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Benthic communities: Use in monitoring point-source discharges

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FOREWORD

Following a request from the ICES Advisory Committee on Marine Pollution (ACMP), the Benthos Ecology Working Group (BEWG) undertook to report on the utility of field surveys of benthic fauna for monitoring the impact of point-source inputs (dumping or discharge of wastes). The ACMP identified four particular areas on which advice was being sought, namely:

a) the types of inputs that might elicit a response from the benthos and the nature of the response(s);

b) the time scales of these responses;

c) the types of benthic community responses that would lend themselves to sufficiently accurate and precise measurement to identify spatial or temporal trends in impacted waters; and

d) the degree to which the significance of benthic community responses can be evaluated in terms of effects on other components of the marine environment, in particular, in terms of effects on fish stocks.

In response, the BEWG have so far provided guidelines for benthic monitoring around discharge points, along with examples of effective applications (ICES, 1989 a,b). The present report revises and extends the coverage of these documents to include both a general appraisal of the utility of benthos studies in pollution monitoring, structured in line with the earlier ACMP request, and a detailed review of the use of meiofauna, in view of relatively recent developments in this field. Though largely concerned with the methodology for detection of the effects of waste discharges, a number of the topics covered have a bearing on the general conduct of benthic investigations.

We wish to acknowledge the many constructive comments received from members of the BEWG and ACMP, during the drafting of sections of this report.

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ABSTRACT

The response to a request from the ICES Advisory Committee on Marine Pollution (ACMP) for advice on this topic has been structured along the following lines:

Section A - a general review of benthic studies in relation to pollution. This was felt to be important in view of increasing interest in the application of a variety of biological effects techniques, as benthic communities represent only one of many possible targets in the study of the effects of point-source discharges. The account also addresses the issues raised concerning the nature and significance of observed responses;

Section B - guidelines for the conduct of benthic monitoring programmes around point-source discharges;

Section C - examples of effective survey design; and

Section D - a review of the use of meiofauna in pollution studies; this is a relatively new area of application, and was felt to warrant separate treatment.

The benthic biota possess a number of important attributes which justify their inclusion in the majority of marine monitoring programmes concerned with biological impact. For example, they are intimately associated with the seabed for much for their life-cycle; this environment is an appropriate target, because sediments act as the ultimate sink for most contaminants discharged into the sea. Added to this, their relative immobility (and consequent comparative ease of quantitative sampling), and long life span (commonly exceeding one year) requires that they must adapt to repeated additions of waste inputs.

The monitoring of benthic communities, especially over several years, represents the most suitable direct method presently available for assessing changes in coastal marine ecosystems. However, the application of alternative measures of impact at a variety of levels of biological organisation deserves continuing encouragement, because no single measure has yet been devised which can be used to predict effects on the marine ecosystem as a whole. Such measures should, as far as possible, be integrated with field studies of benthic communities.

Assessments as to the wider significance of benthic changes may embrace aesthetic and conservation interests, as well as those relating to fishery resources. Clearly, the science of benthic ecology cannot in isolation address all these matters, but it can make a valuable contribution to the decision-making process. For example, many studies show that effects on the benthos are not widespread, but are confined near to inputs. Such 'negative' findings, which may be unappealing as a scientific outcome, do much to assuage concern over the wider impact of controlled waste disposal to sea, in as much as a limited change in a benthic community implies a similarly limited change in dependent biota, such as demersal fish. However, important gaps still remain in our general understanding of the interactions of benthic communities with other ecosystem components, and improvements in this area would heighten confidence in predictions of the consequences of benthic changes.

The types of benthic community responses presently suitable for the measurement of impact have been identified, along with those having future potential but requiring further methodological research. The precise and accurate measurement of such responses is governed by decisions regarding the type of equipment to use, the number of replicates, and the sampling design. These are invariably site-specific and, as a result, the account provides detailed guidance, but not a general 'blue-print', for the conduct of benthic surveys around point-source discharges.

Close attention to field sampling strategy is, therefore, critical to the success of monitoring programmes; this is underscored by the fact that most of the cost attached to benthic surveys occurs at the stage of sampling and (with the added constraint of time) in the laboratory analysis of these samples. Such cost limitations do not generally apply to data analysis, especially given the wide availability of computers and tailored software, so that the application of a range of complementary measures of data structure could be recommended.

SECTION A. ASSESSMENT OF THE UTILITY OF BENTHOS STUDIES IN POLLUTION MONITORING PROGRAMMES

H L Rees and M M Parker

1 INTRODUCTION

1.1 Historical Background

The acknowledged starting point for the quantitative ecological study of the benthic fauna was the work of Peterson and co-workers in Danish waters at the turn of this century (e.g., Peterson, 1918). Though aimed at quantifying the role of benthos as fish food, this work led to the development of the concept of **communities** of marine organisms inhabiting discrete zones of the sea bed. The concept subsequently found wide (though not uncritical) application, culminating in the classic paper by Thorson (1957) which dealt with structural parallels between regionally separated level-bottom communities in physically comparable zones.

Since then, there has been a continuing emphasis on structural attributes of the benthic fauna (exemplified by measures of 'diversity') which has mirrored developments in terrestrial plant ecology. In the last 20 years or so, with heightened concern about the consequences of effluent discharges, attention has been focussed on benthic organisms as indicators of environmental changes, largely because of their relative immobility and ease of quantitative sampling. During this period, the functional role of the benthos in marine ecosystems, and in particular the nature and significance of the link with commercial fish populations, has received comparatively less attention.

1.2 Categories of Benthic Organisms

The benthos comprise animals or plants living in, on or in close association with the sea bed. Conventionally, these organisms are sub-divided on the basis of size (see Table 1, from McIntyre, 1978). These divisions, though somewhat arbitrary, identify the major functional groups of organisms, each of which requires different approaches to sampling and analysis. To these groups may be added the 'hyperbenthos', i.e., free-swimming species - especially crustaceans - which tend to be associated with the water column immediately above the sea bed.

The following account will concentrate mainly on the meio- and macro-benthic components, which, for a variety of reasons, have been the most common targets in field investigations of the effects of discharges.

Table 1. A scheme for classifying benthos by size (from McIntyre, 1978)

Category	Size	Biological features	Sampling techniques	Taxonomic position
Microbenthos	Pass finest sieves	High rates of respiration and reproduction	Plating and culturing. Cores of <2 cm diam.	Bacteria, viruses yeasts, fungi actinomycetes blue-greens Most protozoa Some algae
Meiobenthos	Pass 0.5-1 mm sieves	Medium respiration rates. Two or more generations per year	Cores of 2-10 cm diam.	Large protoza Small metazoa
Macrobenthos	Retained on 0.5-1 mm sieves	Low respiration rates. Two or less generations per year. Mostly infauna	Grabs sampling at least about 0.1 sq.m	Medium-sized metazoa
Megabenthos	Handpicked from samples	As above, mostly epifauna	Towed gear, trawls, dredge	Large metazoa

2 BENTHIC SPECIES AND COMMUNITIES AS INDICATORS OR SENTINELS

The main reasons why the benthic macrofauna are an appropriate target in many investigations of marine pollution are as follows:

a) seabed sediments represent the ultimate sink for most contaminants discharged to the sea;

b) most macrofauna species are relatively long-lived (> 1 year) and sedentary, and so can provide an indication of the integrated effects of discharges over time;

c) they are relatively easy to sample quantitatively. Generally, plankton or fish populations are less amenable to quantitative study on a scale appropriate to the delineation of localised effects of most discharges and, in the latter case, they also have the ability to avoid contaminated areas. No such option is available for sedentary benthic species;

d) they are well-studied scientifically, compared with other sediment-dwelling components (e.g., meio- and micro-fauna), and taxonomic keys are available for most groups;

e) there may be direct links with valued resources, e.g., fish (via feeding) and edible molluscs; and

f) macrofauna community structure has been shown to respond to pollutants in a predictable manner (thus, the results of changes can be interpreted with some degree of confidence).

Use has been made of a wide range of biological responses in assessments of the effects of contaminant inputs; these range from biochemical changes at the subcellular level to the responses of whole communities, and many have been described in McIntyre and Pearce (1980), Bayne

(1985), Bayne *et al.* (1985), Bayne *et al.* (1988), and Addison and Clarke (1990). Such biological assays are particularly important in the case of complex effluents, where the range of contaminants may be too great to allow accurate predictions of effects from chemical analyses alone.

Certain long-lived sedentary benthic species, for example, the bivalve *Mytilus*, which are widely distributed, are ideally suited as 'sentinel' organisms for the monitoring of trends in contaminant levels or biological responses to contaminants (e.g., Phillips and Segar, 1986; Bayne *et al.*, 1979).

3 TYPES OF INPUT WHICH MAY INITIATE A RESPONSE

Controlled point-source inputs enter the sea directly via discharges from pipelines or ships, and indirectly via the efflux of contaminated river water. Disposal from ships occurs at discrete intervals, while pipeline discharges are usually continuous, though the quantity and composition may vary with time (e.g., sewage discharges). Waste inputs may be of constant and well-defined composition, e.g., from a single industrial process, but more commonly - again, as in the case of many sewage discharges - consist of the end-product of some form of treatment of a multiplicity of urban and trade wastes.

The major point-source inputs to the sea may be categorised as follows:

a) sewage and organic matter; sewage sludge;
b) liquid/solid industrial wastes of known origin;
c) oil;
d) dredgings;
e) thermal effluents;
f) radioactive wastes; and
g) mixed inputs of unknown composition.

The major routes of point-source inputs to the sea are via:

1) pipeline;
2) dumping from ships;
3) oil and gas exploration/production;
4) coastal engineering works;
5) ship wrecks; and
6) operational discharges from vessels, including tank washings.

(For the present purpose, indirect or diffuse inputs of contaminants via river discharge, land run-off or the atmosphere are excluded from consideration.)

4 NATURE AND DETECTION OF RESPONSE

The nature (and time-scales) of the earliest response to waste inputs of benthic organisms, at various levels of biological organisation, can be summarised as follows:

1)	sub-cellular (e.g., enzyme systems)	hours-days
2)	cellular (e.g., gametogenesis)	hours-days
3)	tissue (e.g., tumour)	hours-days
4)	individual (e.g., growth/avoidance)	mins-days

5) population (e.g., recruitment/mortality) generation times
6) community (e.g., diversity) seasons-years

Any changes occurring in benthic communities due to waste discharge may thus be viewed as end-points of a hierarchical sequence of adaptive or degenerative responses, and it is the nature and time scales of changes occurring at this level that are likely to be of greatest significance in terms of effects on other resources.

An important goal of research into biological effects techniques is the elucidation - and quantification - of links in this sequence. Thus, measures of responses occurring at, e.g., the level of the individual may ultimately have predictive value, but this is not presently feasible and so cannot be substituted for population and community studies.

Gray (1989a) has recently reviewed the effects of stress on marine communities (especially the benthos), noting that, of a variety of known responses, reduced diversity, retrogression to opportunist species and reduction in size are those which have been best documented.

Regarding the most frequently encountered waste inputs (i.e., routed via pipelines or from ships), it will be helpful to consider the nature of the responses to three main types:

1) to a particulate waste, such as may arise from dredging projects. The disposal of stones or rock will give rise to predictable physical effects on benthic communities, arising from the blanketing of sediments, and subsequent recolonisation of new surfaces; fine particulates will have comparable effects if concentrated locally, but these will generally be less severe if the particulates are more widely dispersed by water movements (e.g., Probert, 1975; Bamber, 1984). The nature and time-scale of the recolonisation sequence will depend not only on the suitability of the new substrate in a particular locality (e.g., McCall, 1977), but also on the frequency of inputs; regular additions may result in the benthos being maintained at an early successional stage giving rise to enhanced productivity (see, e.g., Rhoads *et al.*, 1978, and below). Clearly, the response to particulate matter reaching the sea bed - whether from dredgings, or urban or industrial discharges - may be significantly modified by the presence of associated contaminants;

2) to a potentially toxic liquid chemical waste where, intuitively, one might expect a decline in populations of all macrobenthic species, with increasing waste concentration. Such a response would seem entirely plausible within the immediate mixing zone of an effluent discharge, but on the periphery of influence, survival of species will be a function of tolerance or adaptation (see, e.g., Gray, 1982) and hence analogous to the organic enrichment model, described below;

3) to an organic waste which is not directly toxic, but which may induce alterations in sediments, e.g., through enhanced microbial activity, with consequent effects on the macrofauna. Classically, differences between species in their tolerance, or in their ability to adapt to an altered environment through modification of life-history strategy, lead to successional changes in populations in time or space in response to the waste input (Pearson and Rosenberg, 1978). The empirical model is, in this case, more complex since it involves a progression from 'benign' to 'toxic' effects as organic matter builds up in the sediments, but it has been found to be widely applicable.

That this model fits changes observed in both time and space is, of course, significant, since it can be applied both to 'once-off' surveys of effects along a spatial gradient of contamination from an existing source, and to surveys of temporal changes at one or more sites in response to a new discharge. (Further details of this model are given in Section C.)

Clearly, a potential difficulty of applying this model to mixed discharges is that there may be interaction between responses to directly toxic and non-toxic (especially inert particulate and organic matter) components. From investigations in Norwegian fjords, Rygg (1985) established a significant linear relationship between declining diversity and increasing contaminant concentrations (especially copper) in sediments, which he presumed to be a direct response to toxicity; however, it was evident that successional changes in benthic species were occurring along much of this gradient. Comparable changes have been observed in the benthos around many oil platforms (Kingston, 1987). In neither case was it possible to dismiss the influence of an elevated organic component in the receiving sediments. At present, there is insufficient evidence available to provide a model of field responses to chemical contamination that is substantially different in nature from that of the generalised enrichment model described above. However, early manifestations of the enrichment process would not be expected in response to a toxic discharge.

4.1 Populations/Communities

Pearson and Rosenberg (1978) drew on the notable parallels in the results from widely separated regions in order to construct their generalised model for successional changes in the benthos in response to organic inputs. These changes could be summarised by plots of numbers of species, numbers of individuals, and biomass of samples along enrichment gradients. The same opportunistic taxa were recorded in high numbers at centres of contamination, thus confirming their 'indicator' value. However, successful colonisation is not confined to sites receiving anthropogenic inputs, and their presence in numbers can be more generally interpreted as a response to <u>disturbance</u> of a habitat, natural or otherwise (see Section 5 'Response rates', below). The relevance of this concept to marine benthos has been highlighted by Warwick (1986). As with Pearson and Rosenberg's model, it allows for non-linear changes along gradients, and the expression of the highest community diversity at <u>intermediate</u> levels of disturbance/pollution. (See also Section D, for further discussion of disturbance hypotheses.)

In general, retrospective observations on the predominance of pollution-tolerant species to the exclusion of others offers an imprecise monitoring approach other than for the identification of gross effects (e.g., Gray, 1989a). The importance of the above general model for organic enrichment is that it allows the interpretation of changes from field data at the community level, <u>before</u> the onset of gross effects (at least in stable areas).

Gray and Mirza (1979) contended that departure from a log-normal distribution of individuals among species - representing the natural or equilibrium state in benthic communities - could be used to identify changes due to pollution; moreover, since this departure could be ascribed initially to changes in populations of species at intermediate levels of abundance according to their adaptive capabilities, then this could provide a method both for detecting more subtle effects, and for identifying sensitive target species for follow-up investigation (see also Pearson *et al.*, 1983). This model has been fitted to a variety of data from polluted situations, though its general validity as a frequency distribution appropriate to samples of benthic fauna has been subject to question (e.g., Lambshead and Platt, 1985).

Warwick (1986) and Warwick *et al.* (1987) adapted the graphical method of Lambshead *et al.* (1983) to generate ranked curves both for the abundance and biomass of individual species within a sample in order to assess anthropogenic effects. A shift from low numerical and high biomass dominance to a situation of high numerical and low biomass dominance, as short-lived and smaller-sized 'opportunistic' species become favoured over the fewer larger-sized but less tolerant species, signals a change from unpolluted (or undisturbed) to polluted (or frequently disturbed) conditions, with the cross-over point of the respective curves representing an intermediate stage.

Interpretation depends on subjective assessment of the position of the abundance curve relative to that of biomass for each sample, so that in principle there should be no need for 'control' samples from elsewhere for comparative purposes. Recently, Clarke (1990) has presented a method for statistical evaluation of the abundance/biomass curves.

The above models deal with the inter-relation of numbers (or biomass) of individuals and species along pollution gradients, expressed graphically, and all depend for their successful application on the occurrence of successional changes in benthic species. An alternative means of expressing such trends is provided by the work of Arntz and Rumohr (1986), who demonstrated that the amplitude of seasonal and annual numerical changes (in individuals, species or diversity) provided a good indication of the status of a benthic community. Large and erratic fluctuations occurred in the early successional stage following a perturbation, while, at a later stage of recovery, a greater regularity in seasonal oscillations, reduced amplitude of fluctuations, and little change in dominance rankings signified a change to a more stable community.

Structural changes in benthic communities have traditionally been explored through the calculation of diversity indices (see Washington, 1984 for a useful review of the application of several measures). The most widely used diversity index is the Shannon-Wiener information function H' (Shannon and Weaver, 1949) and its accompanying measure of evenness (Pielou, 1966). These can provide a convenient way of summarising the responses of benthic communities along environmental gradients. Conventionally, high values for diversity/evenness have been associated with stable environmental conditions, where biological interactions are well developed, while low values for diversity/evenness have been associated with unstable conditions, where species are not in competitive equilibrium (e.g., Sanders, 1968). However, this interpretation requires modification in the light of the 'intermediate disturbance' hypothesis (see above). In nature, gradients in physical stability may be determined by the degree of influence of wave or tidal current action at the sea bed, but analogous changes may be expected along gradients of chemical or organic pollution.

