



REGIONAL SEAS

UNITED NATIONS ENVIRONMENT PROGRAMME

JUNE 1995

Statistical analysis and interpretation of marine community data

Reference Methods For Marine Pollution Studies No. 64

Prepared in co-operation with



FAO



IMO

UNEP 1995

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INTRODUCTION

Aim

This manual describes a strategy for the statistical analysis and interpretation of biological data on community structure, consisting of abundance or biomass readings for a set of species and a number of samples. The latter usually consist of one or more replicates taken:

- a) at a number of sites at one time (spatial analysis),
- b) at the same site at a number of times (temporal analysis),
- c) for a community subject to different manipulative "treatments" (laboratory or field experiments),

or some combination of these. The species-by-samples arrays are typically large, and patterns in community structure are often not readily apparent. Statistical analysis therefore centres around reducing the complexity of these matrices, usually by some graphical representation of the biological relationships between the samples. This is followed by statistical testing to identify and characterise changes in community structure in time or space and relate these to changing environmental or experimental conditions.

Emphasis

Of principle concern are the biological effects of contaminants, though, since the same analysis techniques are appropriate to wider studies of community structure, a number of examples are included which are not pollution-related. In general these illustrate some important aspect of the methodology which is applicable to pollution studies. The scope of the examples is specifically marine (though the techniques have wider application) and, though the examples range over different community types (benthic infauna, corals, plankton, fish etc.), there is a bias towards soft-sediment benthos, reflecting both the authors' own research interests and the widespread use of such community data in pollution monitoring.

Scope

There is a vast array of sophisticated statistical techniques for handling species-by-samples matrices, ranging from their reduction to simple diversity indices, through curvilinear or distributional repre-

sentations of richness, dominance, evenness etc., to a plethora of multivariate approaches involving clustering or ordination methods. This manual does *not* attempt to give an overview of all the options, or even the majority of them. Instead it presents a strategy which has evolved over several years, within the Community Ecology group at Plymouth Marine Laboratory, and which has a proven track record in published analysis and interpretation of a wide range of marine community data. The manual attempts to explain *how* and *why* the selected techniques work, to a level of understanding sufficient to appreciate when they are (and are not) applicable, and to interpret their outcome. It is aimed at ecologists with no more than an introductory background in statistics, who need to apply these statistical techniques to answer specific questions about changes in community structure.

This volume is also not a software manual, describing how to use a particular computer program or package to carry out the analyses discussed here, though the advocated approach is mirrored in the software package PRIMER (Plymouth Routines In Multivariate Ecological Research), developed at the Plymouth Marine Laboratory and available commercially. Footnotes in the text make brief reference to the PRIMER modules which have been used to obtain the analyses presented. The PRIMER package has been used throughout (though the figures have in many cases been subjected to further annotation etc. using a presentation graphics program, Harvard Graphics). Note, however, that PRIMER is not the only option for computation. The major statistical packages such as SAS, BMDP, SPSS, GENSTAT, etc. have always included multivariate options, as do some PC packages such as SYSTAT, STATGRAPHICS etc. In addition, more specialised software, such as CLUSTAN, the Cornell Ecology programs, KYST, CANOCO, PATN etc., is fairly widespread. None of these packages will offer precisely the combination of options discussed here, and some take a rather different (but equally valid) approach to the problems posed. An over-riding thrust of the current exposition is, however, to retain as great a simplicity of explanation and transparency of interpretation as is possible in what, conventionally, has been regarded as a difficult area for practitioners lacking a strong statistical background.

Genesis

The arrangement of topics, and the level of exposition, have benefited from a series of research and training programmes undertaken by an *ad-hoc* group of the Intergovernmental Oceanographic Commission (IOC) of UNESCO, the Group of Experts on the Effects of Pollutants (GEEP), a component of the IOC programme on Global Investigation of Pollution in the Marine Environment (GIPME). It draws on experience of application of these statistical techniques at three practical GEEP Research Workshops on the Biological Effects of Pollutants:

- a) the Oslo Workshop, September 1986, reported in summary form in IOC Report No. 53, and in full as a Special Volume of Marine Ecology Progress Series (Vol. 46, 1988, 278 pp);
- b) the Bermuda Workshop, September 1988, summarised in IOC Report No. 61, with the full findings again available in the open literature: a Special Volume of the Journal of Experimental Marine Biology and Ecology (Vol. 138, 1990, 166 pp).
- c) the joint IOC/ICES N. Sea Workshop (Bremerhaven, March 1990), published as a further Special Volume of Marine Ecology Progress Series (Vol 91, 1992, 361 pp).

In addition, much of the material presented here has formed the basis of seven Training Workshops on "Statistical Analysis and Interpretation of Marine Community Data", held in Piran, Yugoslavia 1988, Athens, Greece 1989, Split, Yugoslavia 1990, Alexandria, Egypt, 1991, Tel Aviv, Israel, 1992 and Trieste, Italy, 1993, organised under the joint auspices of FAO, IOC and UNEP, as part of the MED POL programme, and under IOC organisation in the WESTPAC region, Xiamen, China, 1992. The advocacy of these techniques thus springs not only from regular use and development within the Community Ecology and Biodiversity programmes of the Plymouth Marine Laboratory, but also from valuable feedback from a series of workshops in which practical data analysis was central.

