevated on stalks or stipes frequently hide specimens beneath. From a point of view of species diversity, specimens are required for accurate identification in most cases, additionally there is a rather large lower size limit for identification from photographs. Nevertheless, photographs often reveal the presence of specimens which for some reason were not observed *in situ*.
Below depths accessible to SCUBA divers for reasonable time periods, sampling of this type of benthos becomes totally impractical on a quantitative basis and collection of specimens is limited to those that can be grasped with a mechanical device.

Detailed sampling methods will be discussed below.

Methods

General considerations

The ultimate objective of biodiversity monitoring is not only the primary step of determining what kind of organisms inhabit a particular area but also to detect any spatial or temporal change in the fauna in addition to that due to natural variability, and then to attribute the change to its cause. The community to be investigated should be selected for comparative studies, where a pristine reference site relatively free of manmade
influences is selected to evaluate natural diversity and variability, and compared to other more impacted sites established to monitor pollution or other anthropogenic input. In selecting sites, nuisance variables (Gray et al 1992) which complicate comparisons between sampling sites must be minimized to reduce variance in the results. Two of the most significant natural environmental variables that affect species composition of the benthic macrofauna are depth and sediment grain size. Thus sampling should take place at comparable depths and within a narrow range of grain size whenever possible. Proper site selection is therefore crucial. A pilot survey can be an important step in final site selection.

For marine benthic studies methods that use quadrats (simple square method) or transects, as used in terrestrial environments, are feasible for estuarine, intertidal and hard bottom areas (Štirn 1981). These methods are not feasible for soft bottom substrates.

The majority of subtidal marine habitats are within soft substrates. For these a site may be defined as an area with relatively homogeneous habitat from which adequate replicates may be taken. The size of the sampling area will depend on the size of natural limits of the area with a particular habitat and on the size and number of samples being taken. Typically a site or sampling grid may be defined as a 100X100 (Gray et al 1992) or 200X200m (Elliot 1971) area.

From a point of view of checking sub-tidal hard-bottom benthic communities over time, this habitat has some advantages over soft bottoms. Since transects can be used, the ends of transects can be permanently marked by tags attached to the rock (Hatfield et al 1992). A weighted transect line marked at frequent intervals can be laid between the end markers and the transect filmed on video, using the same method each time. Since the photographic sample size is large, and can be sub-sampled in many ways, the method is sensitive to relatively small changes.

Marine sampling operations other than in the intertidal zone invariably require the use of some type of vessel which will often influence survey procedures and choice of gear to a large extent. For example, operating in deep waters increases travel time and time to obtain samples, as compared to shallower near-shore surveys. The choice of gear also depends on the questions and resulting sampling strategies that drive a particular investigation. However, for benthos monitoring programs, there are two major types of objectives with an increase in effort required. The simplest objective aimed at knowing what types of animals are present in a given area, would require only qualitative sampling from different types of habitat. For this dredges are commonly used. If information is required on the relative abundance of species over time, or to estimate

the number or biomass per unit area, then quantitative sampling using devices such as grabs and corers are obligatory, and rigorous planning of a sampling program is necessary.

To design a sampling program for a given area all available bathymetric, geomorphological, sedimentological, oceanographic and biological data should be gathered (see Štirn 1981). In many cases it is useful to gather additional preliminary observations in the form of a pilot study to map out the extent of various types of habitat within the area. Depending on the habitat this may include qualitative dredging on soft bottoms, direct diving observations, and collecting on hard bottoms. The divers' information can be supplemented by underwater photographs or videos.

Once a relatively homogeneous habitat has been located, and the size of the sampling area is determined, a sampling strategy is required. Typically, benthic sampling stations are selected by means of stratified random sampling (e.g. Elmgren et al. 1984). The type of sediment is a major determining factor in the distribution of macrobenthos (Gray et al. 1992) and thus sampling in that case should be stratified for grain size. In order to sample with equal intensity on each type of bottom, samples have to be allocated proportionally according to the relative coverage of each sediment type (see Elliot 1971 for more details). These sampling stations should occur at comparable depths.

In biodiversity studies the number of replicate samples required to adequately sample species can be determined by plotting a species-area curve giving the cumulative number of species against the total number of samples taken. From the shape of the curve an estimate can be made of the number of replicates necessary to obtain an acceptable percentage of the total number of species present (Štirn et al. 1975). This point is at the transition from gradient slope to the asymtote level (Štirn 1981). In practice this means that for macrobenthic sampling on soft bottoms a minimum of 2-3 samples (Gray et al. 1992) should be taken. A minimum surface area of 0.3-1.0 m^2 (Štirn, 1981; Eleftheriou and Holme 1984; Longhurst, 1959; Boudouresque, 1974) should be sampled randomly at each station, requiring a minimum of 3-10 replicate samples with a 0.1 m^2 grab. Species that are scarce, with patchy distributions, or capable of escaping may still be missed, and may require other techniques such as visual diver observations or underwater photography. For hard-bottom benthos the same principles apply and species-area curves are most useful in determining the minimum effective sample size. However, there are more constraints on sample size than with soft bottom communities. To get adequate definition of small species, closeup photography of very small areas, typically 0.01m^2, is required. In this case 20 or more images may be needed for an adequate sample. On the other hand, taking a photograph needs only seconds, compared to minutes for grab samples, so the time involved for hard-bottoms may in fact be less.