An alternative to the use of 'classical' diversity indices as summary measures is the biotic index approach which has been widely applied in freshwater systems (see review by Metcalfe, 1989) and is exemplified in marine areas by the work of Word (1979, 1980). He derived an Infaunal Index from empirical data on the distribution of selected taxa along the Californian coast (USA) in relation to the effects of sewage discharges. In its original form, these taxa were classified into four groups according to their mode of feeding, since it was observed that the response of the benthos to increasing amounts of sewage-derived particulates in the water column, and organic detritus at the sea bed, involved a shift from dominance by suspension-feeding animals (Group 1) to dominance by sub-surface deposit feeders (Group 4). The Index depended for its success on the acquisition of <u>local</u> data; however, a number of the taxa represented are cosmopolitan in distribution. In a recent paper, Ferraro *et al.* (1989) employed a modified form of the Index in the Puget Sound area (USA); of eleven quantitative measures of community structure, the Index ranked first in terms of statistical power.

The Index has the advantage of being grounded in observational ecology rather than mathematical theory in its formulation, thus avoiding some of the controversy surrounding applications of certain other diversity measures, and may find wider application in areas where a sufficient local data-base can be established.

It may be concluded that although predictable changes in diversity may occur in response to pollution - their interpretation again depending on the successional model for change - such indices can be criticised chiefly on grounds of the loss of information resulting from the reduction of multivariate data to single figures. However, they undoubtedly retain a value as summary statistics when used in conjunction with other measures of community structure. (An outline of the various options available for the analysis of marine benthic data is given in Section B.)

Hypotheses concerning the nature of the responses to particular effluents or contaminants may be further tested in the field using experimental enclosures (e.g., Eleftheriou *et al.*, 1982), or alternatively by the maintenance of a small-scale version of the habitat of interest in controlled laboratory conditions (e.g., Gee and Warwick, 1985; Bayne *et al.*, 1988; Pilson, 1990).

4.2 Individuals

Measures of 'fitness' (such as fecundity, growth rate, condition, and certain behavioural and biochemical measures of stress) can be used to determine sub-lethal effects of contaminants at or below the level of the individual organism (see Section 2, above). Such measures may therefore allow the detection of effects of a discharge at a stage prior to the onset of population changes, though the issue as to appropriate choice of sensitive species is itself a controversial one (e.g., Gray, 1980, 1989b; Bayne *et al.*, 1980). Techniques which permit the rapid assessment of relatively large numbers of samples (e.g., the oyster embryo bioassay: Thain and Watts, 1987; see also MAFF, 1990) may also be useful in improving definition of the area of influence of an effluent discharge beyond the immediate mixing zone where changes at the population or community level have already been identified. In principle, the results from field tests can be validated in the laboratory against known dose rates, and *vice versa*. Ideally, test organisms should be selected from a knowledge of the local area of interest (e.g., Pearson and Blackstock, 1983; Bowmer *et al.*, 1986). However, it is more common to use a standard test organism, even at the expense of some 'realism'.

4.3 Concluding Remarks

While an adverse biological effect can often be linked to a contaminant source, it does not follow that the detection of contamination is alone a good predictor of a biological effect, either because the threshold concentration for a response is not exceeded in the locality under investigation, or because only a proportion of the measured contaminant is bioavailable.

Also, in traditional benthic monitoring programmes, a combination of field sampling error and natural variability can limit the ability to detect subtle biological effects of discharges (e.g., Underwood, 1989). This is particularly true in heterogeneous areas where the detection of effects along a gradient of contamination at a distance from the source may, in practice, be severely compromised by gross natural changes in community structure. Further, localities which are naturally exposed to periodic disturbance at the sea bed support benthic populations which by their very nature are likely to be relatively insensitive to low-level contamination.

This leads to the important conclusion that monitoring of benthic population or community responses to the majority of point-source inputs should concentrate on the detection of near-field effects, i.e., including the zone of immediate effluent impact, and beyond this the delineation of their spatial extent within realistic limits imposed by the sampling techniques. In addition, studies should, of course, encompass any zones of long-term accumulation of contaminants at a distance from the point source of interest, where these have been identified, e.g., from a knowledge of the local hydrography, or from the distribution of sediment-bound contaminants.

This last point emphasizes the importance of multi-disciplinary studies, especially at the outset of an investigation where such knowledge may be limited. It should be remembered that determination of the spatial extent of influence of an effluent discharge will depend, *inter alia*, on the analytical detection limits for the chosen chemical or biological measures of contamination, which will not be the same in all cases.

A variety of properties of benthic communities may be employed in identifying 'near-field' effects; these are outlined above and in Section B, below. By contrast, it could be argued that, on practical grounds, the identification of responses at a distance from a discharge ('far-field' effects), especially in heterogeneous areas, is more appropriately directed at the level of the individual. Research into physiological and biochemical norms and ranges of variation in resident species is needed. Such research has already yielded techniques such as the 'Scope for Growth' measurement of Bayne *et al.* (1979); further work is required before they can be recommended as effective and economical alternatives in routine monitoring programmes. For example, it would be necessary to test the presumption that such measures could be used to predict the consequences for peripherally exposed populations. Thus, there is a need to test a range of physical/chemical inducers of response, to calibrate the scale of response, and then to identify relationships between measurable responses and factors of real concern, such as protection of populations (see Section 6, below).

In principle, any test combining the attributes of increased sensitivity and predictive capability over traditional measures could limit the need for field benthic studies around waste discharges. Future research may bring such a possibility closer, and there are already cases where available methods offer sensible alternatives in pollution management, e.g., where liquid wastes are discharged at the surface of deep-water locations, and where there is no prospect of impact at the sea bed other than conceivably via mortality of planktonic larvae of benthic organisms. However, since sediments act as a sink for most contaminants introduced into the marine environment, there is at present no substitute for site-specific studies of the indigenous biota which, if properly designed, should permit effective site monitoring.

Such surveys can be justified not only on scientific and waste management grounds: there is a general public belief in the importance of statements on field conditions as the ultimate measure of success in controlling marine discharges. A scientific parallel may be drawn with fisheries investigations, where there is a continuing requirement for field sampling, and monitoring of commercial catches, in order to validate models of fish stocks and predictions of yield.

A recent tendency towards a polarisation of views on the relative merits of field ecological surveys as against more experimentally orientated approaches to pollution monitoring is not particularly helpful in this debate. Complementary application of a variety of techniques suitable for detecting effects at the individual, population and community level is desirable (McIntyre and Pearce, 1980; ICES, 1986; Bayne *et al.*, 1988), if only as an acknowledgement that no single measure yet exists which can be used to predict the consequences of contaminant effects at all levels of the ecosystem. Such uncertainties are best addressed by widening the scope of impact assessments.

5 RESPONSE RATES

Response rates will depend on the sensitivity and mobility of individuals at different stages of their life-cycle, the nature of the discharge, and the dispersive properties of the receiving environment. Thus, response rates may vary from minutes through avoidance reactions, to years in the case of populations of certain sedentary benthic species.

In nearshore environments, disturbance at the sea bed may occur regularly and predictably in response to tidal currents, or intermittently in response to wave action. 'High energy' conditions commonly result in the maintenance of a superficial soft-sediment benthic fauna at an early successional stage, i.e., favouring short-lived (<1 yr) opportunistic colonisers. (Exceptions include the razor clam *Ensis*, which may avoid surface disturbance by withdrawing into deeper sediment layers.) Opportunistic species may be considered to be less prone to effects of discharges (i.e., more resistant), and the rate of recovery on cessation of disturbance or input faster (i.e., indicating greater resilience), than in the case of longer-lived species characteristic of more stable communities (Boesch and Rosenberg, 1981). Thus, in general, estuarine communities dominated by euryhaline species may be characterized as being both more resistant and more resilient to discharge effects than coastal and, in turn, offshore communities.

Response rates of sedentary benthic communities may be deduced from studies into the path of recovery following reduction or cessation of a discharge (or other human activity). Clark (1989) considers that recovery following acute pollution incidents in temperate regions typically takes place within 6-10 years, while in the tropics recovery may occur within 1-2 years. This is because of relatively short generation times and hence the greater scope for larval recruitment from unaffected areas. Boesch and Rosenberg (1981) predicted that, in general, recovery times for the macrobenthos of temperate regions would be less than five years for shallow waters, including estuaries, and less than ten years for coastal areas of moderate depth.

Communities - such as those occurring on rocky shores - may be dominated by certain long-lived species which through biological interactions have a key role to play in determining the success of others (e.g., Lewis, 1980). Following a pollution event, these may be excluded by competitors in the initial recolonisation phase or, alternatively, may depend for their influence on attaining adult size. Recovery to a pre-pollution state may therefore be prolonged, typically to about 10 years or so in temperate regions, and even more so in the case of, for example, tropical coral reefs. In the Arctic, recovery may be considerably slower (perhaps 100 years) because of naturally infrequent reproductive periods and low fecundity (Clark, 1989).

Examples of studies demonstrating response rates in soft-sediment communities include those of Swartz *et al.* (1986), who detected changes in the benthos along a spatial gradient within three years of a reduction in contaminant loading from Californian deep-water sewage discharges. These changes, interpreted as partial recovery, accorded with predictions from Pearson and Rosenberg's model. In the more quiescent conditions of a Swedish fjord, the benthos took 8 years to fully recover following cessation of an organically rich pulp mill effluent (Rosenberg, 1976).

The rate of sediment infill and recovery of the benthic fauna at previously dredged sites in the Dutch Wadden Sea were examined by van der Veer *et al.* (1985). In tidal channels with fast water flow, conditions approached normality within 1-3 years, while at a more quiescent sandy intertidal site, infilling was incomplete after 15 years. Sediments in the depressions were much finer than nearby, approached anoxia at the surface partly due to entrapment of floating macroalgae, and contained no benthic infauna.

This study illustrates the important fact that the recovery rate is often as much dependent on prevailing hydrodynamic forces determining sediment structure and stability, as on the innate capacity of the benthos to re-colonise new surfaces via larval recruitment or re-distribution of adults.

6 SIGNIFICANCE OF CHANGES IN THE BENTHOS

Effects of waste inputs on marine organisms are generally considered to be ecologically significant only when these effects have consequences at (or above) the level of the population (Anon., 1978; McIntyre and Pearce, 1980; Gray, 1989a).

Most man-made discharges will have <u>some</u> effect on the receiving environment, however small, and it is clearly important to be able to assess both the intensity of any biological effects using currently available techniques, and the spatial scale over which such effects occur and are deemed to be permissible; for large discharges, a regular biological monitoring programme will usually be required in order to ensure containment of such effects (see Section B). In practice, arbitrary limits may have to be set on permissible biological change, as a precautionary measure; it does not follow that outfall-induced changes in the indigenous biota will in all cases have significant implications for commercial or other valued resources.

6.1 Wider Importance of Benthic Communities

The benthos are available in varying degree as food for demersal fish; in addition, certain crustacean and bivalve species may have direct commercial value. The impact on fish populations of any changes brought about by pollution will depend, *inter alia*, on the local importance of the benthos as a food resource and on the geographical extent of any adverse effects, since fish may be able to switch to nearby (unaffected) feeding areas without detriment to the population.

Intertidally, the benthic fauna of sand and mud flats may, in addition, support the nutritional requirements of large populations of birds, especially in estuaries.

The benthos may also have an important role in the cycling of carbon and nutrients (e.g., Patching and Raine, 1983; Graf *et al.*, 1984; de Wilde and Beukema, 1984; Kautsky and Evans, 1987) as well as trace contaminants (e.g., Malins *et al.*, 1986), while the seasonal release of pelagic larvae may influence the structure of planktonic food webs. In the last case, the significance of any interaction is somewhat speculative. Young and Chia (1987) list a wide range of holoplanktonic species which are known to feed on the invertebrate larvae of benthic species, and also cite examples of the potentially important role of fish as predators on benthic larvae. However, in agreement with Strathmann (1974), they consider it unlikely that significant regulation of predator densities occurs as a result of the release of specific larval types.

The significance of feeding by the larvae themselves is even more uncertain, since most studies have tended to concentrate on the activities of holoplanktonic species. Thorson (1946) noted that most pelagic larvae of benthic organisms fed on small-sized phytoplankton. Exceptions include later stages of several decapod larvae (see Strathmann, 1987) and larvae of the polychaete *Nephtys*, which was shown to account for significant mortality of bivalve larvae through predation in the White Sea (Mileikovsky, 1971). Larvae of the polychaetes *Magelona* and *Polydora* are also known to feed on bivalve larvae (Lebour, 1922; Daro and Polk, 1973). Strathmann (*op.cit.*) noted that polychaete larvae appear to have a wider diversity of feeding mechanisms than any other marine invertebrate group.

From work in the Celtic Sea, Warwick *et al.* (1986) argued that the evolution of differences in size structure between dominant members of the holoplankton (especially copepods) and meroplankton (such as benthic larvae) may be partly explained by seasonal partitioning of the phytoplankton food resource, which in spring and autumn are dominated by organisms of different size.

It may be concluded that there is wide scope for interaction between benthic and pelagic communities, including commercial fish species, but the quantitative significance of any interaction can be difficult to ascertain (see below). Finally, the benthos may be valued on scientific or purely aesthetic criteria, e.g., in areas of conservation interest (Gubbay, 1988).

6.2 Assessment of Significance

A biological study around a waste discharge is often considered to be intrinsically more 'relevant' than, for example, a chemical study of contaminant distribution, since its objective is the measurement of a biological effect, and pollution is defined in terms of such effects. In reality, what is normally measured is a biological change and, as with the plankton, so with benthic communities it becomes clear that change occurring on various spatial and temporal scales is not so much an irritating fact of life, but more like a definition of life. Further, while it is sometimes possible to identify the causes of the observed change, e.g., near to an effluent discharge, it is quite another matter to assess its significance. Pollution is defined in terms of the deleterious consequences of contamination; this requires a judgement as to whether or not a change is acceptable in terms of related consequences for valued resources and other facets of the marine environment.

Despite the origins of the science of benthic ecology in the interests of fishery biologists in the feeding habits of fish, the subject has tended to develop somewhat hermetically, and little is known with any clarity of the inter-relationships between the various categories of benthic organisms and the predatory demersal fish. Surprisingly little is known about the relationships between adult benthic organisms and (where present) their own planktonic juvenile stages, especially in terms of control of recruitment, though this matter has been the subject of increasing research effort, with useful results, in recent years (see, e.g., Young and Chia, 1987). Steele (1974), in his attempts at modelling marine ecosystems, pointed to the significant gap in our knowledge of the role of benthic production in fish production. No great advances have been made since then in elucidating this role, although this is clearly a critical issue in attempting to make value judgements about the occurrence of small-scale changes in benthic communities subject to point-source input.

It will be clear from Section 6.1, above, that assessments of the 'significance' of changes in the benthos will differ from site to site, and according to perceptions regarding the importance of a habitat and the need for protection. Although the science of benthic ecology cannot in isolation resolve such differences, nevertheless, the results from many surveys around point-source discharges do much to assuage concern, in as much as they commonly succeed in demonstrating only localised zones of impact at the sea bed. Indeed, it can be argued that, even if relationships between benthos and, for example, local fish populations cannot be readily established, a healthy benthic community (or one subject to only minor change) in the vicinity of a discharge will probably be associated with a healthy fish population, since the most severe effects would initially be felt by the benthos.

The possible effects of discharges on commercial fish stocks are often a primary area of concern, but sampling is unlikely to be feasible on a scale which would throw light on gradients associated with any other than very large or widely dispersed point source inputs (see below). In general, such monitoring is more appropriate to regional scales, where measures of contaminant content (or possibly 'health', e.g., disease prevalence) of fish may provide the ultimate trigger for the imposition of controls on multiple sources (e.g., Preston and Portmann, 1981; Franklin, 1987, 1990). Exceptions to this general rule would be in the case of a commercial shellfish stock in the near vicinity (though this must be a rare occurrence in the case of newly sited discharges), where commercial fish with territorial habits are known to occur in numbers in the disposal area, or where the waste field encompasses a known route for migratory species (e.g., in narrow estuarine reaches).

Fish populations may be directly influenced by discharged contaminants, or indirectly via effects on food organisms, including the benthos. In the latter case, these may arise through changes in the nature or quantity of prey species, or through transfer of certain bioaccumulated contaminants. Both of these areas are amenable to field study and Spies (1984) provides a good example of this approach, applied to large sewage discharges off the Californian coast (USA). There was some evidence to suggest that outfall-induced changes in benthic community type (when occurring over a sufficiently wide area) resulted in changes in fish predator community type.

It may be concluded that, at present, important gaps still remain in our general understanding of interactions between benthic organisms and the wider marine ecosystem, in particular commercial fish species, at all life-cycle stages. Though not a substitute for site-specific studies, such an understanding would greatly facilitate judgements on the significance of localised changes in the benthos in response to waste discharges.

7 DISCUSSION

Effects of discharges on the benthos are in many respects analogous to those following natural disturbance of a habitat, and empirical models have been developed which can be used to predict the effects of waste disposal practices, once a discharge has commenced.

Most surveys around point-source discharges involve the quantitative sampling of benthic organisms by grab, followed by the identification and enumeration of species in the laboratory. The precision and accuracy of the data will depend on several factors, including the number of samples taken, the performance of the sampler, choice of mesh size, patchiness in animal distributions, and heterogeneity in environmental factors such as substrate type (see Section B). The number and type of samples required in order to achieve an acceptable degree of statistical rigour, and the most appropriate sampling design for the determination of spatial or temporal trends, may therefore vary considerably depending on the locality. Thus, it is not possible to provide a general 'blueprint' for the conduct of benthic surveys around waste discharges.