Examples

Throughout the manual, extensive use is made of data sets from the published literature to illustrate the

techniques. Appendix 1 gives the original literature source for each of these 22 data sets and an index to all the pages on which they are analysed. Each data set is allocated a single letter designation and, to avoid confusion, referred to in the text of the manual by that letter, placed in curly brackets (e.g. {A} = Amoco-Cadiz oil spill - macrofauna, {B} = Bristol Channel zooplankton, {C} = Celtic Sea zooplankton etc.).

Literature citation

Appendix 2 lists some background papers appropriate to each chapter, including the source of specific analyses, and a full listing of references cited is in Appendix 3.

Since this manual is not accessible within the published literature, referral to the methods it describes would properly be by citing the primary papers on which it draws; these are indicated in the text and Appendix 2. Alternatively, comprehensive discussion of the philosophy (and many of the details) of the multivariate and univariate approaches advocated can be found in Clarke (1993) and Warwick (1993), respectively.

PREFACE

The Regional Seas Programme was initiated by UNEP in 1974. Since then the Governing Council of UNEP has repeatedly endorsed a regional approach to the control of marine pollution and the management of marine and coastal resources and has requested the development of regional action plans. The Regional Seas Programme at present includes 12 regions and has some 140 coastal States participating in it (1), (2).

One of the basic components of the action plans sponsored by UNEP in the framework of the Regional Seas Programme is the assessment of the state of the marine environment and of its resources and of the sources and trends of the pollution, and the impact of pollution on human health, marine ecosystems and amenities. In order to assist those participating in this activity and to ensure that the data obtained through this assessment can be compared on a world-wide basis and thus contribute to the Global Environment Monitoring System (GEMS) of UNEP, a set of Reference Methods and Guidelines for marine pollution studies is being developed as part of a programme of comprehensive technical support which includes the provision of expert advice, reference methods and material, training and data quality assurance (3). The methods are recommended to be adopted by Governments participating in the Regional Seas Programme.

The methods and guidelines are prepared in co-operation with the relevant specialized bodies of the United Nations systems as well as other organizations and are tested by a number of experts competent in the field relevant to the methods described.

In the description of the methods and guidelines the style used by the International Organization for Standardization (ISO) is followed as closely as possible.

The methods and guidelines, as published in UNEP's series of Reference Methods for Marine Pollution Studies, are not considered as final. They are planned to be periodically revised taking into account the development of our understanding of the problems, of analytical instrumentation and the actual need of the users. In order to facilitate these revisions the users are invited to convey their comments and suggestions to:

Marine Environmental Studies Laboratory
IAEA Marine Environment Laboratory
B.P. No. 800
MC-98012 MONACO Cedex

which is responsible for the technical co-ordination of the development, testing and intercalibration of Reference Methods.

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- (1) UNEP: Achievements and planned development of the UNEP's Regional Seas Programme and comparable programmes sponsored by other bodies. UNEP Regional Seas Reports and Studies No. 1, UNEP, 1982.
 - (2) P. HULM: A strategy for the Seas. The Regional Seas Programme: Past and Future, UNEP 1983.
 - (3) UNEP/IAEA/IOC: Reference Methods and Materials: A Programme of comprehensive support for regional and global marine pollution assessments. UNEP, 1990.

The present manual describes a strategy for the statistical analysis and interpretation of biological data on community structure, consisting of the abundance of biomass readings for a set of species and a number of samples. The arrangement of topics, and the level of exposition, have benefitted from a series of research and training programmes undertaken by an *ad-hoc* group of the Intergovernmental Oceanographic Commission (IOC) of UNESCO, the Group of Experts on the Effects of Pollutants (GEEP), a component of the IOC programme on Global Investigation of Pollution in the Marine Environment (GIPME).

This document was compiled and edited by **Drs. K.R. Clarke and R.M. Warwick** of the Plymouth Marine Laboratory, U.K.

The manual has also been published as IOC (of UNESCO) Manuals and Guides No. 22, 1994.

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Special thanks are due to Mr. M.R. Carr, who has had the principal responsibility for implementing and supervising the coding of the PRIMER package, used throughout the manual, and to the Natural Environment Research Council and the Department of the Environment, U.K., for support of the development of the analysis strategy outlined in this manual.

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PREFACE

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CHAPTER 1: A FRAMEWORK FOR STUDYING CHANGES IN COMMUNITY STRUCTURE

The purpose of this opening chapter is twofold:

- a) to introduce a few of the data sets which are used most extensively, as illustrations of techniques, throughout the manual;
- b) to outline a framework for the various possible stages in a community analysis¹.

Examples are given of some core elements of the recommended approaches, foreshadowing the analyses explained in detail later and explicitly referring forward to the relevant chapters. Though at this stage the details are likely to remain mystifying, the intention is that this opening chapter should give the reader some feel for where the various techniques are leading and how they slot together. As such, it may serve both as an introduction and a summary.

Stages

It is convenient to categorise possible analyses broadly into four main stages.

1) *Representing communities* by graphical description of the relationships between the biota in the various samples. This is thought of as "pure" description, rather than explanation or testing, and the emphasis is on reducing the complexity of the multivariate information in typical species/samples matrices, to obtain some form of low-dimensional picture of how the biological samples interrelate.

2) *Discriminating sites/conditions* on the basis of their biotic composition. The paradigm here is that of the hypothesis test, examining whether there are "proven" community differences between groups of samples identified *a priori*, for example demonstrating differences between control and putatively impacted sites, establishing before/after impact differences at a single site, etc.