The frequency of sampling depends on the objective and on the amount of information already available. In the absence of background knowledge an area under study should be sampled monthly over a year, or at least four times a year corresponding to the seasons, to ascertain seasonal changes to the assemblage. If seasonal changes are known and predictable changes from year to year can be determined by sampling once per year when the lowest abundances occur (mostly winter) (Gray et al. 1992). In the case of hard bottoms one difficulty is that, since sampling has been so limited in the past, our knowledge of natural temporal changes on seasonal or other bases, is very limited. Experience in the sub-tropics has demonstrated large temporal changes on a

non-seasonal basis in subtidal hard bottom benthos (Thomas *et al*. 1992). In temperate situations, more seasonal variation has been observed. If random community structure changes prove to be normal in these communities then long series of samples will be required to establish normal diversity patterns.
The major abiotic factors useful for benthic sampling are salinity, temperature, depth, current speed and direction, as well as sediment grain size.

Sampling

A. General

1. Sample preservation

Chemical fixation of specimens is necessary as soon as possible following collection, preferably after sieving in case of soft-bottom samples, to avoid degradation. Fixation in 4-10 % formalin (2-4% formaldehyde) seawater solution for 2 days is considered adequate (Gray et al. 1992; Eleftheriou and Holme 1984) when using a 3:1 liquid to sample volume ratio. To obtain a 5% formalin solution in a 1 litre jar, 50 ml of concentrated full strength formalin (37% formaldehyde) is added. A buffering agent, such as borax (1 tablespoon per litre or 20 ml of saturated solution), marble chips or hexamine (8g per l of 2% formalin solution), needs to be added to prevent specimen damage from acidification, such as dissolution of calcareous structures. Alcohol is an inadequate fixative (ICES 1994) and should not be used for initial field preservation. However, specimens should be preserved and stored in alcohol (70% ethanol or 50% isopropanol) after fixation. This prevents possible long-term damage to specimens with calcareous structures and also eliminates exposure to toxic (and perhaps carcinogenic) formalin fumes during subsequent sorting. The transfer from formalin into alcohol should include an intermediate water wash and should be performed under well ventilated conditions or with a waste air exhaust system such as a fume hood.

2. Sample labelling

It is essential that samples be properly labeled. Information on the labels should be sufficient to identify the sample with certainty (e.g. include cruise number, date, time, station designation, etc.). Labels, made of heavy weight and chemically resistant paper, should be filled out with a soft carbon pencil which will not fade in Formalin. Filled out labels are place inside the jar containing the specimen and the jar should also be labeled on the outside with a waterproof marker.

3. Sample staining

Samples containing small, inconspicuous specimens in a residue of detritus or other material may benefit from staining to enhance their color contrast. The most commonly used stain is Rose Bengal, 4g/l of 36% formaldehyde (Eleftheriou and Holme 1984) or 1g/dm3 of tap water and 5g of phenol for adjustments to pH 4-5 (Gray et al 1992). Staining in alcohol with Rose Bengal is not possible due to heavy leaching. It is recommended that samples are stained just prior to sorting, after specimens have been transferred from alcohol to water. This prevents leaching of the stain.

4. Taxonomy

Accurate identification of specimens is crucial for any analysis to the valid. Taxonomic competence of personnel must be ensured through training workshops and other regular meetings to verify uniformity of work. Quality assurance procedures must also be implemented to ensure the accuracy of identifications among all personnel (ICES 1994). This includes verification of identifications (5-10% of samples) and documentation (all samples) by a second research technician. Levels of variability must be set (e.g. 5% error rate), which when exceeded during verification, would result in resorting of any affected samples. Keys and guides used for identifications and the taxonomic resolution among the different groups need to be documented. A taxonomic reference collection should also be available for training and verification purposes. A checklist should be established, following a sample from initial sampling to final determination and quantification.

When identifying specimens there invariably will be cases when specimens cannot be identified to species due to damage or unresolved taxonomic problems. In case of doubtful identification the lowest reliable taxonomic level should be given. Following Hällfors and Niemi (1990) uncertainties in identification should be indicated by a question-mark before the second epithet for a species binomen (e.g. *Capitella* ?*capitata*), and before the generic name at the genus level (?*Capitella*). If there is only

one species within the genus, then this is indicated by "sp." following the genus (e.g. *Capitella* sp.), and if it is certain that more than one species is found then this is indicated by "spp." (*Capitella* spp.). Special collective groups may have to be designated when there are difficulties in separation; it is important that they are clearly defined. Marshall et al (1994) give further details concerning unnamable species.

B. Intertidal areas

1. Soft bottoms

Two approaches are possible, first where there is a reasonable tidal range, the area may be sampled by subtidal grab or corer methods (Holme and MacIntyre 1971) as described below; second the habitat can be sampled manually at low tide. The second approach is that most commonly used and has the advantage that sampling can be more tightly controlled. The use of transects is possible and samples can be taken in precise locations. One negative aspect of sampling at low tide is that there is often no ready supply of water for sieving, and samples may have to be carried long distances over soft mud.

Quantitative sampling is normally carried out using quadrats, 0.1m² being the most frequently used and the most practical size. In firmer substrata, a simple metal quadrat is satisfactory but in very soft muds, a metal bottomless box-like quadrat that can be pushed into the substratum is better. In either case, sediment is removed with a square ended spade to a depth to include most biota, and sieved to separate the biota. Five to ten replicates are normally taken at each sampling location.