Criticisms of traditional benthic surveys have sometimes been levelled at their lack of 'relevance', the amount of time required for laboratory processing of samples, and the often equivocal nature of the findings. In response to these criticisms, it may be noted that the benthos have proved to be particularly effective as a monitoring tool in the delineation of 'near-field' effects. In depositional areas, whether near to or distant from discharge points, they present a logical target both in view of the likelihood of accumulation of waste-derived materials, including toxic contaminants, at the sea bed and the requirement of pollution surveys for a statement on biological effects in the field.

Increasing attention is also being paid to improvements in sampling practises, and to the quantification of aspects of change in field populations (other than by means of traditional descriptions of community diversity) which can be used in a more cost-effective manner to identify adverse changes before the onset of gross effects, and which may be more easily interpreted in terms of effects on other resources.

At the same time, it must be acknowledged that significant gaps still remain in our general understanding of the functional properties of benthic communities, and of their interactions with other components of the marine ecosystem. These aspects require a greater commitment of resources for their resolution.

Field benthic studies represent only one of many possible tools in the identification of contaminant effects. There are clear advantages to complementary studies aimed at widening the scope of impact assessments. The relevance of benthic investigations can be enhanced through determining the links with other measures of ecosystem response. Such integration - through field and laboratory study - offers the prospect of future improvements in sensitivity and predictive capability.

The significance of effects of waste discharges on benthic populations notably depends on the existence of trophic links with fish populations, and their commercial importance. Studies of feeding habits can help to identify target organisms for monitoring, which may also represent a major route for the transfer of bioaccumulated contaminants. The actual impact on fish populations will depend on the nature, magnitude, and spatial extent of influence of the discharge. In general, the sampling in adequate numbers of commercial fish is unlikely to throw light on local gradients of biological effect associated with other than very large individual point source inputs, in which case they present an obvious target.

Finally, most effluent discharges to the sea will result in some impact on the local environment. The criteria used in deciding upon the acceptability and extent of any impact may embrace aesthetic and conservation interests, as well as those relating to commercial resources. Field studies of the benthic fauna can make a valuable contribution to the decision-making process.

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SECTION B. PROCEDURES FOR THE MONITORING OF BENTHIC COMMUNITIES AROUND POINT-SOURCE DISCHARGES

H L Rees and C Heip

1 INTRODUCTION

This review of procedures for the monitoring of benthic communities around discharge points is based on recommendations of the ICES Benthos Ecology Working Group. These appeared as guidelines in the 1988 report of the ICES Advisory Committee on Marine Pollution (ICES, 1989), and subsequently in the Monitoring Manual of the Oslo and Paris Commissions (OSPARCOM, 1990a).

Though similar principles apply in the planning of discharge studies both intertidally and subtidally, attention is focussed on the latter, where more sophisticated - and costly - remote sampling methods are usually required. We have also concentrated on soft-sediment communities, which are those most commonly encountered in pollution studies and are generally amenable to routine quantitative study. However, alternatives are also considered.

There are a number of key reference works dealing with the general issues of benthic sampling and analytical methods which should be consulted prior to any benthic survey, notably Holme and McIntyre (1984) and Baker and Wolff (1987). These cover the full range of habitats likely to be encountered in monitoring programmes. On a more specific level, examples of recently produced guidelines for impact studies at the sea bed around particular types of point-source inputs (which follow principles similar to those adopted here) include Rees *et al.* (1990) for UK sewage sludge disposal sites, and OSPARCOM (1990b) for North Sea platforms.

The core of most routine monitoring programmes will involve the study of structural properties of benthic communities (e.g., numbers of species and individuals, 'diversity'), in parallel with relevant physical and chemical measurements of the receiving environment. While there is a case for direct study of the effects of waste discharges on benthic processes (e.g., energy flow), further refinements in methodology are needed before routine applications can be advocated (see Section 5 'Analytical methods for measuring community responses', below).

Gray *et al.* (1980) reviewed the attributes of various components of the marine biota in the context of field monitoring of pollution effects. We concur that the benthic macrofauna, i.e., animals living within or in close association with the sea bed and which are retained on 1 mm (or 0.5 mm) mesh sieves, continue to offer the most suitable target in routine monitoring at the community level, for the reasons outlined in Section A, above.

It should be noted that there is increasing interest in the use of meiofauna as a monitoring tool. Many members of this group - conventionally separated from the macrofauna at a mesh size of 0.5 mm - have the advantage (in terms of pollution studies) of having no pelagic larval stage, and individuals are in intimate contact with the pore water by virtue of their small size. Less field sampling effort is required than for the macrofauna, but their main disadvantage lies in the need for a high level of taxonomic expertise (see Section D).

As further research is done on meiofauna, it is to be expected that additional cost-effective options will become available for routine monitoring. Microfaunal analysis, however, is not sufficiently developed at this stage to provide standard procedures.

2 DESIGN AND IMPLEMENTATION OF FIELD SAMPLING PROGRAMMES

In the case of new waste arisings, it is essential that benthic monitoring programmes commence **before** the onset of discharges, to allow the identification of any effects by direct comparisons of pre- and post-disposal data. However, it is recognised (see below) that a modified strategy will be required in cases where discharges pre-date impact assessments.

2.1 STAGE 1: Desk Study

The starting point in any benthic survey must involve an appraisal of the environment at and around the (proposed) receiving area, entailing:

1) a review of the literature on the biology, physiography and hydrography;

2) a review of human impacts - past and present - including port/harbour construction, offshore structures, dredging/dredgings disposal, other point-source or diffuse waste inputs (e.g., nutrients, possible eutrophication), fishing practices;

3) a review of uses - present and predicted - including waste disposal, commercial fishing, shipping, recreation; and

4) an assessment of potential impact (scale and intensity) of the discharge in the water column and/or at the sea bed.

The outcome of this review will determine the degree of monitoring effort, if any, which will be required to meet the objectives of waste disposal management. In the event of **any uncertainty** regarding the predicted outcome of waste discharge, a programme of biological monitoring is advocated. In stages 2-5 below, it is assumed that there are grounds for anticipating some interaction between discharge products and the sea bed biota.

On average, a macrofauna sample will take 1-2 days to analyse by an experienced individual, but there may be considerable variation outside this range (see Section 4 'Laboratory processing of samples', below). Without prior knowledge of the sampling area, it will be difficult at this stage to assign costs to samples; this will depend on the outcome of stage 2, below. Other important cost considerations include site accessibility - and therefore ship-time and size - and time allocated to data processing/reporting.

2.2 STAGE 2: Planning a Sampling Programme

If existing local information on the benthic biota and supporting habitat is adequate, then the programme may proceed to stage 4, below. If not, then systematic sampling over an appropriate spatial scale is required, using a grid of stations.

The area covered will depend on tidal and residual movements, as well as the nature and quantity of the waste. As a general rule, the **minimum** area covered should enclose the zone of initial sea-bed impact, if known, or alternatively an area defined by at least one tidal excursion from the discharge point. Sampling should also encompass nearby depositional areas, if any, within which there may be a possibility of accumulation of any persistent contaminants in the longer term.

The nature of the sea bed will determine the most effective type of sampling gear. In hitherto unworked areas, a pilot survey using a range of devices will be required in order to resolve any uncertainty regarding the nature of the substrate. This will have the additional benefit of allowing an assessment to be made of the relative importance of the biota living within, on or just above the sea bed. Deployment of still and video cameras, either remotely or by divers, can provide useful data during this phase of sampling (see, e.g., Holme, 1984; George *et al.*, 1985; also Rhoads and Germano, 1986, and O'Connor *et al.*, 1989, for application of a remotely operated sediment profile camera).

In areas of rough ground which are unsuitable for grabs, samples of the biota within or on the sea bed may be obtained by dredging, but usually at the cost of accurate quantification. Alternative methods, such as the use of video or still photography, may be necessary for routine monitoring of such areas (see Section 3 'Field sampling methods', below).

For soft substrates, a grab of standard design conforming with the Day or Van Veen type can be used (see Eleftheriou and Holme, 1984, and Rumohr, 1990, for descriptions; also Section 3 'Field sampling methods', below). These should sample a standard area of 0.1 m^2, should allow access - on retrieval - to the surface sediment to allow sub-sampling, and should have stainless steel buckets to minimise the risk of trace metal contamination, if such determinations are to be made. An estimate of the retained sediment volume should be routinely made; volumes of less than 5 litres on muddy ground, and less than 2 $^1/_2$ litres on hard-packed sandy ground, would normally be discarded.

A sieve mesh of 1 mm can be recommended for field extraction of samples of the benthos from sediments in this type of survey, prior to fixation and return to the laboratory, though local circumstances may occasionally dictate the use of coarser or finer meshes. The implications of mesh size for sample processing and interpretation are dealt with by Eleftheriou and Holme (1984), Bishop and Hartley (1986), Hartley *et al.* (1987), Bachelet (1990), and stage 4, below.

The primary aim of this initial survey is to describe the distribution of the benthos, and to relate this, as far as possible, to habitat type. It should also be possible to identify any major impacts of existing discharges. For soft substrates, samples of the benthic biota should be accompanied by sediment sub-samples for analysis of particle size and a range of physical or chemical contaminant tracers appropriate to the outfall(s) under consideration.

The importance of integrating physical, chemical, and biological approaches to sampling must be strongly emphasised, since changes in the biota near to waste discharges invariably provide only circumstantial evidence for effects. Such evidence can be considerably strengthened by a knowledge of waste transport pathways and the distribution of contaminants, as well as natural environmental variability, though absolute proof will rarely be attainable without recourse to follow-up laboratory investigation.

Because of this descriptive emphasis, and the need for adequate spatial coverage, the information content of such surveys can be maximised - relative to the resources available - by sampling singly at several stations, rather than repetitively at a reduced number.

2.3 STAGE 3: Analysis and Interpretation of Data

At this stage, analysis will be required in order to identify patterns in the spatial survey data, and to allow selection of stations for regular follow-up monitoring (see below). A variety of methods may be employed, either for the analysis of spatial or temporal trends, and these are outlined in Section 5 'Analytical methods for measuring community responses', below.

2.4 STAGE 4: Rationalisation of Sampling Design for Regular Monitoring

The number of sampling stations will largely be governed by spatial heterogeneity at the sea bed, predicted dispersal pathways, and cost considerations. Replicate sampling at a minimum frequency of three per site or stratum may be recommended to allow statistical comparisons between stations in space and/or time. Additionally, the choice of mesh size will have a considerable bearing on sampling accuracy. Because the costs of benthos surveys are usually a function of laboratory processing time, the decisions taken concerning numbers of stations and replicates, along with the timing and frequency of sampling, will be critical to cost-effectiveness.

Local circumstances and, especially, the nature of the waste will dictate the sampling effort required. The simplest case which can be envisaged is the disposal of an inert solid waste with minimum dispersion, the immediate effect of which is the elimination of all biota; clearly the sampling effort required in order to delineate such an effect will be minimal. This is very different from the case of a complex effluent of uncertain composition, where the early onset of any adverse change may be of interest. Here, for example, it may be necessary to use finer sieve meshes (0.5 mm) for extraction of the benthos, along with increased replication, to facilitate the detection of subtle effects.

Important considerations at this stage are the efficiency with which taxa and individuals are sampled, and the proportion of animals retained on different mesh sizes. Upon these will depend the number of samples required to achieve a particular level of precision, which will in turn depend on the survey objectives.

These can be investigated during a pilot survey, or at selected sites on the first occasion at which this sampling stage is implemented. It should be remembered that the cost of collecting additional samples in the field is usually small in comparison to that of laboratory processing, so that those which eventually turn out to be surplus to requirements can be discarded.

A detailed account of these aspects of sampling design is given in McIntyre *et al.* (1984) and Hartley and Dicks (1987). Other relevant sources of information include Andrew and Mapstone (1987), Bros and Cowell (1987), Caswell and Weinberg (1986), Cuff and Coleman (1979; see also Green, 1980, and Cuff, 1980), Green (1979, 1984), Hurlbert (1984), Kingston and Riddle (1989), Millard and Lettenmaier (1986), Saila *et al.* (1976), Skalski and McKenzie (1982), Stewart-Oaten *et al.* (1986), and Walker *et al.* (1979).

In the case of new or existing discharges, a judgement is required as to the degree and spatial extent of degradation of the habitat, if any, which can be accepted. This represents a logical progression from stages 1 to 3, above. Subsequent monitoring will then have as its primary aim the establishment that there is no worsening trend in intensity or extent of impact with time. Clearly, the facility to detect and quantify such changes will be determined both by the adopted sampling strategy and the chosen measures(s) of biological response (see Section 5 'Analytical methods for measuring community responses', below).

It will be noted that, while the emphasis is on the monitoring of temporal trends, the incorporation of a **spatial** element is necessary. In its simplest form, a stage 4 strategy will involve sampling at two sites which are comparable in all respects save for the influence of the discharge. However, in practise, local heterogeneity will invariably demand a greater sampling effort.

The objective at this stage can, therefore, be met by selecting a limited suite of stations which represent those areas of interest for regular follow-up monitoring. These may include zones of waste impact identified from the physical, chemical and/or biological data generated from stage 2 (above), or locations deemed to be potentially at risk.

It should be remembered that the sensitivity of different analytical measures of contamination may vary widely. The presence of detectable levels of contamination does not necessarily imply a biological effect and *vice versa*. Such factors must be considered in weighting the contribution of contaminant data to sampling design.

The sampling design should aim to minimise the influence of extraneous environmental variability; this may be relatively straightforward if, for example, the main dispersal pathway is aligned with depth contours, and environmental conditions along this line are similar. More complicated situations may arise, e.g., where dispersal is across depth contours; in shallow water, this is often accompanied by gradients of substrate type caused by the natural sorting processes of wave and tidal action.

A further complication near to urban or industrial areas is the potentially confounding influence of other discharges in the vicinity. In both these cases, sampling should be designed so as to adequately represent the major strata identified from the descriptive survey.

As a general rule, the frequency of sampling will be greater at the onset of a discharge, in order to allow for: 1) any uncertainty in predicted impact, and 2) stabilisation of impact (intensity and extent) with time. However, many studies of the responses of benthic communities to discharges suggest that only exceptionally will there be a need to sample at a frequency of more than once per year.

Initially, annual sampling at the same time each year is therefore adequate. Ideally, sampling should be conducted within a period (often spring) which will avoid seasonal maxima in larval recruitment, since the transient presence of many of the latter may obscure quantitative trends in adult populations which have been exposed to discharges for longer periods. This can have an additional benefit, since the identification of juvenile stages is often problematic and can add significantly to processing time. The frequency of sampling may subsequently be reduced if there is evidence of stability in the response to the contaminant(s).

The choice of mesh size, as well as timing, will in many cases affect the proportion of juveniles and adults in samples (e.g., Rees, 1984; Bachelet, 1990), and these factors must be taken into account when interpreting the results from regular surveys. At one extreme, it may be noted that small size at settling and slow growth of some macrofauna species may result in failure to recruit to even the finest (0.5 mm) sieve mesh commonly in use within the one- to two-year period that might elapse between surveys (see Buchanan *et al.*, 1986).

2.5 STAGE 5: Establishment of Routine

This will involve adherence to standard protocols for sampling and analysis, and these must include continued monitoring of the relevant physical and chemical parameters. However, some flexibility must be allowed for, e.g., in response to changes in the quantity of waste discharged. The continued validity of the rationalised sampling design should be checked by periodically repeated stage 2 grid surveys.

3 FIELD SAMPLING METHODS

Box-core samplers (such as the Reineck box-core, see Eleftheriou and Holme, 1984) have the potential advantage over grabs of digging deeper into bottom substrates, and creating less disturbance at the sediment surface as a result of a frontal pressure wave which has been associated with the latter (see Eleftheriou and Holme, 1984, and Hartley and Dicks, 1987). However, because of their size and weight, they require larger vessels and relatively calm conditions for their efficient deployment, and so are less versatile than grabs.

Remote grab samplers of the Day or Van Veen type can, therefore, be recommended for routine monitoring of soft sediments. Non-standard equipment should not be deployed. If comparisons are to be made between data sets obtained by different gear, then calibration of performance against recommended designs will be necessary (Rumohr, 1990).

Regarding vertical distribution within sediments, most studies show that the majority of benthic organisms occur in the surface 5-10 cm, and will be adequately sampled by grab. However, the distribution of biomass may be different, in that older individuals, especially of bivalves, may live at depths considerably greater than this. Such occurrences, and their significance to the outcome of monitoring programmes, can only be tested by comparisons of the results of grab and deeper-penetrating core samplers.

Protocols for field sampling, including extraction of the benthos on sieves, and preservation, are given in Eleftheriou and Holme (1984), Hartley and Dicks (1987), Hartley *et al.* (1987), and Rumohr (1990).

Areas of hard ground (e.g., rocks, coarse gravel) present particular problems for quantitative sampling at the community level, as there may be considerable uncertainty as to the sampling efficiency of dredging devices. However, this can be overcome in areas accessible to divers. For example, non-destructive quantitative assessment of the fauna and flora of rock faces has been carried out over several years using stereo photography (Lundalv and Christie, 1986).

Also in shallow subtidal rocky areas, the fauna inhabiting kelp (seaweed) holdfasts, collected by divers, has been used as a monitoring tool (see Moore, 1973). Recent reviews of survey approaches for both inter- and sub-tidal rocky habitats are given by Gamble (1984), Baker and Crothers (1987), and Hiscock (1987).