3) *Determining levels of "stress"* or disturbance, by attempting to construct biological measures from the

community data which are indicative of disturbed conditions. These may be absolute measures ("this observed structural feature is indicative of pollution") or relative criteria ("under impact, this coefficient is expected to decrease in comparison with control levels"). Note the contrast with the previous stage, however, which is restricted to demonstrating differences between groups of samples, not ascribing directionality to the change (e.g. deleterious consequence).

4) *Linking to environmental variables* and examining issues of *causality* of any changes. Having allowed the biological information to "tell its own story", any associated physical or chemical variables, matched to the same set of samples, can be examined for their own structure and its relation to the biotic pattern (its "explanatory power"). The extent to which identified environmental differences are actually *causal* to observed community changes can only really be determined by manipulative experiments, either in the field or through laboratory/mesocosm studies.

Techniques

The spread of methods for extracting workable representations and summaries of the biological data can be grouped into three categories.

1) *Univariate methods* collapse the full set of species counts for a sample into a single coefficient, for example a *diversity index*. This might be some measure of the numbers of different species for a fixed number of individuals (species richness) or the extent to which the community counts are dominated by a small number of species (dominance/evenness index), or some combination of these. Clearly, the *a priori* selection of a single taxon as an *indicator species*, amenable to specific inferences about its response to a particular environmental gradient, also gives rise to a univariate analysis.

2) *Distributional techniques*, also termed graphical or curvilinear plots (when they are not strictly distributional), are a class of methods which summarise the set of species counts for a single sample by a curve or histogram. One example is *k-dominance curves* (Lambshhead *et al.*, 1983), which rank the species in decreasing order of abundance, convert the values to percentage abundance relative to the total number of

1. The term *community* is used throughout the manual, somewhat loosely, to refer to any assemblage data (samples leading to counts or biomass for a range of species); the usage does not necessarily imply indigenous structuring of the biota, for example by competitive interactions.

individuals in the sample, and plot the cumulated percentages against the species rank. This, and the analogous plot based on species biomass, are superimposed to define the *ABC (abundance–biomass comparison) curves* (Warwick, 1986), which have proved a useful construct in investigating disturbance effects. Another example is the *species abundance distribution* (sometimes termed the *distribution of individuals amongst species*), in which the species are categorised into geometrically–scaled abundance classes and a histogram plotted of the number of species falling in each abundance range (e.g. Gray and Pearson, 1982). It is then argued, again from empirical evidence, that there are certain characteristic changes in this distribution associated with community disturbance.

Such distributional techniques relax the constraint in the previous category that the summary from each sample should be a *single* variable; here the emphasis is more on diversity *curves* than single diversity indices, but note that both these categories share the property that comparisons between samples are not based on particular species identities: two samples can have exactly the same diversity or distributional structure without possessing a single species in common.

3) *Multivariate methods* are characterised by the fact that they base their comparisons of two (or more) samples on the extent to which these samples share particular species, at comparable levels of abundance. Either explicitly or implicitly, all multivariate techniques are founded on such *similarity coefficients*, calculated between every pair of samples. These then facilitate either a *classification* or *clustering*² of

samples into groups which are mutually similar, or an *ordination plot* in which, for example, the samples are “mapped” (in two or three dimensions) in such a way that the distances between pairs of samples reflect their relative dissimilarity of species composition.

Techniques described in detail in this manual are a method of *hierarchical agglomerative clustering* (e.g. Everitt, 1980), in which samples are successively fused into larger groups, as the criterion for the similarity level defining group membership is relaxed, and two ordination techniques: principal component analysis (PCA, e.g. Chatfield and Collins, 1980) and non–metric multi–dimensional scaling (NMDS, usually shortened to MDS, Kruskal and Wish, 1978).

For each broad category of analysis, the techniques appropriate to each stage are now discussed, and pointers given to the relevant chapters.

UNIVARIATE TECHNIQUES

For diversity indices and other single–variable extractions from the data matrix, standard statistical methods are usually applicable and the reader is referred to one of the many excellent general statistics texts (e.g. Sokal and Rohlf, 1981). The requisite techniques for each stage are summarised in Table 1.1. For example, when samples have the structure of a number of replicates taken at each of a number of sites (or times, or conditions), computing the means and 95% confidence intervals gives an appropriate *representation* of the Shannon diversity (say) at each site, with *discrimination between sites* being demonstrated by one–way analysis of variance (ANOVA), which is a test of the null hypothesis that there are no differences in mean diversity between sites. *Linking to the environment* is then also relatively straightforward, particularly if the environmental variables can

2. These terms are interchangeable

Table 1.1. *Univariate techniques. Summary of analyses for the four stages.*

| Stages | Univariate examples | |
|------------------------------------|---|---|
| | Diversity indices (Ch 8) | Indicator taxa |
| 1) Representing communities | <i>Means and 95% confidence intervals (CIs) for each site/condition (Ch 8, 9)</i> | |
| 2) Discriminating sites/conditions | <i>One–way analysis of variance, ANOVA (Ch 6)</i> | |
| 3) Determining stress levels | <i>By reference to historical data (Ch 14, 15)</i> | |
| | <i>Ultimately a decrease in diversity</i> | <i>Initial increase in opportunists</i> |
| 4) Linking to environment | <i>Regression techniques (Ch 11); for causality issues, see Ch 12</i> | |

be condensed into one (or a small number of) key summary statistics. Simple (or multiple) regression of Shannon diversity as the dependent variable, against the environmental descriptors as independent variables, is then technically feasible, though in practice rarely very informative given the over-condensed nature of the information utilised.