2. Hard bottoms

Since the community structure on rocky shorelines is invariably stratified (Lewis(1964),

Mathieson and Nienhuis(1991), Stephenson and Stephenson (1972)), sampling designs must take account of this. Sampling is frequently carried out at a series of standard tidal levels (Thomas 1983, 1994b) for example at 10% increments of the tidal range. At any rate the sample design should be such that all zones are adequately sampled. The most frequently used method is to establish a transect at right angles to the shoreline, running from extreme low tide level to the top of the supralittoral fringe. Because, marine communities extend well above high tide level on exposed shorelines (Stephenson and Stephenson 1972) sample design must be based on the distribution of the zones themselves. Quadrats can then be used to sample at specific height increments along the transect. This method has the advantage that the top of the transect can easily be marked and the precise location re-sampled in future. Because sub-habitats such as tidal pools, crevices, overhangs etc, are common on rocky shores and have markedly different communities, they should be sampled separately from the general transects. Samples are usually scraped from the rock surface using a knife or scraper. The collected material can be pooled for later species identification and processing, however, delicate specimens are best separated in vials. A special problem arises in the case of crustose animals and plants. Many of these cannot be scraped off the substratum in a condition that permits accurate identification. Such species must be collected on a portion of the rock which is chiselled off. In collecting in the intertidal, it should be borne in mind that many non-sessile species are adapted to hide in crevices, such locations within sample areas should be carefully sub-sampled. Care should also be taken to note epibiotic species living on the surface of others.
Species importance is difficult to measure in an environment where part of the community is markedly three-dimensional and part is encrusting. Measuring biomass in the latter group is virtually impossible. A satisfactory alternative to biomass is 'surface area occupied' which can be estimated within a clear plastic 0.1m² quadrat, marked with 100 randomly-spaced dots, laid on the community (Menge 1976, Thomas 1983). This may need to be done twice, once with the community as found and a second time after the canopy species are removed. (in the intertidal the main canopy algae frequently occupy close to 100% of the surface area, hiding the encrusting community beneath.)

C. Subtidal areas

1. Soft bottoms

Ships and shipboard equipment
For most subtidal work ships larger than 10m length are required that are equipped with cranes and winches capable of hauling wire ropes for dredges, grabs and corers. For trawling and dredging warps of 12-24 mm diameter are used with lengths of about 2.5-3 times the depth of water being required. For operating grabs of 30 -150 kg, galvanized steel wire of 6-8 mm is appropriate (Gray et al 1992) but rope of appropriate rating has also been successfully used. The ship must be fitted with relevant navigational facilities and a suitable echosounder for bottom determination. In addition sufficient deck space and running seawater must be available for handling samples.

Sampling: gear operation and field data recording

Winching operations are crucial to sample integrity and depend on the type and size of sampling gear. The objective is to obtain representative samples. During sampling the vessel should be at a full stop and the wire should be kept as vertical as possible to ensure vertical set down and lift up of the grab at right angles to the bottom. It is recommended that the final 5 m of descent be at a rate less than 0.5 m/s to minimize shock bow wave disturbance (ICES 1994). A good sample should show a distinguishable undisturbed surface layer often including loose flocculant deposits and there should be no sign of sediment leakage, such as from incompletely closed buckets.
All data and information needs to be recorded by hand during field operations to allow for quality checks later. For this the use of predesigned field work sheets is encouraged. These data sheets with predesignated consecutive station numbers should be available prior to the beginning of sampling operations and a preprinted station list should be also be available together with corresponding sampling coordinates. The type of data to be recorded should include the date, time, position, crew, temperature and salinity (surface and bottom).

Types of gear

Qualitative sampling: Dredges and trawls
Towed gear such as dredges and trawls provide qualitative and sometimes semiquantitative
material by standardizing the condition and duration of towing (Gray et al.
1992) dredges are heavy metal box frames which may have digging edges and are fitted with a bag or coarse net. Trawls are of lighter design, including beam trawls of various types which all consist of a long net with a mouth that is held open by a rigid beam with metal runners at each end. Otter trawls have the net spread open by two otter boards or doors. For benthic studies these types of relatively simple gear are useful in providing an initial indication of the general nature of the fauna and flora in a habitat. However, they are not as appropriate as other gear for quantitative assessments and are thus not further discussed here.

Quantitative sampling: Grabs and corers
Grab samplers have been used for quantitative study of benthic infauna since Petersen (1911). Lowered vertically on a warp, the jaws of the grab 'bite' out a volume of sediment. In contrast to grabs, corers consist of tubes which penetrate the deposit and thus retain a plug of sediment. Since corers are usually smaller than grabs (0.008 - 0.05; typically 0.015m_2) they are more suitable for meiobenthos. For macrobenthos, corers are prone to exhibit edge or boundary effects, which are disturbances of the sample caused by the edge of the sampler (Gray et al. 1992; Hessler and Jumars 1974), and larger organisms are less likely to be caught. Box corers with a sampling area of up to 0.25 m_2 (Hessler and Jumars 1974) are an exception but these also very large, heavy, expensive and difficult to operate, and require the facilities of a large ship. Weston 1990 used a 0.06 m_2 spade corer which may be appropriate for macrofauna but corers are not further considered here.

Type of grab

There are numerous types of grabs being used for benthic sampling. Eleftheriou and Holme (1984) review 14 different types, in addition to other sampling gear. A choice of grab type should first be determined by requirements related to a particular study. Choice also depends on working conditions and the particular environment under which the gear is being operated and to some extent the size of the desired sample. Cost, simplicity, and ease of use are also determining factors. Eleftheriou and Holme (1984) concluded that if moderate weight and adequate depth penetration are prime factors of consideration, then van Veen, Smith-McIntyre and Day crabs are well suited. Among these the van Veen grab with its long arms is not as optimal under open-sea conditions as the Smith-McIntyre grab. The Day grab has the advantage of simpler and safer construction and is now widely used (Gray et al 1992). Other simple and robust allpurpose benthos samplers, such as the all-purpose Ponar grab (Pohle et al 1994) and versions of the original Petersen grab have also shown to operate with predictable consistency and are thus also recommended.
It has been shown that the construction of a given grab type can also affect sampling. The size of net covered windows, located on the upper side of the grab to diminish shock wave in front of the grab, does affect sampling efficiency in a van Veen grab (Andersin and Sandler 1981). To reduce the shock wave it is suggested that the windows cover at least 30% of the upper side of a grab and that the rate of descent be controlled (see elsewhere).
Satisfactory performance of a particular type of grab is also important. This includes proper sealing, straight descent and taking a relatively undisturbed sample of adequate depth. To obtain an adequate sample the grab should penetrate to a digging depth of at least 10 cm (20 better) and contain about 4 l of sediment in a 0.1 m^2 grab (Gray et al 1992). Proper weighting and operation of the grab is thus essential.
Grabs usually perform poorly in coarse sediments such as gravels. In these substrates anchor dredges have been mostly used in the past and are still useful for initial semiquantitative assessments of the benthos. The only effective quantitative coarse sediment sampler appears to be the specialized Hamon grab because it can sample a constant surface area with greater success than comparable devices used in surveys of soft sediments (Kenny and Rees, 1994).