Monitoring strategies in such localities may benefit from a consideration of the role of individual resident species (especially sedentary bivalves) as indicators of biological effects and/or contaminant bioaccumulation at the population level (e.g., Rees and Nicholson, 1989).

4 LABORATORY PROCESSING OF SAMPLES

The laboratory undertaking will depend on the objectives of data analysis (see Section 5, below), but typically will require the identification and enumeration of all taxa encountered in preserved samples. In most cases, identification to the level of species can be achieved by reference to standard taxonomic keys. The time - and therefore cost - required to achieve this will vary considerably according to:

1) the expertise and continuity of staff;

2) previous knowledge of the area. Familiarity gained from initial surveys, and/or access to historical reference collections of taxa encountered, can considerably enhance the speed and efficiency of processing;

3) mesh size. In general, the larger the mesh size used in sampling, the easier and faster will be the rate of processing, since juvenile stages and a range of adult species of small size will tend to be omitted. Identification of the former, in particular, can be problematic;

4) the nature of the samples. Samples containing large quantities of residual material in addition to the benthic fauna can create special problems at the sorting stage. For example, fine organic detritus, often found in association with muddy depositional areas, may extend the sorting time to several days, in contrast to a fine sandy sediment, where the separation of sediment from the fauna may be virtually complete at the field sampling stage.

Extraction of the biota in the presence of quantities of inorganic material, such as coarse sand or gravel, can be speeded up by simple decantation, or more sophisticated procedures such as Barnett's fluidised sand bath (see Eleftheriou and Holme, 1984; also Pauly, 1973), provided that thorough checks are made on the efficiency of the procedures.

If sub-sampling is considered to be necessary, it should be remembered that while this may be acceptable for species counts, only exceptionally will this account for the full range of taxa present. Thus, sorting of the entire sample is required, unless this deficiency can be tolerated in subsequent comparisons of the data. Such a compromise can impose severe limitations, especially when making comparisons with studies elsewhere and, if possible, should be avoided.

Details of laboratory procedures are given in Eleftheriou and Holme (1984), Hartley *et al.* (1987), and Rumohr (1990). Regarding biomass determinations, these should be expressed as ash-free dry weight using appropriate conversion factors (Rumohr *et al.*, 1987), or following procedures outlined in the report of a recent ICES intercalibration exercise (Duineveld and Witte, 1987; see also Rumohr, 1990).

It will be appreciated from the above that a major proportion of the resources committed to benthic monitoring programmes will be taken up at the laboratory processing stage. Model sampling strategies must be translated into the reality of local routines, and some compromises are invariably necessary. It is, therefore, imperative that this aspect is taken into account at the survey planning stage and adequate resources allocated.

5 ANALYTICAL METHODS FOR MEASURING COMMUNITY RESPONSES

The purpose of this account is to outline the main classes of techniques available for the analysis of benthos data, rather than to provide an exhaustive literature review, which may be found elsewhere (see, for example, Burd *et al.*, 1990, for recent coverage of this topic). Further details on several of the techniques, together with examples of their application to field data on the macrofauna and meiofauna, can be found in Sections A and D.

5.1 Numerical Analysis of Primary and Derived Variables

5.1.1 Primary variables

Following quantitative sampling, determinations of species composition, **densities**, **weight** and preferably **size**, fulfill a basic requirement of most routine benthic monitoring programmes. Any subsequent rationalisation, e.g., selection of target organisms for single-species studies, or identification to the level of higher taxa only, should proceed only after the establishment of a sound baseline of knowledge of the biota in the receiving area.

The variables of total abundance, number of species, and biomass can be surprisingly robust indicators of environmental changes, and have been shown to respond predictably along organic enrichment gradients (see Pearson and Rosenberg, 1978). Moreover, these are explicable in terms of functional responses of the biota to alterations in the benthic habitat. They may be expressed graphically, or by simple mapping techniques, depending on the sampling design.

5.1.2 Derived variables

The ability to detect gradients or trends using the primary variables is often limited. The application of classical univariate or bivariate statistical techniques may even be misleading. A variety of summary statistics and ordering techniques have been developed which may be used for the further elucidation of structure in the data, and to aid the formulation of hypotheses concerning effects of discharges. They may also provide an objective basis for rationalising subsequent sampling programmes (Clarke and Green, 1988).

It should be remembered that a number of these methods employ sophisticated mathematical techniques, a sound appreciation of which is required for the correct interpretation of the output. Heip *et al.* (1988a) provide a critical review of several commonly used methods for the analyses of marine benthic data. Further details are provided in Section D, in the context of meiofauna investigations.

These techniques take three main forms:

a) graphical displays of species-area or species-abundance relationships

Many different methods exist which are variants of ranked species abundance (RSA) curves and k-dominance curves in which species are ranked according to abundance. Species abundance distributions are frequency distributions with a logarithmic or linear ordinate. When geometric abundance classes are used, a log-normal distribution is often found.

These graphs and distributions are useful tools for presenting the data, but since these are empirical relationships, it is difficult to detect causality when changes occur.

b) diversity

Two aspects are recognised: species richness, i.e., the number of species, and equitability, i.e., the distribution of individuals among the species.

Many different indices have been proposed. A coherent system may be found in the diversity numbers of Hill (1973), which include S (the number of species), H' (the Shannon-Wiener index) and Simpson's index, among others. Diversity indices are useful and may be compared statistically. The different diversity numbers in the Hill series cover different structural aspects and thus more adequately represent overall community structure. However, full species-abundance plots contain more information (Heip *et al.*, 1988b).

c) classification and ordination

These are techniques capable of synthesis and ordering of the data. Classification involves arranging the sites or species into groups (clusters), setting them apart from the members of other groups. Ordination attempts to place sites or species in a space defined by one or more axes in such a way that knowledge of their position relative to the axes conveys the maximum information about them.

Classification and ordination should be standard practice to analyse abundance or biomass data obtained in surveys involving many species and stations. The definition of a standard protocol of data analysis is still awaited (but see Gray *et al.*, 1988; Clarke and Green, 1988). However, the general availability of personal computers and software packages brings these methods within easy reach. A bibliography of selected references on this topic is given at Annex 1.

5.1.3 Conclusions

Many options are available within each of the above categories of data analysis, and no single measure can be recommended as suitable in all cases. Rather, there is merit in the application of a variety of different measures of community structure, a procedure which is facilitated by the wide availability of statistical packages.

5.2 Other Measures of Community Properties

5.2.1 Investigations at higher taxonomic levels

The traditional taxonomic unit in marine pollution investigations is that of the species. Recently, a number of workers have demonstrated that gradients of response can often be adequately described at higher taxonomic levels (Warwick, 1988a,b; Heip *et al.*, 1988a,b; Herman and Heip, 1988). Warwick (1988b) argues that natural variables, such as water depth and substrate type, tend to result in species replacement within taxonomic groups as a result of evolutionary adaptation. However, the advent of man-made pollution is too recent for such adaptation, and hence within-taxon species changes in response to natural variability will tend to be overridden by a uniformity in response at higher taxonomic levels, e.g., at the level of the family or above.

Such an approach could also result in significant reductions in time spent identifying rare species; this might facilitate the processing of more samples, often a practical limitation in pollution surveys.

Presently, validatory work has concentrated largely on the retrospective analysis of data from well-defined pollution gradients. While the technique clearly has promise, a wider programme of testing of its efficiency is required before its routine adoption can be recommended.

5.2.2 Trophic groups

For most species, the predominant feeding mode can be deduced from the literature, and such information may be used as a measure of the response of the benthic fauna to waste discharges. For example, a shift in dominance from suspension-feeding to surface deposit-feeding may indicate excessive turbidity, or increased accumulation of organic matter at the sea bed (e.g., Pearson, 1971).

One limitation which should be borne in mind is that some species have the ability to switch from one feeding mode to another (e.g., Buhr, 1976). Dauer (1984) provides a critique of the utility in impact studies of classifying polychaetes into feeding guilds, as far as current knowledge permits.

5.2.3 Vertical distribution of fauna

Soft sediments present an array of habitats suitable for faunal colonisation both within and on the substrate, including, for smaller-sized species, the interstices between particles. Effects of discharges may be manifested through a reduction in habitat suitability, e.g., due to sediment accretion, or the development of anoxic conditions at depth as a result of excessive organic matter accumulation.

Periodic examination of the vertical distribution of the fauna (e.g., from undisturbed sediment cores), along with measures such as redox potentials, may therefore be useful in assessing the progress of any degradation process within sediments (e.g., Pearson, 1987).

5.2.4 Size spectra

The response of benthic communities to pollution may be expressed in terms of changes in the frequency distribution of body size, e.g., for the macrofauna, smaller 'opportunistic' species are commonly found to predominate near to discharges, at the expense of larger, slow-growing species (Pearson and Rosenberg, 1978; Pearson, 1987; see also Warwick *et al.*, 1986). This approach requires exhaustive treatment of samples if carried out over the full size range of the benthic biota (e.g., Schwinghamer, 1983), and - though a promising area of research - could not be advocated for routine monitoring.

Recently, Warwick (1986) and Warwick *et al.* (1987) have proposed a method of pollution detection based on the relation between ranked abundance and biomass curves of the macrofauna at individual sites (see Section A). The technique may prove to be useful, but presently requires further empirical testing.

5.2.5 Community metabolism

Impairment of community function in response to waste inputs may have consequences for the supply of energy (as food) to higher trophic levels, and may be expressed through changes in, e.g., oxygen uptake, ETS (electron transport activity), ATP concentrations, and heat production.

These may be measured *in situ* or from sediment sub-samples and can be converted into units of carbon or energy flow. As rate functions, they can contribute to models of ecosystem energy flow, and thus have potential application in assessments of assimilative capacity, or vulnerability to contaminant effects (e.g., Pamatmat *et al.*, 1981; Graf *et al.*, 1984; de Wilde *et al.*, 1986).

Presently, methodological problems and uncertainty regarding the accuracy of some of the measured responses preclude their routine application. However, the general approach is considered to have potential value as a monitoring tool, for use in conjunction with traditional studies of community structure.

5.2.6 Annual production calculated from growth and mortality rates

This entails separate examination of each of the dominant species using methodology outlined by Crisp (1984), followed by summation to obtain an estimate of community production. The approach has the advantage of providing data on: 1) the performance (e.g., growth rates) of individual species in proximity to discharges; 2) the 'carrying capacity' of the receiving environment, in energetic terms; and 3) the nature and quantity of biomass available as food for fish.

The main disadvantage is that the sampling and initial analytical effort can be time consuming; at least seasonal sampling is required. It may be possible to overcome this using annual P:B (production:biomass) ratios for species studied elsewhere, but this may not always be valid, since the ratios may vary substantially from one region to another. The approach cannot presently be advocated for routine monitoring programmes, but the potential for future application should improve as the database expands (see Brey, 1988).

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SECTION C. EXAMPLES OF EFFECTIVE BENTHIC SURVEY DESIGN

H L Rees

1 INTRODUCTION

A strategy for the conduct of benthos sampling programmes around point-source discharges was outlined in Section B. In the following account, the principle features of this strategy are illustrated by reference to an idealised waste discharge. This is followed by discussion of a framework for the interpretation of benthic changes, and then examples from the pollution literature are described, in order to demonstrate the importance of effective benthic survey design.

1.1 Sampling Plan for a Hypothetical Discharge

Figure 1 summarises the use of guidelines for an idealised point-source discharge to a uniform area of sea bed. These range from an initial desk study, to exploratory and quantitative baseline surveys, and then to regular monitoring, in accordance with the account given in Section B.

For brevity, attention is confined to sediment quality and the benthic infauna, but this is not to underrate the potential value of widening the scope of impact assessments to include other biota (e.g., epifauna and demersal fish communities).

The frequency at which restricted-scale regular and more intensive baseline-type surveys are conducted will mainly depend on:

1) the nature and prior history (if any) of the waste discharge: a new input may require more frequent sampling until an acceptable steady state has been reached regarding the intensity and spatial extent of impact;

2) alterations to the nature and rate of an existing discharge: clearly, unanticipated changes occurring at regular or baseline stations as a result of alterations in a discharge may necessitate modification to the survey design or frequency;

3) the perceived sensitivity of the receiving environment, e.g., in relation to commercial fisheries resources or conservation value; such an evaluation may also involve other than purely scientific considerations.

As a general rule, the minimum sampling frequency will be annual for regular surveys on a restricted scale, and every 2-3 years for the more detailed baseline-type surveys. In both cases, these should be conducted at the same time of the year so as to minimise natural variability in the data.

The progression of Figure 1 is towards concentration of effort at a limited number of strategically placed sites, at which replicate samples are taken. In this hypothetical example, the regular survey design may take one of two forms, both of which retain a facility for **spatial** comparisons:

1) the first assumes a good knowledge of the waste transport pathway, but some uncertainty as to the precise nature and lateral extent of benthic changes in relation to the waste plume;

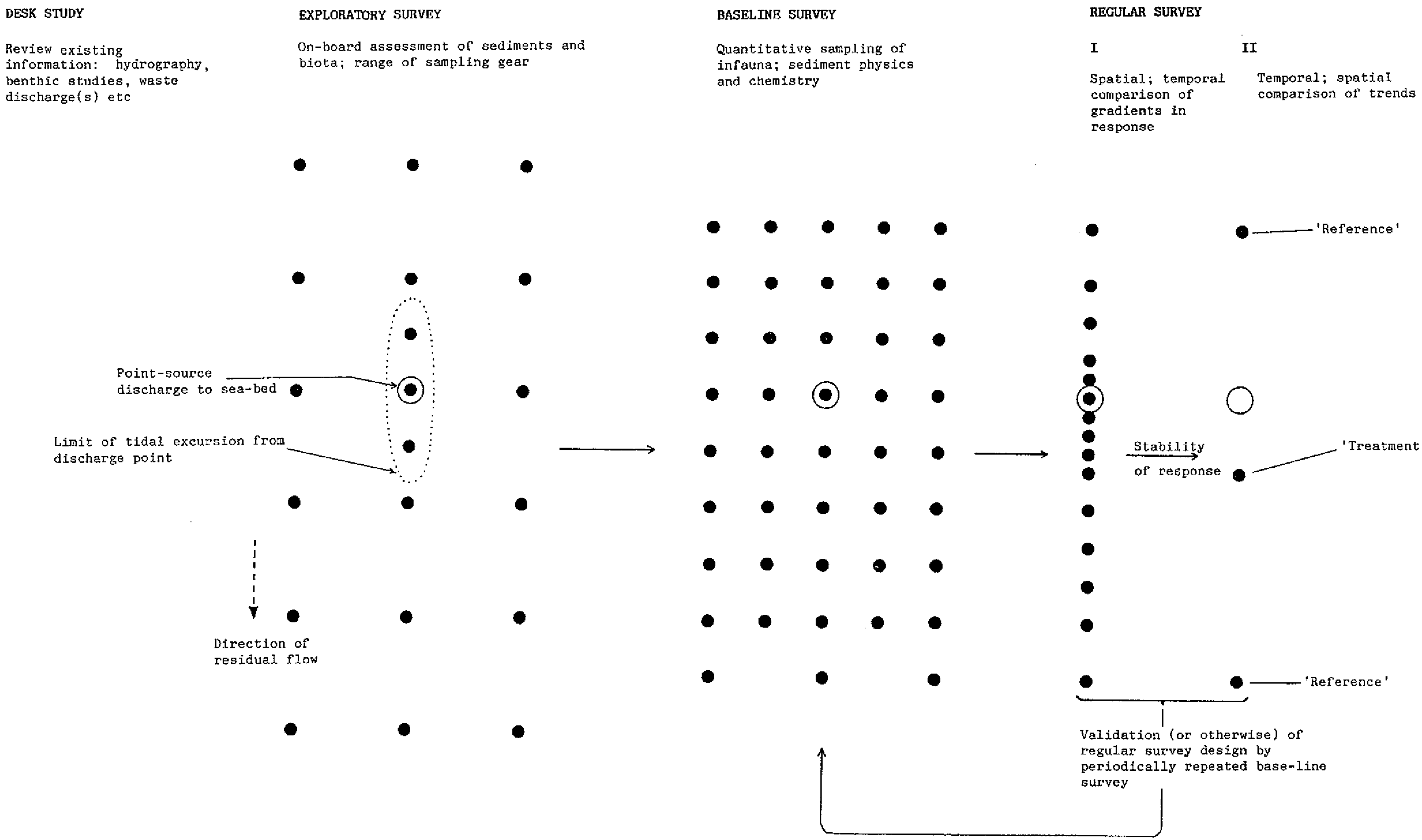


Figure 1. Idealized sampling strategy for benthic monitoring at a point-source discharge.

2) the second assumes a good knowledge of both these factors, following stabilisation of the benthic response (within acceptable bounds) to a discharge of constant rate and composition; in such a case, repeated sampling should provide reassurance as to the nature and extent of discharge impacts and could, therefore, justify a much reduced sampling effort.

Coupled with periodically repeated baseline surveys, the overall strategy should be to detect both predictable and otherwise unforeseen changes (e.g., episodic pollution events), which may warrant an expansion of sampling effort, further controls on the discharge, or both.

The aim is to simplify the interpretation of trends for management purposes. However, it should be emphasised that it is not possible to provide a blueprint for the conduct of surveys of the sediments and benthos at all locations, because of the wide array of environmental conditions, as well as discharge types.