For impact studies, much has been written about the effect of pollution or disturbance on diversity measures: whilst the response is not necessarily unidirectional (under the hypothesis of Huston, 1979, diversity is expected to rise at intermediate disturbance levels before its strong decline with gross disturbance), there is a sense in which *determining stress levels* is possible, through relation to historical diversity patterns for particular environmental gradients. Similarly, empirical evidence may exist that particular indicator taxa (e.g. Capitellids) change in abundance along specific pollution gradients (e.g. of organic enrichment). Note though that, unlike the diversity measures constructed from abundances across species, averaged in some way³, indicator species levels or the number of species in a sample (*S*) may not initially satisfy the assumptions necessary for classical statistical analysis. For the number of species, *S*, the normality and constant variance conditions can usually be produced by transformation of the variable (e.g. $\log S$). However, for most individual species, abundance across the set of samples is likely to be a very poorly-behaved variable, statistically speaking. Typically, a species will be absent from many of the samples and, when it is present, the counts are often highly variable, with an abundance probability distribution which is heavily right-skewed⁴. Thus, for all but the most common individual species, transformation is no real help and parametric statistical analyses *cannot* be applied to the counts, in any form. In any case, it is not valid to “snoop” in a large data matrix, of typically 100–250 taxa, for one or

more “interesting” species to analyse by univariate techniques (any indicator or keystone species selection must be done *a priori*). Such arguments lead to the tenets underlying this manual:

- a) community data is inherently multivariate (highly so) and usually needs to be analysed *en masse* in order to elicit the important biological structure and its relation to the environment;
- b) standard parametric modelling is totally invalid.

Thus throughout, rather little emphasis is given to representing communities by univariate measures, though some possibilities for construction can be found at the start of Chapter 8, some brief remarks on hypothesis testing (ANOVA) at the start of Chapter 6, a discussion of transformations (to approximate normality and constant variance) at the start of Chapter 9, and an example given of a univariate regression between biota and environment in Chapter 11. Finally, Chapter 14 gives a series of detailed comparisons of univariate with distributional and multivariate techniques, in order to gauge their relative sensitivities and merits in a range of practical studies.

EXAMPLE: Frierfjord macrofauna

The first example is from the IOC/GEEP practical workshop on biological effects of pollutants (Bayne *et al.*, 1988), held at the University of Oslo, August 1986. This attempted to contrast a range of biochemical, cellular, physiological and community analyses, applied to field samples from potentially contaminated and control sites, in a fjordic complex (Frierfjord/Langesundfjord) linked to Oslofjord (*IF*), Fig. 1.1). For the benthic macrofaunal component of this study (Gray *et al.*, 1988), four replicate 0.1 m² Day grab samples were taken at each of six sites (A–E and G, Fig. 1.1) and, for each sample, organisms retained on a 1.0 mm sieve were identified and counted. Wet weights were determined for each species in each sample, by pooling individuals within species.

Part of the resulting data matrix can be seen in Table 1.2: in total there were 110 different taxa categorised from the 24 samples. Such matrices (abundance, *A*, and biomass, *B*) are the starting point for *all* the analyses of this manual, and this example is typical in respect of the relatively high ratio of species to samples (always $\gg 1$) and the prevalence of zeros. Here, as elsewhere, even an undesirable reduction to the 30 “most important” species (see Chapter 2) leaves more than 50% of the matrix consisting of zeros. Standard multivariate normal analyses (e.g. Mardia *et al.*, 1979) of these counts are clearly ruled out; they require both

3. And thus subject to the central limit theorem, which will tend to induce statistical normality.

4. It is the authors' experience, certainly in the study of benthic communities, that the individuals of a species are not distributed at random in space (a Poisson process) but are often highly clustered, either through local variation in forcing environmental variables or mechanisms of recruitment, mortality and community interactions. This leads to counts which, in statistical terms, are described as over-dispersed, combined with a high prevalence of zeros, causing major problems in attempting parametric modelling by categorical/log-linear methods.

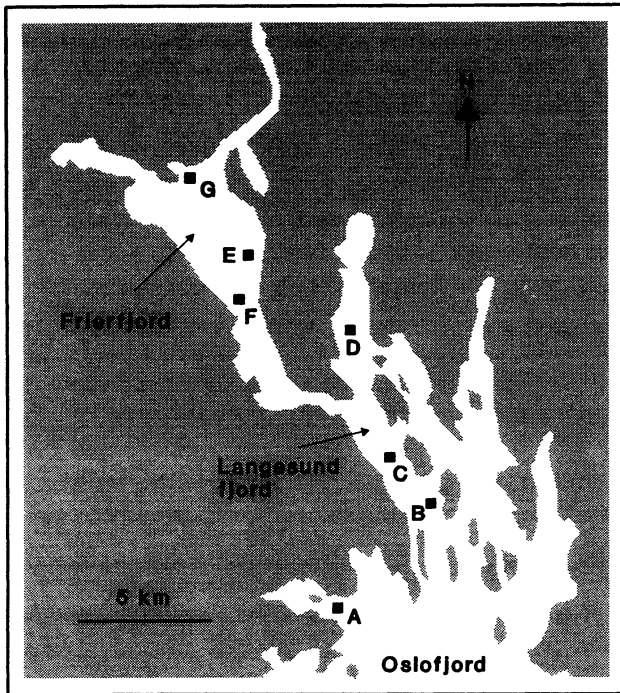


Fig. 1.1. Frierfjord, Norway (F). Benthic community sampling sites (A-G) for the IOC/GEEP Oslo Workshop; site F omitted for macrobenthos.