Size of grab

The commonly used types of grabs cover a surface area of 0.03-0.55 m^2, but 0.1-0.2 m^2 appears to be the most commonly used size (Eleftheriou and Holme 1984; Riddle 1989). For sampling in the Baltic Sea a 0.1 m^2 van Veen grab has been designated as the standard sampling instrument for macrobenthos (Dybern et al. 1976). Economic considerations usually limit the size of the grab, with larger and heavier grabs (some over 410 kg) necessitating large vessels for deployment. Smaller samplers are also advantageous in view of the general sampling rule that many small samples are better than few large ones. The reasons for this are that many small samples result in greater coverage of the sample site, a better estimate in spatial dispersion and a greater number of degrees of freedom for statistical analysis. Consequently grabs with a surface area of 0.05-0.1 m^2, weighing about 30-50 kg, are being recommended here.

Sample treatment

Initial handling

A stable platform, such as a stool or table, is required to unload the grab. The condition of the sample needs to be ascertained and recorded before emptying the contents into a container. Samples can be emptied into appropriately marked or labeled buckets of proper size (incl. one label with sample) until further processing is possible (e.g. during operations with short time intervals between sample taking).

Screen and mesh size

Benthic samples need to be sieved to separate the animals from the substrate. Screens used are made from stainless steel, bronze or brass gauze attached to the bottom of a sturdy frame 15-25 cm high. The free surface of the screens should be about 30X 30 cm. If a series of screens are being used a frame in the form of drawers may be constructed into which the screens can be fitted similar to a rack-like stand, so that the screened material can be shaken down at once into a bottom tray.. The pore size of the screen used will greatly affect the numbers and types of animals retained and correspondingly the labour time to separate specimens . The sieve mesh sizes used range from about 3 mm (e.g. Wildish et al 1972) to 0.1 mm (see Gray et al. 1992), depending on the type of study. It is now generally accepted that the maximum sieve pore diameter size should not exceed 1 mm (ICES BEWG,1994:93). The most commonly used mesh sizes for macrofauna are 0.5 and 1 mm (Powilleit and Kube 1994; Elmgren et al. 1984; Wildish et al. 1977). Benthic ecologists in the Baltic Sea have standardized mesh size to 1 mm, with the recommendation that 0.5 mm mesh should be used in addition whenever possible (Dybern et al. 1976; Ankar et al. 1979). Most deepsea studies commonly employ finer screens ranging from 0.3 to 0.5 mm (Hessler and Jumars 1974). Jumars (1975) employed 0.42 mm screens which have also adopted for other studies (e.g. Pohle et al. 1994). Screens smaller than 0.3 mm will generally retain nematodes, harpacticoids and ostracods which are considered components of the permanent meiofauna (McIntyre, 1969). A comparison of specimens retained by different sieve meshes (Eleftheriou and Holme 1984) shows that a 1 mm screen poorly retains some of the wormiform macrofauna. Retention could be as low as 1% for some polychaetes (e.g. *Cossura*), while a 0.5 mm screen would retain 77 % of the same taxon. The use of 0.5 mm screens is thus recommended for macrobenthos sampling.

Processing

Before fixation the collected grab or core samples need to be sieved to separate specimens from the substrate. This should be accomplished soon after sample collection, on board ship if necessary, to avoid sample degradation or having to fix the sample before further processing. Sieving is accomplished by washing the sample in some type of washing tank by washing the material with gentle jets of seawater, shaking by hand and separating agglomerations. Hoses with fixed sprinklers are adequate. Immersing screens with samples in waterbaths while gently shaking have also proven successful. Eleftheriou and Holme (1984) show various washing table equipment. Consecutive sieving in sieves with larger to smaller pore diameter is recommended, especially in samples with coarse deposits. Sieved specimens and any remaining material (except large stones) are transferred into an appropriate (preferably

glass) container by washing contents from the finest sieve into a fine mesh net, inverting the net into a container and rinsing the net down with seawater. Unless immediately processed, fixation of samples in formalin is necessary. In the field, buffering the solution by adding marble chips is adequate. Subsequent transfer to 70% ethanol or 50% isopropanol is required, if samples are stored before further processing. Otherwise the formalin will dissolve structures such as mollusc shells.

Sample sorting

Sorting is probably the step that requires the most time. Thus, before a sample is sorted the preserving liquid should be drained off and specimens rinsed because the fumes can be irritating. Most animals are sorted in a medium of fresh water in small Syracuse watch glasses or divided Petri dishes. Sorting is done under a binocular dissecting microscope using fine stainless steel forceps and/or probes for manipulation. A small amount of the sample to be sorted is processed. This process is repeated until the entire sample has been sorted completely. The data is recorded on a sorter worksheet. Five to ten percent of the samples should be checked by a another qualified person to ensure proper and complete sample processing.

2. Hard bottoms

Ships, boats and vessels

The type and size of tender for subtidal hard-bottom sampling varies greatly with the method to be employed. At the top end of the scale, sampling with a manned submersible in deep water requires a large, appropriately equipped vessel and a well trained crew.