1.2 Description of an Empirical Model for Interpreting Benthic Changes in Response to Point-Source Discharges

A framework for the interpretation of any changes which occur in response to a waste discharge is clearly fundamental to the successful outcome of any monitoring programme, as noted in Section A. An empirical model developed by Pearson and Rosenberg (1978) for changes in the benthos in response to organic enrichment - probably the most commonly encountered manifestation of discharge effects - has proved to be particularly useful in this respect. As it has a bearing on the examples given below, a brief description of the model is appropriate.

A summary of the changes which are predicted to occur along an enrichment gradient is shown in Figure 2. A general enhancement in species numbers and biomass at low input levels is followed by proliferation of small-sized and short-lived 'opportunists' at the expense of larger, less tolerant species, as organic matter builds up and sediments become increasingly anoxic with depth. At this stage, total abundance markedly increases, but species numbers and biomass decline. Further accumulation, leading to conditions of anoxia even at the sediment surface, results in the elimination of all macrofauna species.

The model has the advantage of being equally applicable to trends in space and time; this can be illustrated by reference to a discharge which is decreasing in quantity. The response in space along a series of stations away from the outfall, sampled on succeeding occasions, will be a compression of the sequence of changes shown in Figure 2 towards the right, i.e., there will be a reduction in the **spatial extent** over which changes will occur. The **response in time** at a single location near to a discharge will precisely mirror the sequence from right to left as shown in Figure 2. Actual examples of its application in both dimensions are given below.

2 EXAMPLE 1: SEWAGE DISCHARGES OFF THE COAST OF CALIFORNIA (USA)

The following account draws selectively from a voluminous literature concerned with the monitoring of sewage discharges along the Californian coast, much of which was carried out under the auspices of the 'Southern California Coastal Water Research Project' (e.g., Bascom, 1979; Mearns, 1981; Mearns and Word, 1982), and more recently by the US Environmental Protection Agency (Swartz *et al.*, 1985, 1986). This work has employed a variety of sampling designs to meet different objectives, and follows a progression from wide-scale spatial grids extending well beyond any zones of impact, to line transects targeted at the examination of localised effects. The latter involved repetitive sampling at a limited number of stations, to facilitate a statistical comparison of trends.

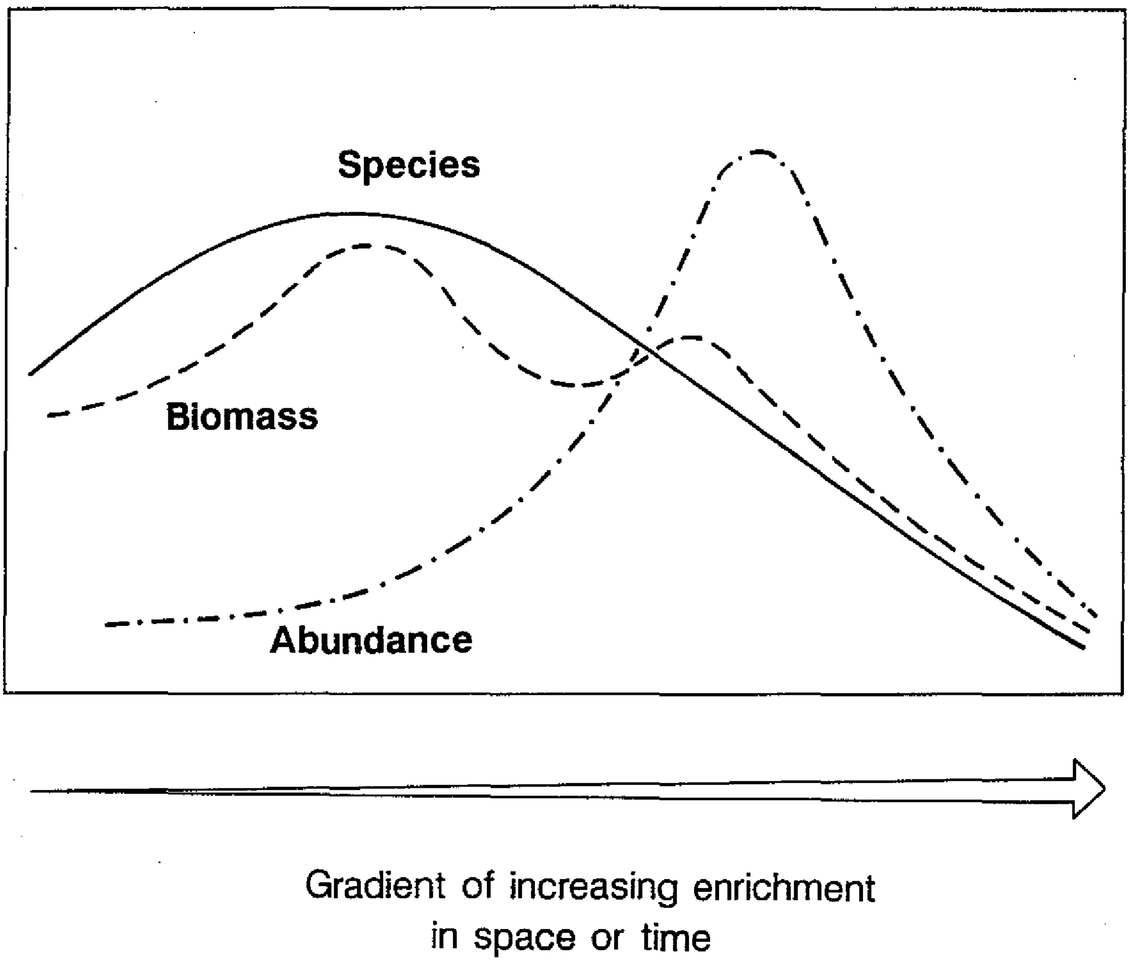


Figure 2. Empirical model for changes in the macrobenthos in response to organic enrichment (after Pearson and Rosenberg, 1978).

2.1 Baseline-Type Surveys and the Establishment of 'Control' Stations

The main pipeline sewage discharges are located at depths of at least 60 m, and the predominant near-field dispersal pathway for particulates at the sea bed is parallel to the coastline. Figures 3a and 3b show spatial trends in a variety of sediment contaminants and faunal indices at stations sampled along the 60 m contour. Two 0.1 m^2 Van Veen grab samples were taken at each station, one for sediment quality and the other for benthos using a 1 mm mesh sieve. The effect of these outfalls, especially off Palos Verdes, can be clearly discerned.

The information from this and other sources was used to define 'normal' conditions for a range of chemical and biological variables in the depth range 20-200 m, in the latter case employing a locally derived 'Infaunal Index' (see Word, 1979). The findings were used to interpret the data from a survey employing 300 stations (also sampled singly for the benthos) spanning this depth range; the region off Palos Verdes is shown in Figure 3c as an example.

Contours identify a 'degraded' area in the immediate vicinity of the outfalls, along with a 'changed' fauna with a notable north-westward extension. These contours agree with observations on a range of trace metal contaminants, e.g., zinc (Figure 4, from Hershelman *et al.*, 1981).

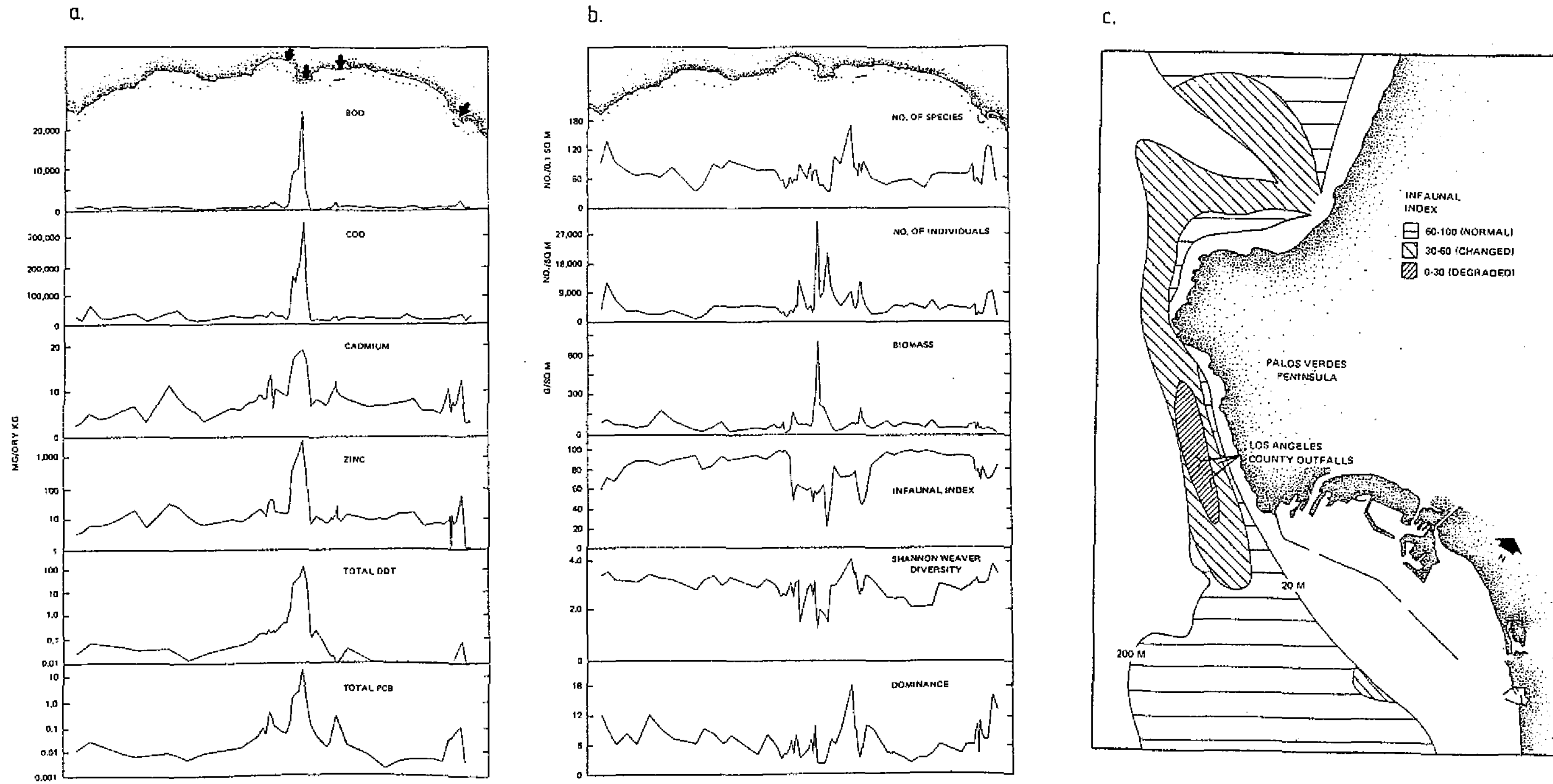
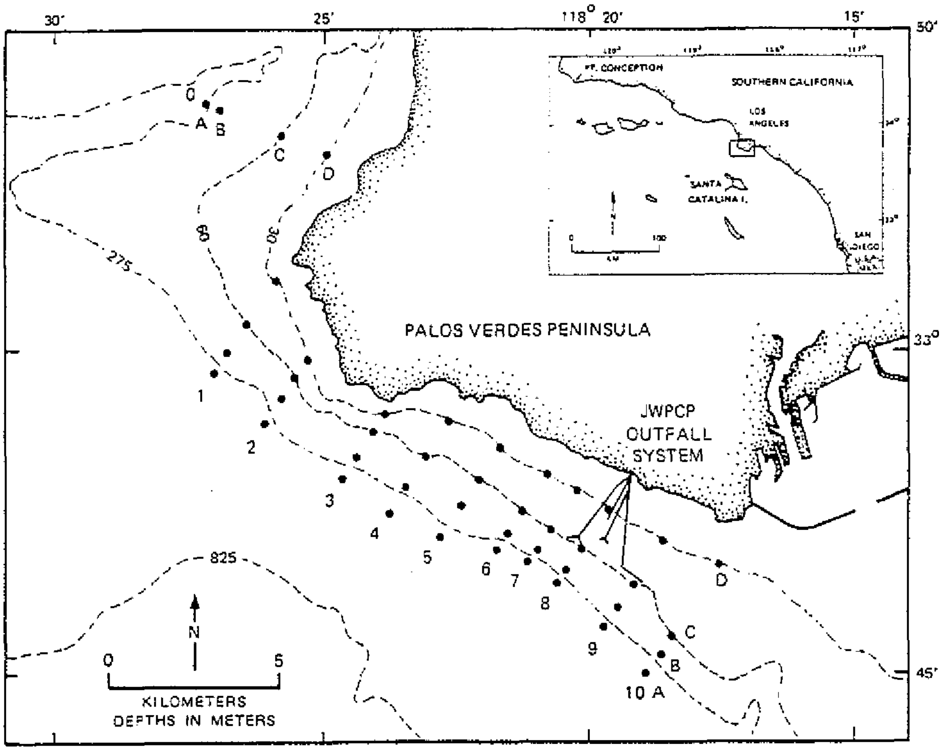


Figure 3. Trends in sediment contamination (a) and faunal indices (b) along the 60 m depth contour (arrows show the location of the major deep-water sewage discharges) (from Word and Mearns, 1979); zonation of the benthos determined from a grid of stations sampled off Palos Verdes (c) (from Bascom, Mearns and Word, 1979). Reproduced with permission from the Southern California Coastal Water Research Project Authority.

a.



b.

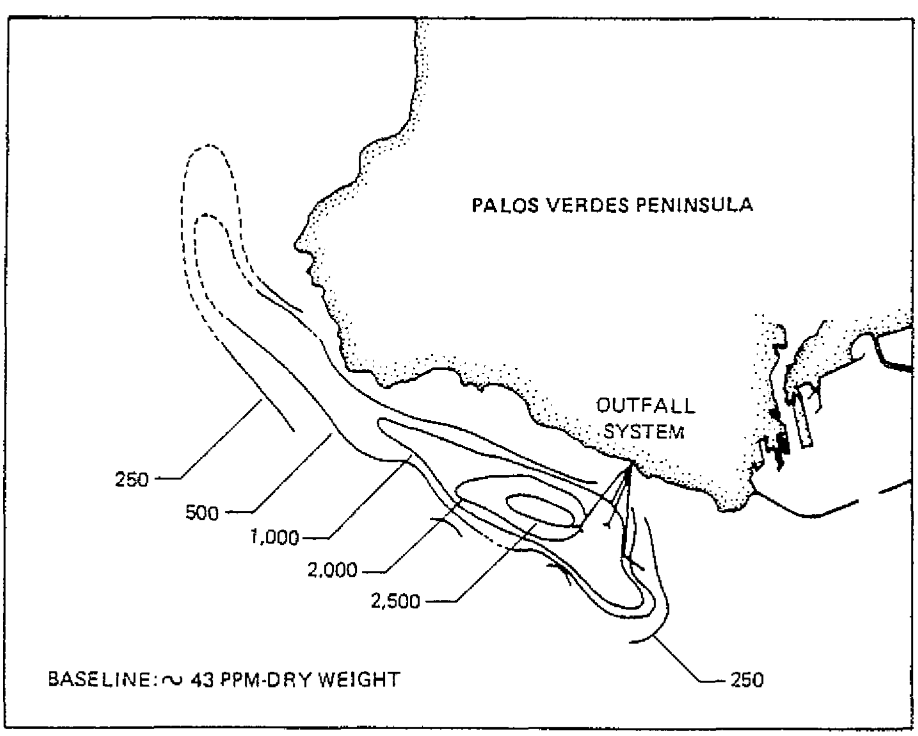


Figure 4. Location of sediment sampling stations (a) and contours for zinc concentrations (b). (Reprinted with permission from Marine Pollution Bulletin, Vol. 12, Hershelman, G.P., Shafer, H.A., Jan, T.-K., and Young, D.R., Metals in marine sediments near a large California municipal outfall. Copyright 1981. Pergamon Press PLC).

2.2 Rationalised Sampling Design for Determining Effects Along a Main Gradient of Contamination

Knowledge of the main dispersal pathway, contaminant distributions, and impacts on the benthic fauna off Palos Verdes allowed Swartz *et al.* (1985) to restrict the sampling effort to a limited number of stations westward of the discharges at 60 m depth, including one station representative of 'normal' conditions as defined by the above baseline study. In this survey, ten Van Veen grab samples were taken at each station: five for the benthic infauna, using a 1 mm mesh sieve, and five for physical, chemical, and toxicological analyses. Sampling was conducted in 1980 and 1983 (Swartz *et al.*, 1986), thus allowing statistical comparisons between stations and between years, during which time the contaminant loading from the discharges was estimated to have decreased by about 20%.

One of the main objectives of this work was to compare changes along the contamination gradient with the empirical model of Pearson and Rosenberg (1978), which predicts a sequence of successional events in the benthos in response to organic enrichment, as described above.

Trends in numbers of individuals, species, and biomass along the Palos Verdes station transect (see Figure 5) were broadly in agreement with the model. For example, in 1980, stations close to the outfall were strongly dominated by the polychaete *Capitella*, a common indicator of disturbed or enriched sediments. Dominance by this species was much reduced in 1983, and this was accompanied by significant increases in the number of species and in biomass. Other differences included a significant reduction in species number, densities and biomass at intermediate stations, and in a number of sediment contaminants. There were no significant differences in these variables at the distant control site.

Sediments sampled near to the outfall in 1980 were acutely toxic to an amphipod crustacean in a bioassay experiment, but there was no evidence for toxic effects in 1983.