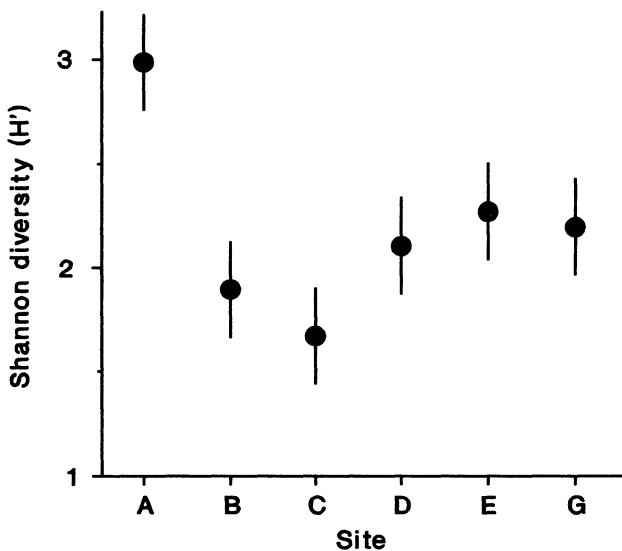


Fig. 1.2. Frierfjord macrofauna (F). Means and 95% confidence intervals for Shannon diversity (H'), from four replicates at each of six sites (A-E, G).

that the number of species (variables) be small in relation to the number of samples, and that the abundance/biomass values are transformable to approximate normality: neither is possible.

As discussed above, one easy route to simplification of this "high-dimensional" complexity is to reduce each

Table 1.2. Frierfjord macrofauna (F). Abundance and biomass matrices (part only) for the 110 species in 24 samples (four replicates at each of six sites, A-E, G); abundance in numbers per 0.1m², biomass in mg per 0.1m².

| Species | Samples | | | | | | | |
|---------------------------|---------|----|-----|----|----|----|----|----|
| | A1 | A2 | A3 | A4 | B1 | B2 | B3 | B4 |
| Abundance | | | | | | | | |
| <i>Cerianthus lloydi</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Halicryptus sp.</i> | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| <i>Onchnesoma</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Phascolion strombi</i> | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| <i>Golfingia sp.</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Holothuroidea | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Nemertina, indet. | 12 | 6 | 8 | 6 | 40 | 6 | 19 | 7 |
| Polychaeta, indet. | 5 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| <i>Amaena trilobata</i> | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| <i>Amphicteis gunneri</i> | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 |
| Ampharetidae | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| <i>Anaitides groenl.</i> | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 |
| <i>Anaitides sp.</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | | | | | | |
| Biomass | | | | | | | | |
| <i>Cerianthus lloydi</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Halicryptus sp.</i> | 0 | 0 | 0 | 26 | 0 | 0 | 0 | 0 |
| <i>Onchnesoma</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Phascolion strombi</i> | 0 | 0 | 0 | 6 | 0 | 0 | 2 | 0 |
| <i>Golfingia sp.</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Holothuroidea | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Nemertina, indet. | 1 | 41 | 391 | 1 | 5 | 1 | 2 | 1 |
| Polychaeta, indet. | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Amaena trilobata</i> | 144 | 14 | 234 | 0 | 0 | 0 | 0 | 0 |
| <i>Amphicteis gunneri</i> | 0 | 0 | 0 | 0 | 45 | 0 | 0 | 0 |
| Ampharetidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Anaitides groenl.</i> | 0 | 0 | 0 | 7 | 11 | 0 | 0 | 0 |
| <i>Anaitides sp.</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | | | | | | |

column of the matrix (each sample) to a single, univariate description. Fig. 1.2 shows the results of computing the Shannon diversity (H' , see Chapter 8) of each sample⁵, and plotting for each site the mean diversity and its 95% confidence interval, based on a pooled estimate of variance across all sites from the ANOVA table, Chapter 6. (An analysis of the type outlined in Chapter 9 shows that prior transformation of H' is not required; it already has approximately constant variance across the sites, a necessary prerequisite for standard ANOVA). The most obvious feature of Fig. 1.2 is the relatively higher diversity at the "control" location, A.

5. Using the PRIMER program DIVERSE.

Table 1.3. *Distributional techniques. Summary of analyses for the four stages.*

| Stages | Distributional examples | |
|------------------------------------|--|---|
| | ABC (<i>k</i> -dominance) curves (Ch 8) | Species abundance distributions (Ch 8) |
| 1) Representing communities | Curves for each site/condition (or preferably replicate) | |
| 2) Discriminating sites/conditions | ANOVA on univariate summaries (e.g. <i>W</i> , Ch 8), or: ANOSIM test (Ch 6) on "distances" between every pair of curves | Test for commonality of distributions (e.g. chi-squared), if valid |
| 3) Determining stress levels | Biomass curve drops below numbers curve under disturbance | Species abundance distribution has "longer tail" with disturbance |
| 4) Linking to environment | Difficult, except for univariate summaries of the curves (by regression) (Causality: see Ch 12) | |

DISTRIBUTIONAL TECHNIQUES

A less condensed form of summary of each sample is offered by the distributional/graphical methods, outlined for the four stages in Table 1.3.