Most sampling, however, is carried out in water less than 100m deep, relatively close to shore using SCUBA divers. In most cases back-up facilities for the divers, such as compressors and changing facilities, are located on-shore in a nearby establishment. However, if sampling is to be carried out in remote locations all these facilities must be in the tender. The working and safety equipment for the SCUBA divers will vary somewhat with the rules currently in force in the parent establishment. However, stress on diver safety is currently increasing across Canada and throughout the world. Hard bottom sampling operations should ensure that all the men, material and training required to satisfy Federal, Provincial and supporting establishment rules are in place and in proper working order. In many cases this will include, diver to diver and diver to surface communication, life lines, standby divers etc. In some situations provisions for surface supplied air are needed. The minimum working team is two divers, backed up by a fully suited and equipped stand-by diver on the vessel. Frequently three or more divers are needed in the water, and more back-up divers may be required. A "diving safety officer" is normally required either at the parent establishment or with the diving team. In any event he/she will have to be familiar with all the operations being carried out and the conditions at the dive site, and will have to authorise each dive.

For safety and other reasons the tender must be moored close to the working site. This may present problems in areas with relatively smooth rock bottoms, especially where current velocities are high. Special mooring arrangements may be needed. Where the tender cannot be moored very close to the dive site, an appropriate smaller vessel, such as a large inflatable boat should be present at the dive site. It should be so

equipped and manned that it can provide all the immediate support services needed and could accommodate the entire dive crew at once, if needed. Obviously, in some cases, more than one support boat may be required.
If suction dredging or similar back-up operations are to be used, appropriate air or water pumps, and sufficient hose for the situation must be in place.

Sampling gear

Cameras. Almost all hard-bottom benthic sampling relies heavily on the use of underwater cameras, both video and still, hand-held or remote. A great many different types are available in all categories but some have proved to be the most reliable under the difficult conditions involved.

Hand-held still cameras

For hand-held still camera work, 35mm format camera-flash combinations, with integral waterproofing have been shown to perform better than cameras and flash units in waterproof housings. The latter are much more bulky, and consequently catch more current rendering them more difficult to move and position. They also have fewer useful attachments and are more leak prone. The "Nikonos" series of cameras and flashes are the best and most widely used; there is a wide range of available lenses, including extreme wide angle and auto-focus is available on the latest models. Where there are problems with water clarity, wide angle lenses have the advantage that they reduce the camera to object distance, thereby minimizing interference. In most situations focussing is a problem and yet must be critical for accurate species identification. For this reason the use of close-up attachments and framers is prevalent. In this case the camera is preset for focus and the frame, which is in the plane of focus, is used to surround the sample. This method has the added advantage that it ensures that a specific area of community is included in the photograph. Close up equipment which give an image of 1:1 (3.5 cm across the full frame) up to those giving an image at 10:1 (35cm across the full frame) are frequently used according to the size of specimens involved, and the detail of analysis required. Multiple contiguous exposures within a specially marked quadrat may be needed where an area larger than the close-up frame must be photographed. It is frequently useful to take photographs of the same site at different scales so that the overall situation as well as the detail is recorded. In some situations, larger format cameras, for example 10 x 10cm format, may offer advantages, but they are much more costly, are larger and all require a housing.
Flash units with sufficient power to allow the use of 100 or less ASA film rating are essential. Those camera-flash combinations with automatic exposure control capability are best. In some situations, where relatively large areas (1m² or more) are being photographed, multiple fash units may be needed. Those with ‘slave’ capability reduce complex wiring problems. The placement of the flash-head relative to the camera is important, particularly where there is much suspended material in the water. Flash units at an obtuse angle to the camera accentuate particles in the water, but on the other hand those with their axis of illumination close to the axis of the camera, produce rather a flat image with limited contrast. In practise a flash angle that gives good image quality with minimal interference from suspended particles must be determined by experience. Stereo-pair photography although more complex may offer advantages in assessing three dimensionality and has been used by some European workers with success

(Lundalv 1971, Torlegard and Lundalv 1974, Rorslett *et al*. 1978, Svane 1988).
Film choice is important since accurate colour rendition may be very important in the identification of specimens. Additionally, fine-grain, low ASA films have superior definition to faster films. For close-up work, good flash units are sufficiently powerful to allow the use of ASA 25 film, which has both the best colour rendition and very fine grain. 100 ASA films are grainier and colour rendition is less accurate, but, they have the advantage of faster speed for situations where lighting is less bright. In non close-up situations 100ASA film offers the best compromise.

Remote still cameras
Remote still cameras are almost always custom made. They normally consist of a frame equal to the area being photographed, surmounted by a camera and one or more flash-heads, at the correct distance for accurate focus. The camera is usually fired by a trigger actuated by bottom contact. Cameras equipped with automatic film advance and cocking, have the advantage that they do not have to be raised to the surface after each exposure. Larger-format cameras such as those with 10 x 10 cm image size, in a housing have advantages in this type of situation since extreme close-ups are normally not attempted and specimen images are small on the film. Additionally, large format cameras are capable of using large film spools to give 100 or more images. Being large and bulky, a winch is required for deployment and the camera and frame is quite subject to deflection by water currents. The information below on grab operation is also appropriate to these cameras.