This decrease in the effects of enrichment, along with any which might be attributable to chemical contaminants, led the authors to conclude that there had been a significant improvement in conditions between 1980 and 1983. This was ascribed to a reduction in contaminant loading during this period, though the disturbing effect of severe storms may also have contributed to improved sediment quality. It may also be noted that Mearns and Word (1982) established a significant **spatial** relationship between emission rates of suspended solids at five Southern California sewage discharges and the area of sea bed occupied by an altered benthic fauna.

3 EXAMPLE 2: OIL PLATFORM DISCHARGE OFF THE UK COAST

The second example, selected from the large body of literature concerned with monitoring around oil platforms, is that of the Beatrice oilfield in the UK sector of the western North Sea. The Beatrice field was discovered in 1976; its development since then, along with an account of sea-bed monitoring, is described by Addy *et al.* (1984), Addy (1987), and Hartley and Bishop (1986), among others.

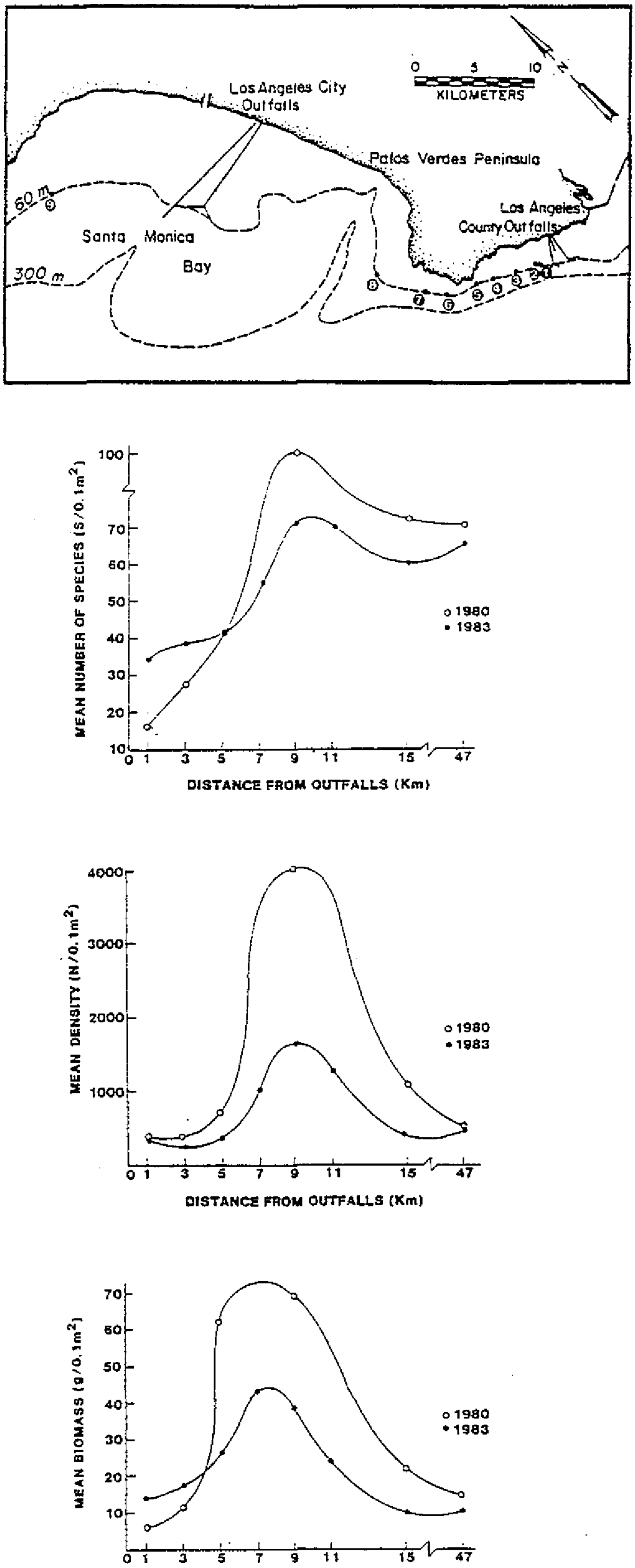


Figure 5. Station locations and trends in the main faunal indices (from Schwartz *et al.*, 1986).

3.1 Baseline Surveys

Pre-production surveys involved sampling on a grid of widely spaced stations encompassing the oil platforms. The sampling grid in 1981 is shown in Figure 6a (from Levell *et al.*, 1989). Most of these stations were sampled singly for the benthic macrofauna, using a 0.1 m^2 Day grab, and a 1 mm mesh sieve to retain the animals. Additional sediment samples were taken for physico-chemical analyses, including particle size distribution and hydrocarbon content. These surveys allowed the faunal distributions to be related to natural environmental factors (Hartley and Bishop, 1986); there was no apparent influence due to industrial activity (Addy *et al.*, 1984).

3.2 Rationalised Sampling Design

Drawing on the knowledge gained from the baseline surveys, and with an expectation that effects on the benthos would arise principally from the disposal of drilling discharges in the near-vicinity of platforms, subsequent investigations employed a limited number of stations located on inter-crossing transects (Figure 6b, from Addy *et al.*, 1984). Note that the innermost stations are much more closely spaced than on the baseline grid; also that the NNE-SSW transect is aligned with the predominant direction of tidal flow.

At each station, two Day grabs were taken for the benthic macrofauna, also using a 1 mm mesh sieve. A further grab was taken to obtain sediment sub-samples for physico-chemical analyses.

The concentrations of aliphatic hydrocarbons in the sediments are shown in Figure 6c, on a logarithmic scale; as might be anticipated, the highest values occurred very close to the platform. Away from this, generally higher values were found along the NNE-SSW transect. Note also that higher overall concentrations after February 1982 could be accounted for by a switch from water-based to 'low toxicity' oil-based drilling muds.

Counts of the opportunistic polychaete *Capitella* (Figure 6d) provide a useful illustration of local perturbation following the use of oil-based muds: the highest densities occurred in the immediate vicinity of the platform, and elevated counts extended over a greater distance on the NNE-SSW transect. These changes were accompanied by a marked reduction in the numbers of taxa close to the platform.

In this example, an integrated study of the benthos, and sediment physics and chemistry provided the basis for delimiting a localised zone of gross biological effect, which was attenuated along the axis of principal water flow. While it appears that sampling along this axis might profitably have been extended to a greater distance from the discharge point (and indeed subsequent work has taken this into account), the main effect could be attributed to a combination of physical disturbance in the immediate vicinity of the discharge, organic enrichment (including some changes analogous to the Pearson and Rosenberg model of Figure 2), and toxicity.

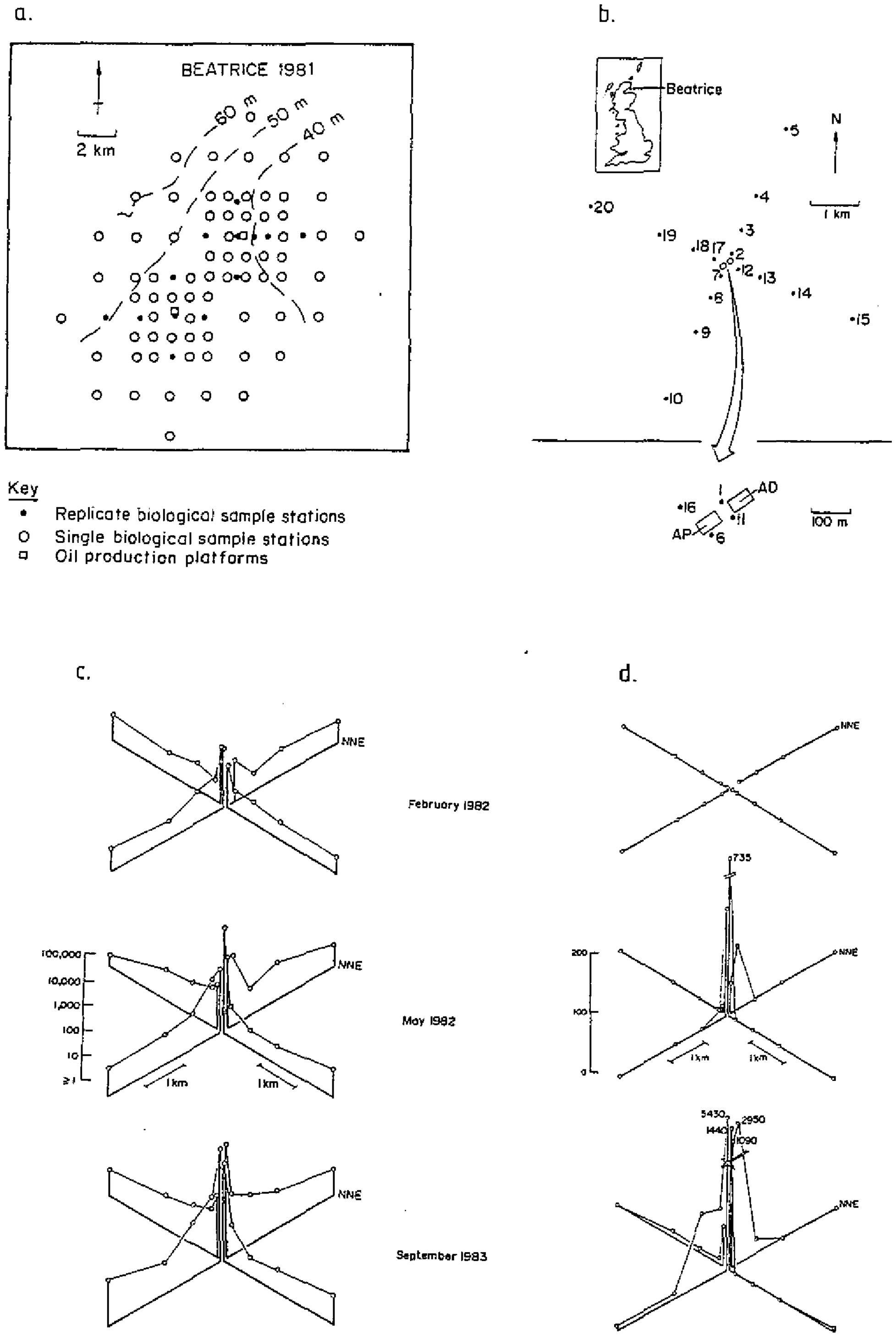


Figure 6. a. Grid of stations employed in a 1981 survey of the Beatrice oilfield (from Levell *et al.*, 1989; reproduced with permission of J. Wiley and Sons and acknowledgement of the Institute of Petroleum);

b. intersecting transects for studying 'near-field' effects (AD = drilling platform; AP = production platform);

c. concentration of aliphatic hydrocarbons (ppm) in sediments;

d. estimated numbers of *Capitella* per m^2.

(b, c, and d reprinted with permission from Marine Pollution Bulletin, Vol. 15, Addy, J.M., Hartley, J.P., and Tibbetts, P.J.C., Ecological effects of low toxicity oil-based mud drilling in the Beatrice oilfield. Copyright 1984. Pergamon Press PLC).

4 EXAMPLE 3: PULP MILL DISCHARGE IN A SWEDISH FJORD

This example is drawn from survey work in a Swedish fjord, aimed at investigating the effects of an organically rich pulp mill waste (Rosenberg, 1976). The sampling area, and station locations, are shown in Figure 7a. At each station, five samples were taken with a 0.1 m^2 Smith-McIntyre grab, and the macrofauna were retained on a 1 mm mesh sieve. Sediment sub-samples were analysed for particle size and organic carbon.

Note that the extensive amount of previous work on the water, sediments, and biota of this area (reviewed by Rosenberg, 1976), along with the narrowness of the fjord location, allowed the selection of representative stations for an examination of longitudinal trends away from the discharge.

4.1 Spatial Gradients and the Value of Temporal Continuity

Changes in space along a gradient of decreasing organic enrichment from left to right (Figure 7b) closely parallel those predicted by the model of Figure 2, although it is of interest to note bi-modality in the curve for abundance. Figure 7c is particularly useful in highlighting the value of continuity in sampling, allowing the progress of temporal changes to be evaluated. In this case, predictable changes occurred in response to the cessation of the discharge at a site in close proximity; the recovery time was about eight years.

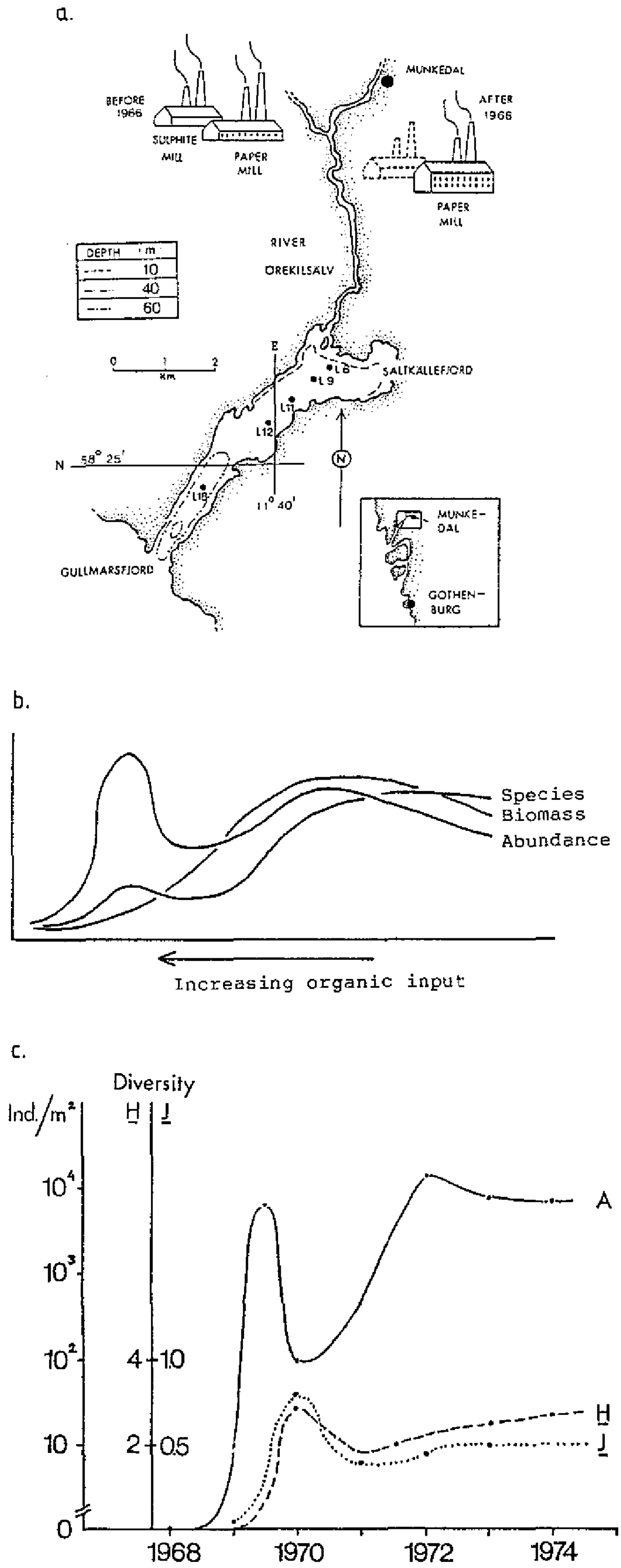


Figure 7. a. Station positions in the Saltkallefjord, W. Sweden (from Rosenberg, 1976; reproduced with permission of Oikos);
b. summary of spatial changes in the benthos;
c. temporal changes in abundance (A), diversity (H), and evenness (J).

(b and c from Pearson and Rosenberg, 1978; reproduced with permission of the Oceanography and Marine Biology Annual Review, Aberdeen University Press).

5 CONCLUSIONS

It should be noted that the above examples were chosen from among the many available for two reasons: a) because of the amount of published information provided over many years, and b) the manner in which impacts and their spatial extent were established through multidisciplinary study, employing appropriate sampling design.

These examples together demonstrate the importance of:

1) geographically extensive 'baseline' surveys to establish zones of waste impact and the broad boundaries of natural spatial variability;

2) integration of studies, especially on hydrography, sediment physics and chemistry, and biology;

3) rationalised sampling designs, where possible, in order to simplify the interpretation of trends across the main zones of impact identified from baseline surveys;

4) sample replication, facilitated by the reduction in effort implicit in 3), allowing statistical analysis of trends in space and/or time;

5) continuity in sampling;

6) predictive models for the responses of the benthos to waste discharges (which are commonly non-linear along gradients of contamination) against which observed changes can be assessed.

Finally, while a survey strategy which succeeds in accurately detecting impacts on the benthos can provide a valuable management tool, it should be emphasised that, at present levels of understanding of ecosystem responses to waste discharges, this will rarely obviate the need for a value judgement as to the wider significance of observed changes.

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SECTION D. THE USE OF MEIOBENTHOS IN POLLUTION MONITORING STUDIES: A REVIEW

M Vincx and C Heip

1 INTRODUCTION

The potential of the meiobenthos for pollution monitoring has previously been discussed by Marcotte and Coull (1974), Pequegnat (1975), Gray *et al.* (1980), and Heip (1980).

From a practical point of view, the sampling of meiobenthos in intertidal as well as in subtidal areas is relatively simple and is possible on a small scale: only small amounts of sediment are necessary to elucidate the structure of the meiobenthic communities (using cores). However, because of their small size, meiobenthos species are laborious to sort and to identify; only hard-bodied taxa (especially copepods and nematodes) are recommended in a monitoring context because highly specialized techniques are needed to identify soft-bodied forms such as Turbellaria.