Representation is by curves or histograms (Chapter 8), either plotted for each replicate sample separately or for pooled data within sites or conditions. The former permits a visual judgement of the sampling variation in the curves and, as with diversity indices, replication is required to *discriminate sites*, i.e. test the null hypothesis that two or more sites (/conditions etc.) have the same curvilinear structure. The easiest approach to testing is then to summarise each replicate curve by a single statistic and apply ANOVA as before: for the ABC method, mentioned earlier, the *W* statistic (Chapter 8) is a convenient measure of the extent to which the biomass curve "dominates" the abundance curve, or vice-versa. This is effective in practice though, in theory, it simply amounts to computing another diversity index and is therefore just a univariate approach. A more general test, which honours the curvilinear structure, could be constructed by the ANOSIM procedure (described later under multivariate techniques), computed between every pair of replicate ABC curves.⁶

The distributional/graphical techniques have been proposed specifically as a way of *determining stress levels*. For the ABC method, the strongly polluted (/disturbed) state is indicated if the abundance *k*-dominance curve falls above the biomass curve throughout its length (e.g. see the later plots in Fig. 1.4):

the phenomenon is linked to the loss of large-bodied "climax" species and the rise of small-bodied opportunists. Note that the ABC procedure claims to give an *absolute* measure, in the sense that disturbance status is attributable on the basis of samples from a single site; in practice however it is always wise to design collection from (matched) impacted and control sites to confirm that the control condition exhibits the "undisturbed" ABC pattern (biomass curve above the abundance curve, throughout). Similarly, the species abundance distribution has features characteristic of disturbed status (e.g. see the middle plots in Fig. 1.6), namely a move to a less "J-shaped" distribution by a reduction in the first one or two abundance classes (loss of rarer species), combined with the gain of some higher abundance classes (very numerous opportunist species).

The distributional/graphical methods may thus have particular merits in allowing recognition of "stressed" states (Chapter 14), though they have the disadvantage of being more difficult to work with statistically,

6. This is somewhat esoteric and is not pursued in this manual; for details see Clarke (1990). Similarly outside the current scope are tests of equality for two or more observed histograms arising from species abundance distributions. Again, the most straightforward approach to testing is probably to summarise each distribution by two or three measures (of location, spread, skewness etc.) and carry out ANOVA on the summary statistics for each replicate. Another possibility is a chi-squared test (or some form of Cramer-von Mises approach), for testing equality of two or more frequency distributions, but this is unlikely to be valid given the species interdependencies in a single sample.

Table 1.4. Loch Linnhe macrofauna (L). Abundance/biomass matrix (part only); one (pooled) set of values per year (1963-1973).

| Species | 1963 | | 1964 | | 1965 | | 1966 | | ... |
|--------------------------------|------|-------|------|-------|------|------|------|-------|-----|
| | A | B | A | B | A | B | A | B | |
| <i>Scutopus ventrolineatus</i> | 0 | 0 | 0 | 0 | 11 | 0.05 | 0 | 0 | |
| <i>Nucula tunuis</i> | 2 | 0.01 | 13 | 0.07 | 16 | 0.10 | 6 | 0.04 | |
| <i>Mytilus edulis</i> | 0 | 0 | 0 | 0 | 5 | 0.09 | 0 | 0 | |
| <i>Modiolus sp. indet.</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| <i>Thyasira flexuosa</i> | 93 | 3.57 | 210 | 7.98 | 28 | 1.06 | 137 | 5.17 | |
| <i>Myrtea spinifera</i> | 214 | 27.39 | 136 | 17.41 | 2 | 0.26 | 282 | 36.10 | |
| <i>Lucinoma borealis</i> | 12 | 0.39 | 26 | 1.72 | 0 | 0 | 22 | 0.73 | |
| <i>Montacuta ferruginosa</i> | 1 | 0 | 0 | 0 | 4 | 0.02 | 0 | 0 | |
| <i>Mysella bidentata</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| <i>Abra sp. indet.</i> | 0 | 0 | 0 | 0 | 12 | 0.26 | 0 | 0 | |
| <i>Corbula gibba</i> | 2 | 0.13 | 8 | 0.54 | 9 | 0.27 | 2 | 0.13 | |
| <i>Thracia sp. indet.</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | | | | | | | | | |

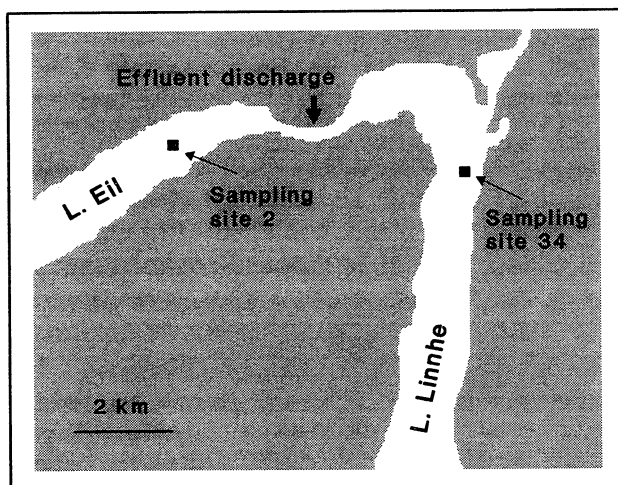


Fig. 1.3. Loch Linnhe and Loch Eil, Scotland (L). Map of site 34 (Linnhe) and site 2 (Eil), sampled annually over 1963-1973.

for example in *linking to environmental variables*, where the only viable course again seems to be reduction of the curve(s) for each sample to a summary statistic (such as *W*), which can be regressed on particular abiotic variables.