Hand-held video cameras
Video cameras have several advantages over still cameras for the photography of hardbottom benthos but are very limited in other aspects. First, video cameras allow the filming of a continuous strip of bottom, in effect a belt transect. This can later be analysed at any level of detail required from single frames on up. Secondly, video cameras can operate satisfactorily at very low light levels and at high light levels have better depth of field than still cameras. On the negative side the detail available in the image is greatly reduced in comparison with still cameras and the control of the filming operation is more difficult. Additionally, the size of the cameras is larger, they all need waterproof housings, they catch more current and are generally more unwieldy.
The best compromise for size, availability of suitable housings, image detail and accurate colour rendition is offered in Hi 8 type cameras. Sony cameras in Amphibico housings are very reliable and versatile and relatively easy to use.
For general underwater photography, to record the general nature of communities, or the detail of individual species, the video camera has considerable advantages over the still camera. Because of the sensitivity of the system the camera aperture is normally very small, giving great depth of field. In many cases objects from 1 meter away to infinity will be in sharp focus. The diver can therefore do both panoramic shots and reasonable close-ups without having to re-focus. Where extreme close up photography is needed, the cameras can be focussed much closer than with still cameras. In water of poor visibility, the image from a video camera is better than that seen with the naked eye or with a still camera. Where some scale is needed a second diver can be included in the image, or a weighted prominent scale can be placed in the field-of-view.

For the best quantitative results, video cameras may be used to film a belt transect marked by a weighted rope marked at frequent intervals. For example, short distance markers could be at 10-25 cm increments and numbered tags at meter increments would show distance along the transect. These transect lines must be custom-made. The basic line is lead-cored "sinker" line; marks can be made from short lengths of brightly coloured twine woven through the main line. It helps in analysis if 50cm marks contrast with shorter interval marks. The best meter (or more) increment makers are cattle neck tags. These large, inexpensive, tough tags of brightly coloured plastic have large white numbers on both sides, thus a number is almost always visible to the camera. These tags are available in numbers from 1 to at least 100. Such lines can either be laid at specific locations by a diving team or can be laid from a moving boat and then straightened by a diver before use. For repetitive surveys of exactly the same location the line can be laid between permanent markers. In some situations, transect lines can conveniently be laid straight down a slope in others they can be laid at constant depth following contours. In the first situation, depth can conveniently be recorded on the film by the diver placing his depth gauge in the field of view.
Transects are normally filmed with the camera held vertically at a constant distance above the transect line. The camera can be at any distance above the bottom and used at fixed focus, but in practice it aids in analysis if a minimum of two distance markers are always in the field of view at any one time. Keeping at an absolutely constant height above the bottom is virtually impossible, but since the depth marks on the transect line are visible, the scale can easily be worked out. With a wide angle lens, a working distance of about 50cm gives reasonable detail of small specimens. The speed of swimming must be reasonably slow, in the range of 5-10m/min, or image detail will suffer. Metal video tapes give the best definition but metal particle tapes are somewhat more robust and still satisfactory. Due to the sensitivity of the system the need for supplementary lighting is minimised. In clear water, good image quality is maintained down to at least 20m and much deeper in some cases. Where light levels are very low, or where the colour balance is critically important, continuous supplementary lighting must be used. However, this uses a great deal of electrical power and necessitates the carrying of a large, heavy battery housing in addition to the camera.

Methods of Analysis

Collections of Biota. In the laboratory samples collected in the field are normally sorted to species using appropriate standard identification guides and keys for the taxonomic groups and geographic locations of the collections. Reference specimens should be preserved according to accepted practice for the type of specimen and meticulously labelled on waterproof card which can be inserted in the container. In most work this is followed up by a count of individuals per species and a determination of species biomass on a fresh weight, dry weight or ash-free dry weight basis. Processing for biomass beyond the fresh weight basis is destructive and if important specimens are present in the collections they should be removed before this stage. A correction for lost biomass is normally based on calculated fresh weight-dry weight regressions based on specimens that need not be retained. In the case of a single specimen of a species it should not be processed beyond the fresh weight stage.
Species-area curves (Štirn et. al. 1975) should be constructed for all sets of samples to

check whether the full array of species has been collected (see p. 7 for details). If it has not, then either further samples should be collected or as a second best, the total diversity can be reasonably estimated from the species/area curves.
Photographs. Species identification from photographs is more difficult than from specimens and in critical situations, collections will be required to supplement the photographs. In many cases identification to fairly broad taxonomic groups may be all that is possible from photographs. However, with increasing experience of the total array of biota involved, very reliable species identifications are possible from close-up photographs. Photographs are just as amenable to species-area analysis as are collections. Species counts may also be made with good accuracy although difficulty is presented in the case of colonial species such as some tunicates. Such colonies are usually treated as individuals (Noble et al. 1976) although this does introduce errors into estimations of relative abundance and dominance.
Areal coverage of species can be made basically as would be done in the field by placing a random dot overlay (Menge 1976) over the projected image. The point sampling method described by Sutherland (1974) may be used instead of the random dot overlay.
In the context of the EMAN mandate methods in assessing biodiversity should address two issues: establishing an inventory and understanding changes in community structure over time and space, i.e. differences among assemblages of marine benthic species. Benthic assemblages contain large number of species, often well over 100 species within a single sample. A variety of techniques have been employed to simplify the resulting large data sets which fall under five main headings discussed below. For all these procedures various pre-processing of data is necessary before testing of structure. Clarke and Green (1988) summarize these steps, including other aspects of statistical design.

1. Univariate methods

These are generally used to extract universal features of communities which are not the function of specific taxa, i.e. these methods are species independent. Thus they are not sensitive to spatio-temporal variations in species composition, such that assemblages with no species in common can theoretically have equal diversities (ACMRR/IABO). In comparison to multivariate methods they are obtained more easily but, as graphical/distributional methods, tend not to be as sensitive as multivariate methods (Warwick and Clarke 1991) in terms of detecting changes.