Nematodes and copepods are abundant in almost every marine habitat (normally ranging between 500 and 10 000 individuals/10cm^2), making them suitable for ecological and statistical analysis. Nematodes are particularly notable for their persistence as a taxon, and are found in all environmental conditions that can support metazoans. The high diversity of meiobenthic organisms has been used in the past as an argument against the use of these organisms for biomonitoring purposes, because of the difficulties encountered in identifying the numerous species. The wider use of meiofauna in pollution monitoring studies was therefore facilitated by the publication of comprehensive taxonomic works which made identification much easier for the ecologist (for copepods: Wells, 1971, 1978, 1979, 1981; Bodin, 1979; for nematodes: Gerlach and Riemann, 1973, 1974; Tarjan, 1980; Lorenzen, 1981; Platt and Warwick 1983, 1988).

Another particular advantage of nematodes and interstitial copepods is their conservative life cycle (i.e., the absence of highly mobile pelagic life stages), so that local contamination effects are not hidden by immigration. They have a rapid turnover compared to the macrofauna; they also have a short life-span and are in intimate contact with pore water. Thus, they should demonstrate a fast response to pollution.

Usually, eutrophication and organic pollution will lead to increased food supply and a rise in the total number of benthic organisms. Other types of pollution do not increase the food supply. The difference is important because changes in community structure induced by organic pollution and toxic pollution cannot be identical.

2 DETECTION OF POLLUTION-INDUCED DISTURBANCES

Several methods for the detection of pollution-induced disturbances have been proposed. They all take into account the changes observed in the structural aspects of the community caused by pollution:

1) taxon diversity of the meiofaunal components;

2) relative abundance of higher taxa of the meiobenthos (e.g., the ratio of nematode to copepod abundance);

3) species diversity of dominant taxa (indices, graphical methods); and

4) species distribution patterns.

The impact of pollution on the functional aspects of meiobenthic communities (e.g., respiration, productivity) has not been well studied.

2.1 Taxon Diversity

Taxon diversity of the meiofaunal phyla has been proposed as a possible tool for the assessment of pollution effects by Van Damme and Heip (1977) and by Herman *et al.* (1985). Taxon diversity is lower in polluted conditions; this is caused mainly by the disappearance of the rare taxa (e.g., Ostracoda, Gastrotricha, Halacarida, Hydrozoa, Tardigrada). However, because of small sample size, sampling techniques are often not adequate to provide an accurate density estimate of these rare taxa, which sometimes occur in numbers as low as 1 to 10 individuals/10 cm^2; the diversity of the meiobenthic community is then much influenced by the presence or absence of these rare taxa. It is well known that sediment composition is also very important in the determination of taxon diversity. Herman *et al.* (1985) examined taxon diversity of the meiobenthic communities at 18 stations along the Belgian coast; in the sandy stations up to seven different higher taxa were found, while in more than 50% of the other stations only one or two taxa (i.e., nematodes and copepods) occurred.

Amjad and Gray (1983) also found a decrease in the number of meiofaunal taxa along an organic enrichment gradient, which was similar to the gradient in the nematode-copepod ratio (see below).

Aissa and Vitiello (1984) examined the meiofauna of the lagoon of Tunis which is influenced by the discharge of sewage arising from the urbanized area. Densities decreased according to an increasing gradient of organic pollution (and a rise of the redox potential discontinuity layer). Nematodes and polychaetes were the most resistant meiobenthic organisms; indeed, nematodes were the only surviving metazoans at the most severely affected sites.

Keller (1984, 1985) described the effect of domestic sewage on the structure of the meiobenthic communities along a transect off Marseille, France. She differentiated three areas: (1) a heavily polluted coastal zone, where sediments were devoid of macrobenthic animals, and supported a relatively poor meiofauna (nematodes, copepods and acari). The copepods were uncommonly large in size and constituted most of the total benthic biomass; the nematodes were mainly freshwater species; (2) an intermediate zone, which was much richer in meiofauna and also more diversified. Polychaetes increased in number, while acari became scarce and copepods decreased in size. 0.4-1 km away from the outfall, where the sediment was strongly polluted, the nematode community consisted of large individuals which contributed greatly to the biomass; (3) an offshore, slightly polluted, zone, where meiofauna densities were reduced and individuals decreased in size with increasing depth. Generally, an enrichment in the meiofauna was evident from the coastal to the intermediate zone. Enrichment induced by urban pollution had been recorded previously, but not at a distance of more than 1 km away from the outfall.

Huys *et al.* (1984) and Smol *et al.* (1986) found that nematodes and copepods were more abundant in a dumping area for waste from titanium dioxide (TiO_2) manufacture; also, that there was a significantly lower taxon diversity of the meiofaunal groups.

Frithsen *et al.* (1985) examined in detail the response of benthic meiofauna to long-term, low-level inputs of fuel oil, such as may be present at the heads of urbanized estuaries and bays. They used mesocosms (outdoor tanks) containing sediment and sea water from Narranganset

Bay, Rhode Island, USA. The abundances of metazoan meiofauna decreased during periods of oil addition; ostracods and harpacticoid copepods were the most sensitive metazoan groups. Abundances of most meiofaunal groups returned to levels similar to those of the controls within 2 to 7 months after the termination of oil additions. However, the abundance of kinorhynchs and halacarids remained depressed for more than 1 year after the last oil addition, presumably due to residual oil in the sediments.

Moore *et al.* (1987) found a sharply reduced density and species richness of all intertidal meiofaunal taxa within 320 m of the discharges of hydrocarbons in the Firth of Forth. However, at 600-900 m, meiofaunal densities were enhanced or depressed, relative to clean sediments, depending upon the seasonal pattern of the redox potential discontinuity layer. Oil platform discharges resulted in strongly reduced nematode densities in the near vicinity. By contrast, copepod densities were greatly enhanced, which was considered to be due to the epibenthic habit of the species involved, enabling them to flourish in conditions of an enhanced food supply and/or low predation and competition.

In a review of field experimentation in meiofaunal ecology, Coull and Palmer (1984) referred to fourteen papers which described pollution experiments in the field or in meso- or microcosms. It was evident from this work that changes in structural parameters of the most important taxa of the meiofauna differed according to sediment type, the nature of the pollutant (oil, sewage, nutrients), sampling techniques and time.

The ability to discriminate between stations along a putative pollution gradient in two Norwegian fjords was just as efficient when the meiofauna data were pooled into taxonomic groups higher than the level of the species (Heip *et al.*, 1988a).

2.2 The Nematode-Copepod Ratio

The use of ratios to observe trends in marine data sets was suggested by Margalef (1975, 1978). He found that a number of ratios describing planktonic ecosystems decreased in response to disturbance (stress, upwelling, and pollution). Amongst the ratios were the numbers of dinoflagellates to diatoms, zooplankton biomass to phytoplankton biomass, and carnivore biomass to herbivore biomass.

Parker (1975) and, in more detail, Raffaelli and Mason (1981) proposed the nematode-copepod ratio as a tool for pollution monitoring using meiobenthic organisms. However, these two studies interpret this ratio in an opposite way.

Parker (1975) compared the subtidal meiofaunal composition of two estuaries in North America: one was polluted by industrial waste from the Dow Chemical Company factory (the Brazos River Estuary) and the other was almost completely undisturbed (the Colorado Estuary). He found that under disturbed conditions 'benthic copepods predominate at their trophic level, while under normal conditions nematodes predominate'. However, only 'surface material from an undisturbed grab sample sufficient to fill a 6-ounce jar' was examined; this may have resulted in overestimation of the copepods, since these animals are mostly restricted to the upper cm of the sediment, while nematodes occur much deeper.

Raffaelli and Mason (1981) compared the response of nematodes and copepods to organic pollution in intertidal areas along the British coast. They sampled to a depth of 35 cm and found that the ratio of nematode to copepod densities was highest where sewage pollution was most obvious. In particular, an increase in the abundance of deposit-feeding nematodes (capable of using the high amount of organic material) was noted, while the copepods decreased in number.

Organic pollution causes an immediate increase in food supply so that, in such areas, extremely high densities of meiobenthos (mainly nematodes) can occur.

Raffaelli and Mason's publication (above) stimulated a large response in the literature concerning the use of meiofauna in pollution monitoring studies, because a very simple, and therefore attractive, tool had been proposed. Most studies were carried out on organically enriched beaches along the British coasts; within these areas, the nematode to copepod ratio increased in response to the presence of large quantities of organic wastes (Warwick, 1981; Raffaelli, 1982; Lambshead, 1984; Shiells and Anderson, 1985). Similar observations were made in the Oslofjord by Amjad and Gray (1983).

The ratio of nematodes to copepods also increased with decreasing particle size, but ratios from polluted sites were always extremely high. Ratios from clean beaches were low and always less than 100, even for muddy sites; all intertidal sites (comprising fine as well as coarse sediments) with ratios exceeding 100 were polluted with organic material (sewage). Some sublittoral ratios from unpolluted sites were high, but never approached the very high values characteristic of polluted intertidal areas. The sublittoral ratios also increased with depth. It is obvious that this ratio must be used with caution as the index is also strongly affected by sediment granulometry.

Coull *et al.* (1981) thoroughly discussed the validity of the nematode/copepod ratio and pointed out that spatial and temporal variations, as well as other ecological processes (such as predation) could alter the ratio independently. The authors rightly pointed out that it is not permissible to reduce the complex meiofaunal community structure to a single ratio.

Warwick (1981) proposed a refinement of the ratio based on trophic dynamic aspects of the meiofauna. He assumed that food is the factor which limits energy flow through the nematode and copepod communities; in that case, the total number of copepods should be proportional to the number of type 2A nematodes (epigrowth-feeders) only, as only 2A nematodes are dependent on the same food source as the copepods. If copepods are indeed more sensitive to the effects of pollution than nematodes, then changes in the proportion of copepods relative to type 2A nematodes might be a useful indicator to separate the effects of pollution from any changes or differences in sediment type. Warwick (1981) suggested that pollution might be indicated by nematode/copepod ratios of around 40 for fine sediments, and 10 for sands. These values are considerably lower than the values of over 100 proposed by Raffaelli and Mason (1981).

Platt *et al.* (1984) and Lambshead (1986) suggested that the ratio should be abandoned as a practical pollution indicator, because (1) it oversimplifies a highly complex set of relationships, and (2) nematode and copepod populations may react independently to a variety of environmental parameters (of which pollution is only one).

Shiells and Anderson (1985) proposed a possible improvement to the ratio whereby only interstitial forms are included, so that only those animals occupying the same micro-habitat are compared.

An increase in copepods due to pollution (as found by Parker, 1975) was recorded by Vidakovic (1983), Moore and Pearson (1986), and Hodda and Nicholas (1986).

Vidakovic (1983) examined Adriatic sublittoral stations, which are constantly influenced by sewage; in this area, the number of copepods increased more than the number of nematodes.

Moore and Pearson (1986) also found an enhancement of copepod density resulting from sewage pollution. They concluded that the nematode to copepod ratio is mainly determined by the availability of high dissolved oxygen levels to the copepod fauna. In both studies (Vidakovic, 1983; Moore and Pearson, *op. cit.*), the overlying water contained high levels of dissolved oxygen.

Coull and Wells (1981) found no relationship between the ratio of nematodes to copepods and pollution. However, they sampled sediments only to 1-2 cm depth. A comparison with data from the Southern Bight of the North Sea (unpublished results) suggests that these authors may, as a result, have missed up to 90% of the nematodes.

Hodda and Nicholas (1985) also found that the ratio was not related to pollution; they examined the meiofauna associated with mangroves in southeastern Australia, which was strongly influenced by inorganic pollution; nematode as well as copepod densities decreased as pollution increased.

In mesocosm experiments with organically enriched sublittoral soft sediments, Gee *et al.* (1985) found that the nematode to copepod ratio was unreliable as a biomonitoring tool. The authors suggested that the differential responses in community structure between the nematode and copepod components of the meiofauna might be a better indication of stress at the community level.

Raffaelli (1987) discussed the variable behaviour of the nematode/copepod ratio in organic pollution studies. It was concluded that differences in the habitat requirements of nematodes, mesobenthic and epi-/endobenthic copepods affected the responses of these groups to organic pollution.

Thus, it is clear that the ratio of nematodes to copepods is not *a priori* a valid tool for pollution monitoring, because the ratio is much influenced by the type of sediment, the nature of the pollution (organic/inorganic), and the location of the habitat (intertidal/subtidal).

We conclude with the following quote from Raffaelli (1987): "Whatever the merits of the nematode/copepod ratio for marine pollution monitoring, it is interesting that so many experienced investigators should feel disposed to test the index, and this has itself highlighted some important relationships in meiobenthic ecology".

2.3 Oligochaeta

Although oligochaetes never dominate in marine meiofaunal communities, it may be noted that Hodda and Nicholas (1985) found that the relative abundance of oligochaetes was significantly correlated to levels of pollution in the mangroves of the Hunter River Estuary.

The oligochaete *Limnodrilus* sp. was found to be overwhelmingly dominant in some heavily polluted estuaries, accounting for up to 70% of all meiofauna (Brinkhurst and Jamieson, 1971; Coull and Wells, 1981).

Coates and Ellis (1980) proposed as 'the most practical index for marine pollution' the percentage of total adult enchytraeids represented by *Lumbricillus lineatus*. Unfortunately, in many marine biotopes, this species is completely absent; it occurs mostly in estuarine conditions.

2.4 Gastrotricha

The gastrotrich genus *Turbanella* is an indicator of organic enrichment on beaches (Gray, 1971; Raffaelli, 1982).

2.5 The Relative Abundance of Species

Changes in the relative abundance of species have been advocated as a useful means of demonstrating pollution effects at the community level. This can be done by the simple use of diversity indices, or by plotting the distribution of individuals among different species.

The nematode and copepod assemblages are commonly studied at the species level, and different analytical techniques have been proposed to detect sublethal effects of pollution on the species distribution within these groups.

2.5.1 Diversity indices

The use of a variety of diversity measurements in order to assess the relative complexity of a community in relation to the degree of pollution has increased enormously during the 1970s. Heip *et al.* (1988b) reviewed the use of the different diversity indices that are available; opinions about the different methods of measuring diversity are almost as numerous as the number of articles discussing them.

A coherent system of diversity and evenness indices is the series of diversity numbers proposed by Hill (1973), which includes species richness (number of species), exp H (H = Shannon-Wiener index) and Simpson's index (see Heip *et al.*, 1988b).

The Shannon-Wiener diversity index has been used to indicate long-term changes in community structure (e.g., Heip, 1980) and generally has lower values in polluted situations. The Shannon-Wiener index is often coupled with a measurement of evenness which, independently of the number of species in the sample, will approach a maximum value when the individuals are divided more evenly among species. Platt *et al.* (1984) remarked that the Shannon-Wiener information function is currently the most popular diversity index among marine biologists. The index is more biased towards the species richness component of diversity than many other popular indices. Since it is dominance (the reciprocal of evenness) which appears to be more relevant in the context of pollution, Simpson's index is preferred, because it weights species by their abundance (Platt *et al.*, 1984).

Gray (1979) has shown that statistically significant changes in diversity indices are associated with only very gross changes in community structure; therefore, the value of using a diversity index in a monitoring context must be questioned.

Lambshead *et al.* (1983) offered a criterion for comparing diversity based on the pattern of dominance of all species in a sample. The method is applied by plotting the % cumulative abundance of species: the so-called 'k-dominance' curves. This method can reveal that some assemblages cannot be compared in terms of diversity or equitability (when the curves intersect): the intrinsic diversity indices are unreliable under these circumstances. The k-dominance curves provide an easily visualized picture of diversity.

2.5.2 Species-abundance distributions

Species-abundance curves can only be drawn if the sample is large and contains many species ($S > 30$) (see Heip *et al.*, 1988b).

The relative abundances of species can be described in 'statistical models', which make assumptions about the probability distributions of the numbers of the species within the community. Heip *et al.* (1988b) discussed the use of several such models. Notable among these are the logarithmic normal (log normal) and log series statistical models of species frequency distributions, which have been found to describe data from natural communities of harpacticoid copepods (Gray, 1978; Castel, 1980; Hicks, 1977; Hockin and Ollason, 1981; Hockin, 1982) and nematodes (Shaw *et al.*, 1983; Platt and Lambshead, 1985).

Gray and Mirza (1979) and Gray (1979, 1981) proposed that unperturbed communities can be identified by the fit of the log normal model to the observed species frequency distribution, while perturbed communities suffering from pollution are fitted by the log series model.

However, other authors (e.g., Kempton and Taylor, 1976) suggest the fit of a log series distribution for stable communities, and a log normal distribution for unstable communities.

Caswell (1976) derived the log series species distribution through application of a neutral model, i.e., a model in which the species abundances are governed entirely by stochastic processes such as immigration, emigration, birth and death, and not by competition, predation or other specific biotic interactions (see Heip *et al.*, 1988b). To date, effort has been mainly concentrated on the fitting of models to field data; the parameter estimates of these models have not been widely used in further analysis. Comparisons of different distributions by means of subsequent statistical testing are the only useful characteristics of these models.

2.5.3 Examples

COPEPODA

Marcotte and Coull (1974) examined the changes in species composition, diversity, and survival strategy of the subtidal harpacticoid community in response to organic enrichment in the North Adriatic. In winter, the copepods numerically dominated the most polluted stations; copepod diversity decreased in response to increased organic enrichment. The harpacticoids nearest the pollution source were dominated by *Tisbe* sp. in winter, and by *Bulbamphiascus imus* in summer. The material was collected from the top 10 cm of the bottom and sieved on a 0.125 mm mesh. Coull and Wells (1981) examined the intertidal meiofauna of muddy substrates in a polluted system, a nearby unpolluted system, and a healthy system in Australian waters. Sampling was confined to superficial sediments above the redox discontinuity layer (ranging from < 1 to 1-2 cm) and the material was sieved on a 0.044 mm mesh. Copepods dominated over nematodes in the first two systems; there was an extremely high percentage of oligochaetes in the polluted system (up to 78%). Copepod diversity was lowest in the polluted area. The healthy system showed a dominance of nematodes, a high abundance of *Echinoderes* aff. *coulli* (Kinorhyncha), and the highest species diversity of all taxonomic groups.