EXAMPLE: Loch Linnhe macrofauna

Pearson (1975) describes a time series of macrobenthic community samples, taken over the period 1963-1973 inclusive, at two sites in a sea loch system on the west coast of Scotland (L, Fig. 1.3). Pooling to a single sample for each of the 11 years resulted in abundance and biomass matrices of 115 rows (species) and 11 columns (samples), a small part of which is shown in

Table 1.4.⁷ Starting in 1966, pulp-mill effluent was discharged to the sea lochs (Fig. 1.3), with the rate increasing in 1970 and a significant reduction taking place in 1972 (Pearson, 1975). The top left-hand plot of Fig. 1.4 shows the Shannon diversity of the macrobenthic samples over this period, and the remaining plots the ABC curves for each year.⁸ There appears a consistent change of structure from one in which the biomass curve "dominates" the abundance curve in the early years, to the curves crossing, reversing altogether and then finally reverting to their original form.

EXAMPLE: Garroch Head macrofauna

Pearson and Blackstock (1984) describe the sampling of a transect of 12 sites across the sewage-sludge disposal ground at Garroch Head in the Firth of Clyde, SW Scotland (G, Fig. 1.5). The samples considered here were taken during 1983 and consisted of abundance and biomass values of 84 macrobenthic species, together with associated contaminant data on the extent of organic enrichment and the concentrations of heavy metals in the sediments. Fig. 1.6 shows the resulting species abundance distributions for the

7. Note that the abundance and biomass matrices have been merged here, using the PRIMER programs JOINFILE and REDUCE to combine the files and arrange the columns. This is no longer a valid input format to the PRIMER analysis programs (which require separate abundance and biomass matrices) but is a useful temporary file to construct as a cross-check on accuracy, making it easier to spot errors in which there is no abundance value corresponding to a biomass value, for example.