Note of WP2
(Please take the time to **also** read the updated articles by Carlo Heip and coll. and Serge Dallot about indices !! Take care to the bias !
Heip C. et al. 2001 – Indices of diversity and eveness. In *Concepts and methods for studying marine biodiversity, from gene to ecosystem*, J.-P. Féral (ed), European TMR / CNRS practical training course. Océanis 24 (4): 61-87 [1998]
Dallot S. 2001- Sampling properties of biodiversity indices. In *Concepts and methods for studying marine biodiversity, from gene to ecosystem*, J.-P. Féral (ed), European TMR / CNRS practical training course. Océanis 24 (4): 89-105 [1998])

a) Species Richness. This is one of the oldest and most basic diversity

measurements, based directly on the total number of species at a site; the term species richness is often preferred since the exact number of species in a community is rarely known. However, this method depends on sample size and does not consider the relative abundance of different species. It thus has limited ecological value. As an ecological concept abundance is another important component of diversity (Hurlbert 1971), which Peet (1974) referred to as heterogeneity, representing the equitability or evenness of allotment of individuals among the species. A greater number of species increases species diversity, and a more even or equitable distribution among species also represents greater diversity. Various indices have been developed in this regard but there is little point in calculating them all, as they are all strongly correlated (Gray et al. 1992). The most commonly used index is mentioned here.

b) Shannon-Wiener's index:
where proportional abundance or percent importance, (p_i) = n_i/N for the it species;
S = total number of species, (n_i) = number of individuals of a species in sample;
N = total number of individuals of all species in sample. Thus the value of H' is dependent upon the number of species present, their relative proportions, sample size (N), and the logarithm base. The choice of the base of logarithm is arbitrary (Valiela 1995) but in comparing indices the base used should be stated and be the same. Marked dominance of one species gives low diversity, while codominance of several species gives high diversity. Because the equation is a biased estimator (Valiela 1995), a corrective term (S-1)/2N should be subtracted from the right hand side of the equation. Formal testing for statistical differences in H' is possible through calculation of the variance of H' (Hutcheson, 1970). Practical computations in applying this index to data are presented in Štirn (1981). Of all indices, Shannon and Wiener's formula is probably the most widely used diversity index using both abundance and richness (e.g. Gray et al. 1990). It appears to be the most consistently useful way of obtaining significant diversity indices which are relatively independent of sample size (Štirn 1981) and by some (e.g. Štirn 1981, Gray et al. 1992) has been recommended the most suitable expression of biotic diversity.

Equitability, or the ratio of observed diversity to that of a completely equal species frequency distribution (range 0-1) can be quantified separately using the Shannon-Wiener Index as J' = H'/H'max, where H' is the observed species diversity and H'max is the logarithm of the total number of species (S) in the sample (ACMRR/IABO 1976, Gray et al. 1992). For example, 2 species with 50 individuals each would represent complete equitability or evenness with a value of 1. Two species with one and 99 individuals each, would score only 0.08.

2. Graphical/distributional methods
These form a class of techniques which can be thought of as intermediate between univariate summaries and full multivariate analysis of the species/samples matrix. Two widely used methods to compare biotic diversity are known.

a) Rarefaction diversity curves. This method was developed by Sanders (1968) and has been widely adopted for the assessment of diversity within ecosystems, such as for pollution studies (Gray and Pearson 1982). The number of species for a given number

of individuals is estimated. It's main advantage is that it is independent of sample size. Assumptions are that species/individuals relationships are similar in communities being compared and that individuals are randomly distributed. Štirn (1981) and Sanders (1968) show how the graphs are derived.

b) K-dominance curves, developed by Lambshead et al. (1983), result from plotting percentage cumulative abundance against species rank *k* on a logarithmic scale, where species assemblage x is more diverse than y if the curve for y is everywhere below or touching that of x. (e.g. see Warwick 1986, Warwick et al. 1990a,b). Clarke (1990) developed a statistical procedure to test for significant differences by an "analysis of similarities" (ANISE), by comparing the variability in k-dominance curves between replicates with that of spatially or temporally separated samples.
A further development of k-dominance curves involves superimposing k-dominance plots for species abundance and biomass (Warwick 1986), known as abundancebiomass comparison (ABC) curves, where the relative position of abundance and biomass on the plot can reveal pollution impacts. Relatively undisturbed sites have biomass curves above abundance curves and vice versa. This method has been widely used (e.g. Warwick and Clark 1991, Clark 1990, Warwick 1988) but for EMAN purposes this may only have limited applicability, since biomass data are, and probably will, not be routinely collected.
In general, both the rarefaction and k-dominance methods will give the same results. Two advantages of k-dominance curves are that the relative dominance of the commonest or rarest species can be determined at a glance and the computations to construct the curves are easier (Lambshead et al. 1983). In agreement with Lambshead et al. (1983), it is recommended that graphical methods be routinely applied to marine biological data before calculating more complex diversity or equitability indices.

3. Multivariate methods

In ecological context these computer-based sorting methods are used to classify taxa or sites showing similar attributes into groups. In themselves they simply indicate the degree of similarity or dissimilarity in species composition between stations, or at the same station over time. Strong correlative evidence of cause and effect, such as from pollutants, can only be obtained when relating station groupings to measured environmental and pollution gradients or some indirect measure of pollution intensity, such as distance from pollution source or time of pollution event (Gray and Pearson 1982, Warwick 1987). Because they are based on formal criteria, these methods appear more objective than others. In contrast to diversity indices, the multivariate methods discussed preserve species identity and are generally regarded as more sensitive in detecting changing community patterns. Thus effects can be detected earlier (Warwick and Clarke 1991; Gray et al. 1990). Multivariate methods, however, also suffer from shortcomings. They are considerably more complex than other methods, involving substantial pre-processing or editing of data, such as transformations, and presently there is no uniform or agreed procedure. The data matrix also needs to be reduced for data processing. Generally this removes rare species from analysis, an intrinsic propertyof all communities which may include some of the defining species (Gray and Pearson 1982). Multivariate methods fall under the

two broad categorizations of clustering and ordination. Before data matrices are subjected to either type of analysis, species abundances and biomass data are commonly -transformed and similarities between every pair of samples computed using the Bray-Curtis coefficient (Bray and Curtis 1957): Cz = 2w/(a+b), where a is the sum of abundances of all species found in a given sample, b is the sum of species abundances for another sample, and w is the sum of the lower of the abundance values for each species common to both samples.