Hockin (1983) examined the effects of organic enrichment on a harpacticoid community on an estuarine intertidal beach (in Great Britain), by means of field experiments. The pollutant used was a suitable nutrient source for the sediment-dwelling microfauna upon which many of the copepods feed. The increased supply of organic matter resulted in an increase in the species richness, a decrease in the dominance diversity and no change in the number of individuals and, by inference, the biomass. It was observed that the log series model adequately fitted most data sets, while the log normal only fitted data drawn from the community inhabiting the organically enriched sediments.

Heip *et al.* (1984) recorded that *Microarthridion littorale* was the dominant copepod in the polluted eastern area of Belgian coastal waters, accounting for 94%, on average, of all harpacticoids. The impoverishment of the harpacticoid fauna from west to east (from less to more polluted) was also reflected in the average diversity, which decreased from H' = 0.87 bits/individual in the west to 0.43 bits/individual in the east, and in the observation that 14 out of 15 stations in the west yielded harpacticoids, against 21 out of 30 in the east.

Van Damme *et al.* (1984) examined the influence of pollution on the harpacticoid copepods of two North Sea estuaries, the Western Scheldt estuary and the Ems Dollard estuary. The Western Scheldt estuary is more loaded with heavy metals (Zn, Cu, Pb) than the Ems Dollard estuary; in particular, copper is continuously present at concentrations which, according to bioassays, would severely affect egg production and larval development of planktonic copepods. The remarkable scarcity of harpacticoid life on the nutrient-rich mudflats of the Western Scheldt is probably due to heavy metal pollution. In the Western Scheldt, two distinct copepod assemblages occur, a mesobenthic assemblage (small, interstitially living grazers, e.g., *Kliopsillus constrictus*, *Paramesochra* sp. A, and *Paraleptastacus espinulatus*) and an endo-epibenthic assemblage (large, burrowing or epibenthic detritus-feeders, e.g., *Canuella perplexa*, *Pseudobradya* spp. and *Tachidius discipes*). In the Ems Dollard estuary, the copepods all belong to the endo-epibenthic assemblage and are found in the pure as well as in the muddy sands.

Keller (1984, 1985, 1986) described the copepod community which was influenced by a sewage outfall off Marseille, France. As was the case with the nematode communities, the copepods could be divided into two main groups: (1) near to the outfall, the community was dominated by the copepod species *Darcythompsonia faviliensis* (which had not previously been reported in marine environments); (2) the second community (from 400 to 4000 m from the outfall) was characterised by the dominance of *Bulbamphiascus imus*, a cosmopolitan species relatively tolerant to pollution. Both species diversity and Motomura's constant (calculated from the log linear model) increased from the outfall to the offshore zone. (Nematodes showed the same trend: see below).

Gee *et al.* (1985) studied the effects of organic enrichment on meiofaunal abundance and community structure in mesocosms containing sublittoral soft sediments. Harpacticoid copepods increased significantly in abundance in the treatment boxes, and showed a general trend towards increased dominance and decreased diversity with increasing levels of organic enrichment. However, in the low-dose treatment, there was also an increase in the number of species present.

Moore and Pearson (1986) examined the impact of sewage sludge dumping on the copepod community of a subtidal muddy deposit off the Scottish coast. Three groups of species were recognized, each characteristic of the different levels of sludge loading. The first group, in the centre of the dumping ground, consisted of only one species (*Bulbamphiascus imus*), which was overwhelmingly dominant there. The second group included those species which were virtually absent from the centre of the dumping ground, but become dominant in the moderately enriched sediments on either side (e.g., *Amphiascoides debilis*, *Typhlamphiascus lamellifer*, *Paramphiascoides hyperborea*). A third group of species only began to appear towards the ends of the transect (e.g., *Pseudameira furcata*, *Pseudameira* sp., *Amphiascoides subdebilis*).

Bodin (1988) examined changes in the meiofauna of three beaches between 1978 and 1984, following pollution by the 'Amoco Cadiz' oil spill. The hydrocarbons exerted a toxic effect on the meiofauna only during the first weeks. Thereafter, the major pollution came from excess organic matter that induced oxygen impoverishment in the environment. Among harpacticoid copepods, 'test' species and groups (sensitive, tolerant, opportunistic, etc.) could be distinguished, which represented bioindicators of an imbalance caused by perturbations due to excess organic matter.

Hockin (1983) concluded that the use of copepods as indicators of environmental quality was presently problematic. The response of natural communities seemed to be dependent both upon the load of organic matter and the composition of the community with respect to the ratio of bacterial- and algal- feeding species. Increased bacterial productivity alone (on organically enriched beaches) may cause increased diversity of the copepod fauna, because of the increase in density as well as in diversity of typically bacterial-feeding species.

Along a putative pollution gradient in two Norwegian fjords, data on copepod distributions provided a better means to discriminate between sites than data on nematodes (Heip *et al.*, 1988a).

NEMATODA

The species distribution of the nematodes has been more extensively studied, because they have proved to be sensitive biological indicators of pollution. Nematodes are very diverse taxonomically and occur everywhere, usually in large numbers which often exceed those of other taxa by an order of magnitude or more (Platt, 1984; Heip *et al.*, 1985).

A decrease in nematode density after contamination with hydrocarbons has been demonstrated in the sediments of several beaches (Wormald, 1976; Giere, 1979; Boucher, 1980), but not in all cases (Green *et al.*, 1974), and not in sublittoral sands (Elmgren *et al.*, 1980; Elmgren *et al.*, 1983; Boucher, 1980, 1981). An obvious decrease in nematode abundance after an oil spill has often been followed by 'explosive development' of a few opportunistic species within one year (Wormald, 1976; Giere, 1979). However, after the 'Amoco Cadiz' oil spill, no such 'explosive development' occurred on the beaches (Boucher, 1980, 1981).

After an oil spill at La Coruña (northern Spain), *Enoplolaimus litoralis* became extremely dominant; many specimens had ingested oil droplets covered with bacteria. In the intestine of *Bathylaimus* sp. and *Tripyloides* sp., oil particles surrounded by clouds of bacteria were found (Giere, 1979).

After the 'Amoco Cadiz' spill, nematode diversity in the sublittoral sands in Morlaix Bay decreased significantly, and most obviously 9 to 12 months after the accident happened (Boucher, 1980). This was due, on the one hand, to an increase of *Anticoma ecotronis*, *Sabatieria celtica*, *Paracyatholaimus occultus* and *Calomicrolaimus monstrosus*, species normally abundant in silty sands and, on the other hand, to a decrease of *Ixonema sordidum*, *Monoposthia mirabilis*, *Rhynchonema ceramotos*, *Chromadorita mucrocaudata*, *Xyala striata*, *Viscosia franzii* and *Rhynchonema megamphidum*, species normally dominant in clean sands.

Renaud-Mornant *et al.* (1981) also examined the same polluted area and found that the mortality 10 days after the oil input was not significant. After one month, density decreased: mortality occurred especially in the surface sand layers while, in the deeper layers, meiofauna was found to be in the process of spring reproduction. After six months, nematodes became extremely dominant and accounted for 90% of the meiofauna.

Gourbault (1984) examined the nematodes from the Bay of Morlaix Channel over a period of two years after the 'Amoco Cadiz' oil spill. A change of species composition was observed, with diversity decreasing in the upper part of the Channel. (Species typical of sediments two years after the spill were: *Sabatieria pulchra*, *Terschellingia longicaudata* and *Aponema torosus*.)

Gourbault and Lecordier (1984) and Gourbault (1987) found that the nematode assemblage data from the Bay of Morlaix Channel could not be fitted with the usual abundance distribution models (log normal, log series, log linear). The Pareto law (y=ax..), used in some economic studies when dealing with partitioning of resources, was found to be more meaningful in this case. The nematode assemblages from the Morlaix estuary were regularly monitored at three sites from October 1978 (half a year after the 'Amoco Cadiz' oil spill) to November 1984. It was concluded that by 1984 the fauna had recovered at all sites to a situation similar to that prevailing in October 1978.

Field monitoring studies of the effects of heavy metal pollution have been conducted by Lorenzen (1974), Tietjen (1977, 1980) and Heip *et al.* (1984), and laboratory studies by Howell (1982, 1983, 1984).

Lorenzen (1974) found no short-term effects on the nematode fauna in a region of the German Bight of the North Sea subjected to industrial waste disposal. (The waste contained 10% sulphuric acid and 14% ferrous sulphate.)

Tietjen (1977) found that heavy metals did not affect nematode populations in Long Island sublittoral muds, although a slight decrease in diversity was evident. Tietjen (1980) examined the nematodes from the New York Bight Apex, a sandy sediment area with high levels of heavy metals and organic carbon. High concentrations of the contaminants in medium sands resulted in lowered abundance of the nematode families which normally live in this kind of sediment, namely Chromadoridae, Desmodoridae and Monoposthiidae. Other species, such as *Sabatieria pulchra*, which are normally associated with finer sediments, increased in abundance. This species is adapted to living in conditions of low dissolved oxygen concentration and/or high organic content.

In Belgian coastal waters, influenced by the impact of the polluted Western Scheldt estuary, Heip *et al.* (1984) found that nematode species richness was significantly correlated with the heavy metal content. Closest to the mouth of the Western Scheldt, the only meiofaunal component which survived in high densities consisted of non-selective deposit-feeding nematodes, albeit with few species per station (*Sabatieria punctata, Daptonema tenuispiculum, Metalinhomoeus* n.sp. and *Ascolaimus elongatus*). A combination of trophic diversity (expressed in a trophic index: see Heip *et al.*, *op. cit.*) and species richness provided a good indication of the influence of pollution along the Belgian coast. The impoverishment of the nematode community along a gradient of increasing pollution from west to east could be explained by the gradual elimination of species, as stress increased. Trends in harpacticoid copepods could also be explained in the same way.

Huys *et al.* (1984) and Smol *et al.* (1986) examined the effects of dumping waste from TiO_2 manufacture on the meiofauna in the Southern Bight of the North Sea. The copepod communities were most diverse outside the dumping area; the samples from the dumping area were characterized by low diversities and high densities of nematodes. A comparison of data on the nematode communities with an interval of ten years between sampling (corresponding with the period before and during dumping, respectively) revealed that a change in nematode composition had occurred. However, the seasonal variability of the communities in this type of sediment is not known; therefore, further research is necessary before a proper interpretation of these results can be made.

Shaw *et al.* (1983) and Lambshead *et al.* (1983) examined several methods for the analysis of monitoring data. (The results from surveys of littoral nematode assemblages in Strangford Lough, N. Ireland, provided the basis for much of this work; see Platt, 1977.) They found that the abundance of the most common species as a percentage of the total sample (i.e., the dominance index) was a good indicator of environmental stress. However, the work did not include measurement of any chemical pollutants for correlative purposes. The use of the dominance index is not suitable in every case study (Platt and Lambshead, 1985; Lambshead, 1986). Platt and Lambshead (1985) found that disagreement with predictions of a neutral model for the distribution of species abundances (Caswell, 1976) provided a method of detecting disturbance or stress. They subjected 98 samples of marine benthic organisms to the neutral model analysis, with some conflicting results. Assemblages of marine organisms may be more (nematodes) or less (macrofauna) diverse when influenced by contaminants, compared with the normal situation. Where disturbance was known to have occurred, spatial or temporal variations in the degree of deviation from the predicted diversity were in accordance with a hypothesis of diversity based on a combination of the 'intermediate disturbance hypothesis' (Connell, 1978) and the 'general hypothesis of species diversity' (Huston, 1979). These state that an undisturbed assemblage of organisms would have a low diversity, due to competitive exclusion of some species, while at moderate levels of disturbance such competitive interactions are prevented, and the diversity rises as more species are able to co-exist. Further increases in the scale of disturbance result in a lowering of diversity, as certain species are eliminated as a result of 'catastrophic' effects.

Vitiello and Aissa (1985) described the nematode communities in polluted sediments of the lagoon of Tunisia; only three nematode species were characteristic of the organically polluted communities, namely *Terschellingia* n.sp., *Sabatieria pulchra* and *Penzencia flevensis*. The mean length of the nematodes was longer in the polluted area, where the predators among the nematodes were almost absent. In the non-polluted region, predators amounted to about 15% of the communities.

As was the case with copepods, the nematode communities near a sewage outfall off Marseille (France) were divided into two assemblages (Keller, 1986): (1) near the outfall, nematodes of the order Rhabditida occurred. These are generally abundant in polluted rivers, and the source of the populations off Marseille appeared to be sewage-derived; it was also known that a polluted stream was diverted into the sewer prior to discharge. Some marine nematodes known for their affinity to polluted sediments, such as *Metoncholaimus pristiurus*, *Sabatieria pulchra* and *Terschellingia* n.sp., were also present; (2) at distances of 400-4000 m from the outfall, sampling stations had different nematode assemblages consisting mainly of deposit-feeders, because of the presence of significant amounts of organic matter in the sediments. Moreover, two heavily influenced nematode associations were detected at the two most distant stations (1.8 km and 4 km from the outfall), one of them being composed of only mud-dwelling deposit-feeders. This showed that the whole study area was perturbed by the presence of the outfall. Keller (1986) concluded that, in general, both species diversity and Motomura's constant increased from the outfall to the offshore zone; this indicated a rise in the number of ecological niches available.

Hodda and Nicholas (1986) studied nematode diversity in mangrove mud-flats adjacent to the steel works and chemical factories in the Hunter River Estuary, Australia. Their results suggested that nematode diversity might not be a good universal indicator of marine pollution. The polluted areas were more diverse taxonomically, though seasonal variations in population density and other environmental factors complicated the comparison.

The most recent example of the problems with this approach to pollution monitoring is given by Lambshead (1986). He reported on an investigation into the effects of contamination, at a 'subcatastrophic' level, on some marine nematode assemblages of beaches in the Clyde Inland Sea area, Scotland. All stations were sampled once (in September 1978), and the median of the sand fraction varied from less than 150 mm for the contaminated stations to more than 150 mm for the uncontaminated stations. Results were consistent with the expectation that, at uncontaminated stations, diversity would be higher, density lower and feeding-type ratio in favour of the epistratum feeders. However, 'k-dominance' curves did not show significant differences between the uncontaminated and the contaminated stations.

Therefore, Lambshead (1986) and Lambshead and Paterson (1986) proposed a new method for the detection of sub-catastrophic contamination at the community level, which involved the application of numerical cladistics to ecological analysis. The presence of a species is coded as the derived character state, while absence is coded as primitive; this means that the outgroup consists of a theoretical station containing no species. All the species used in the analysis must be potentially capable of reaching (if not surviving in) any of the stations examined. This kind of cladistic analysis can only be applied to stations drawn from the same potential species pool. However, reduction in diversity and survival of only the most tolerant species is the only obvious effect of pollution so far detected; therefore, it is difficult to agree that species presence is a derived character, especially when the interest is in pollution effects. The establishment of 'homology' in ecological studies also has some problematic features; thus, the ontogenetic method as well as the outgroup comparison are highly speculative.

3 CONCLUSIONS

This review of the use of meiobenthos in studies of marine pollution has highlighted some of the difficulties and controversies surrounding the interpretation of observed changes. It is usually very hard to distinguish pollution-induced from natural changes as, in most cases, the pre-pollution status of the meiobenthic community and supporting environment is not well known. *A posteriori* studies of the effects of pollutants often involve the use of 'natural experiments', in which the density or species diversity of benthic organisms of a polluted area is compared with an environmentally similar area nearby, usually referred to as a 'comparison' or 'reference' area (see Eskin and Coull, 1984, for a review). Eskin and Coull (*op. cit.*) warned that great caution should be taken when reference areas are selected and compared with 'disturbed' areas; in such cases, a single factor (e.g., pollution) should not be interpreted as causative of observed differences in densities, or in the distribution of abundances. Regardless of the mechanisms controlling distribution, small- and medium- scale (mm-cm) spatial distribution of meiofauna appeared to be so variable and unpredictable that no *a priori* assumption about the similarity of meiofaunal populations at the two comparison sites could be made, despite the apparent visual similarity of the sediment sites. It was, therefore, concluded that larger samples, which obscured the patchy distribution of the meiofauna, were necessary for biomonitoring purposes. In contrast, great stability and spatial predictability of harpacticoid copepods have been observed in other locations (Willems, in preparation), while a recent workshop demonstrated that the ability to discriminate between stations using meiofauna data was as good as, or better than, that using macrofauna data (Heip *et al.*, 1988a).

In general, density is not much affected by pollution, whereas diversity seems to decrease. Pollution is often accompanied by changes in habitat characteristics; both the lethal effect of a pollutant or the change in sediment texture may be responsible for the observed changes. It is known that some nematode species are resistant to high levels of pollution and anaerobiosis. However, the effect of, e.g., heavy metals on nematode population dynamics can only be studied in the laboratory. Despite their significant role in marine sediments, only a few experimental studies into the effects of pollution have been conducted (see Heip *et al.*, 1985, for a review). It is concluded that knowledge of the ecotoxicology of meiobenthos is still very poor, and that much more work remains to be done.

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ANNEX 1

MULTIVARIATE ANALYSIS IN MARINE BENTHIC ECOLOGY: A LIST OF SELECTED REFERENCES

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