Classification methods

This is based on assignment of entities to classes or groups, the input data generally consisting of species abundances in a two-way samples by species data matrix (Gauch 1982). The process of classification is essentially the summarization for each sample, of the information in many numbers (all species abundances), into a single number (the cluster assignment). There are countless ways in which many numbers can be summarized into one number, emphasizing dominant species, minor species, individual species, etc. In an attempt to standardize to a single method, the most common method involves hierarchical agglomerative clustering, where similar stations are fused into larger and larger groups. This grouping is based on group-averaging or nearest neighbour sorting of a matrix of sample similarities, using the Bray-Curtis similarity measure. The results are displayed in a tree-like dendogram. Species having the greatest contribution to the division of sites into clusters can be determined using the similarity percentages (SIMPER) program (Warwick et al. 1990a).

Ordination methods

These methods attempt to present a picture of the relationship between samples in terms of their similarity of species abundance or biomass, where the relative distance apart of any pair of samples is intended to reflect their relative dissimilarity. Clark and Green (1988) define it as an analysis of a data matrix of n samples by p species, whereby a new set of variables is found which optimally predicts the structure in the relationships among the original p variables. Methods differ by the optimality criterion and how the ordination algorithm finds the new axes which represent the new variables. There are several ordination techniques now employed, including Reciprocal Averaging (RA) and Detrended Correspondence Analysis (DECORANA). Warwick (1987) concluded that the selection of the most appropriate technique was largely one of personal choice, the availability of suitable programs and computing facilities. Here, only Principal Component Analysis (PCA) and non-metric Multidimensional Scaling (MDS) are discussed. The latter has certain theoretical advantages (Clarke and Green 1988) and has shown to be empirically more robust (Warwick et al. 1988).

In PCA the amount of variation accounted for by the new axes is maximized, proceeding by way of an eigenanalysis on the p-by-p correlation matrix, where the new axes are uncorrelated. The procedure is relatively simple to perform but the new axes are rarely interpretable as simple environmental factors causing the structure in the species abundance data.

Multidimensional Scaling (MDS) attempts to construct a map of the sites in which the more similar two samples are in terms of species abundance or biomass, the nearer they are to each other on the ‘map’ (Clarke and Green 1988, Gray et al. 1988).

Multidimensional Scaling is popular because it is dependent only on rank information rather than quantitative values, using statements in the form 'Sample 1 is more similar to Sample 2 than it is to Sample 3'. The extent to which these relations can be adequately represented in a 2 dimensional map is expressed as the 'stress coefficient' statistic, low values indicating success (e.g. <0.1). Results are displayed as plots which have arbitrary configurations and scale.
Neither clustering or ordination methods are in competition with each other (Clark and Green 1988) and it has been recommended to do both (e.g. Gray et al. 1988).

4. Indicator species

A review of indicator species by Pearson and Rosenberg (1978) showed that species present in the most polluted areas, such as the polychaete *Capitella capitata*, were those typical of the first stages of succession. However, such species may also occur in high densities in areas other than those showing organic enrichment (Gray and Pearson 1982). Nevertheless, groups of species characterizing various stages of enrichment, do occur in local areas. These groups, however, will vary in different geographical regions and thus may not be universal indicators. General taxonomic groups known to intolerant to pollution include sponges, most cnidarians, gastrotrichs, kinorhynchs, echiurids, sipunculids, stomatopods, cumaceans, scaphopods, most echinoderms and the ascidians (Štirn 1981).
Gray and Pearson (1982) used the distribution of individuals among species to identify critical taxa. Contrary to multivariate methods this is a simple technique that uses the whole data set of common and rare species which identifies groups of indicator species that can be used in reduced scale monitoring programs. The number of species on the y-axis is plotted against the number of individuals per species aggregated into geometric classes on the x-axis. Plots over time or space can then be compared and the changes in the number of species of particular size classes noted. Environmental disturbances are indicated by a decrease of rare species closest to the y-axis. Indicator species are within the classes comprising those groups of moderately common species (usually V and VI) where change is rapid along a spatial or temporal gradient (see Gray and Pearson 1982 p.116-117 and Gray et al. 1990 p.290 for details). Using standard Analysis of Variance (ANOVA), the changes of particular species can be tested for statistical significance (Gray et al. 1990).
For multivariate techniques indicator species having the greatest contribution to the division of sites into clusters can also be determined using similarity percentages (SIMPER), as described in Warwick et al. (1990a).

5. Taxonomic reductions: Aggregation of species data to higher taxonomic levels

Using clustering analysis and MDS ordination Gray et al. (1990) demonstrated that environmental monitoring costs may be significantly reduced by working at taxonomic levels above the species level. By ordering data on species into families the grouping of unpolluted, somewhat polluted and heavily polluted sites remained intact and comparable to the species analysis. With minor changes the overall pattern also remained the same by further lumping data into polychaetes, molluscs, echinoderms and crusteaceans. Using MDS and ABC plots at the family level Warwick (1988) found no loss of information compared to species analyses but there were some differences when using MDS with phyla, depending on the strength of the transformation and

whether abundance or biomass was used. Warwick et al. (1990b) also found some loss of information at the phylum level but little loss of information by aggregation of species data to family level both in univariate (ABC plots) and multivariate analyses (MDS) for the macrofauna component. However, for the nematode component of the meiofauna this resulted in substantial loss of information above the genus level. Warwick et al. (1990b) suggest that the genus level may be the optimum taxonomic level for most efficient discrimination but the family level may be appropriate for macrofauna (Gray et al. 1992). Clearly these results need to be substantiated further before worldwide application of these techniques which promise greatly improved cost effectiveness.

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