8. Computed using the PRIMER program DOMPLOT.

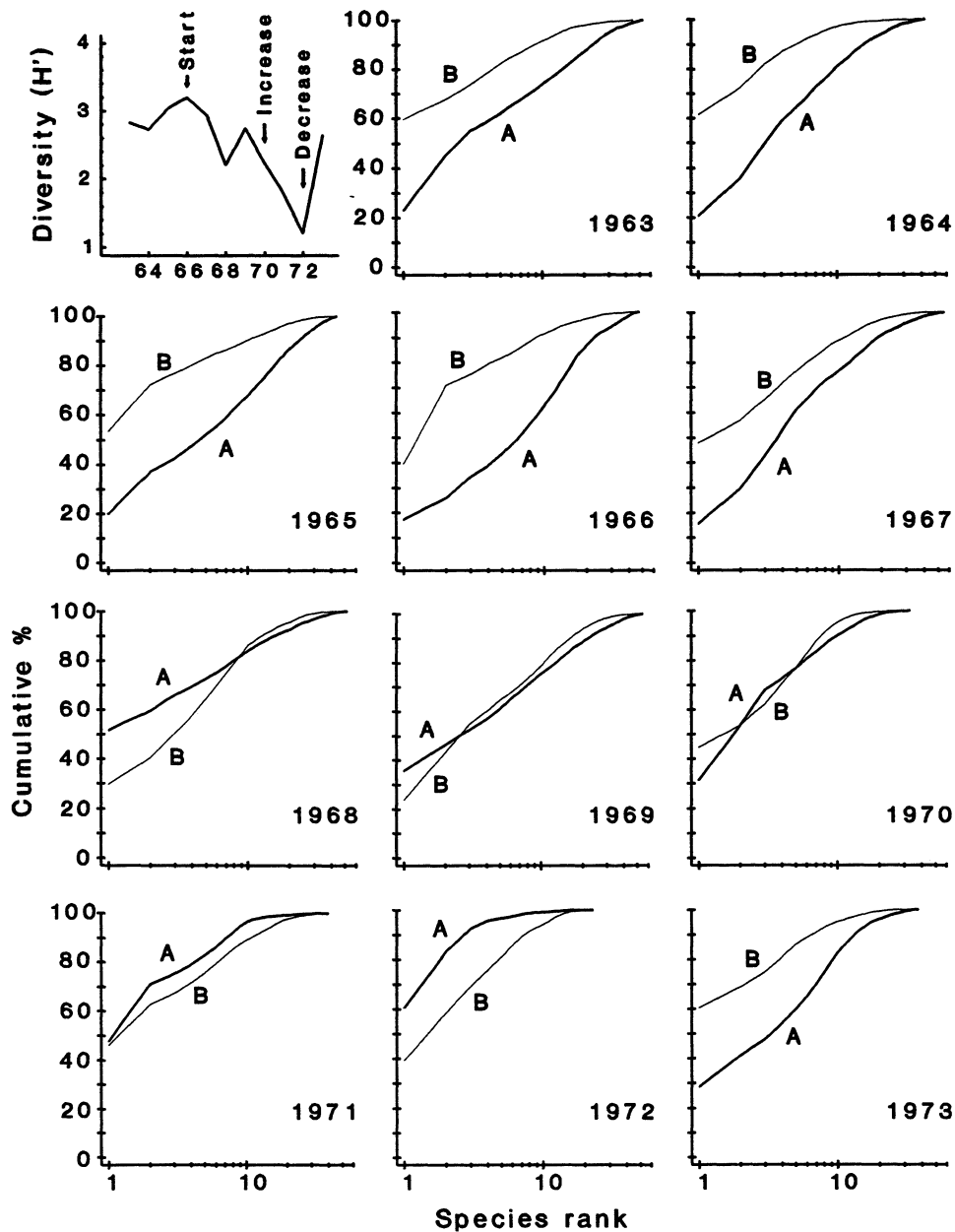


Fig. 1.4. Loch Linnhe macrofauna (L). Top left: Shannon diversity over the 11 annual samples, also indicating timing of start of effluent discharge and a later increase and decrease in level; remaining plots show ABC curves for the separate years 1963-1973 (B = biomass, thin line; A = abundance, thick line).

twelve sites, i.e. at site 1, twelve species were represented by a single individual, two species by 2-3 individuals, three species by 4-7 individuals, etc. (Gray and Pearson, 1982). For the middle sites close to the dump centre, the hypothesised loss of less-abundant species, and gain of a few species in the higher geometric classes, can clearly be seen.

MULTIVARIATE TECHNIQUES

Table 1.5 summarises the analyses possible under the four stages, when adopting one of three multivariate methods: hierarchical clustering (CLUSTER), multi-dimensional scaling (MDS) and principal component analysis (PCA).

The first two methods start explicitly from a triangular matrix of similarity coefficients computed between every pair of samples (e.g. Table 1.6). The coefficient is usually some simple algebraic measure (Chapter 2) of how close the abundance levels are for each species, averaged over all species, and defined such that 100% represents total similarity and 0% complete dissimilarity. There is a range of properties that such a coefficient should possess but still some flexibility in its choice: it is important to realise that the definition of what constitutes similarity of two communities may vary, depending on the biological question under consideration. As with the earlier methods, a multivariate analysis too must attempt to reduce the complexity of the (high-dimensional) community data by taking a

particular (low-dimensional) "view" of the structure it exhibits. A view in which most of the emphasis is on the pattern of occurrence of rare species may be very different than one in which the emphasis is wholly on the handful of species that numerically dominate most of the samples. One convenient way of providing this spectrum of choice, to match the biological imperatives whilst retaining desirable theoretical properties, is to restrict attention to a single similarity coefficient (such as that of Bray and Curtis, 1957) but allow a choice of prior transformation of the data. A useful transformation continuum⁹ (Chapter 9) ranges through no transform, square root, fourth root,

logarithmic and finally reduction of the sample information to the recording only of presence or absence for each species: at the former end of the spectrum all attention will be focussed on the dominant counts, at the latter end on the rarer species.

For the clustering technique, *representation* of the communities for each sample is by a dendrogram (e.g. Fig. 1.7a), linking the samples in hierarchical groups on the basis of some definition of similarity between each cluster (Chapter 3). This is a particularly appropriate representation in cases where the samples are expected to divide into well-defined groups, perhaps structured by some clear-cut environmental distinctions. Where, on the other hand, the community pattern is responding to abiotic gradients which are more continuous, then representation by an ordination is usually more appropriate. The method of non-metric MDS (Chapter 5) attempts to place the samples on a "map", usually in two dimensions (e.g. see Fig. 1.7b), in such a way that the *rank order* of the distances between samples on the map exactly agrees with the rank order of the matching (dis)similarities, taken from the triangular similarity matrix. If successful, and success is measured by a "stress

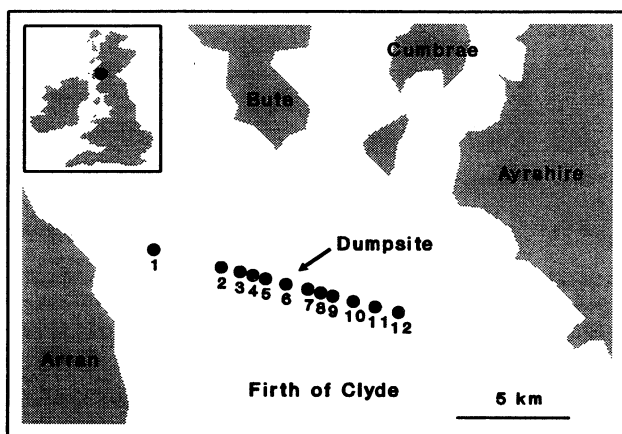


Fig. 1.5. Garroch Head, Scotland (G). Location of sewage sludge dump ground and position of sampling sites (1-12); the dump centre is at site 6.

9. Many of the PRIMER programs, such as the clustering routine CLUSTER, have built-in transformation and selection options. There are also stand-alone programs for these purposes, such as COLOP and REDUCE, the former allowing general algebraic operations on columns.

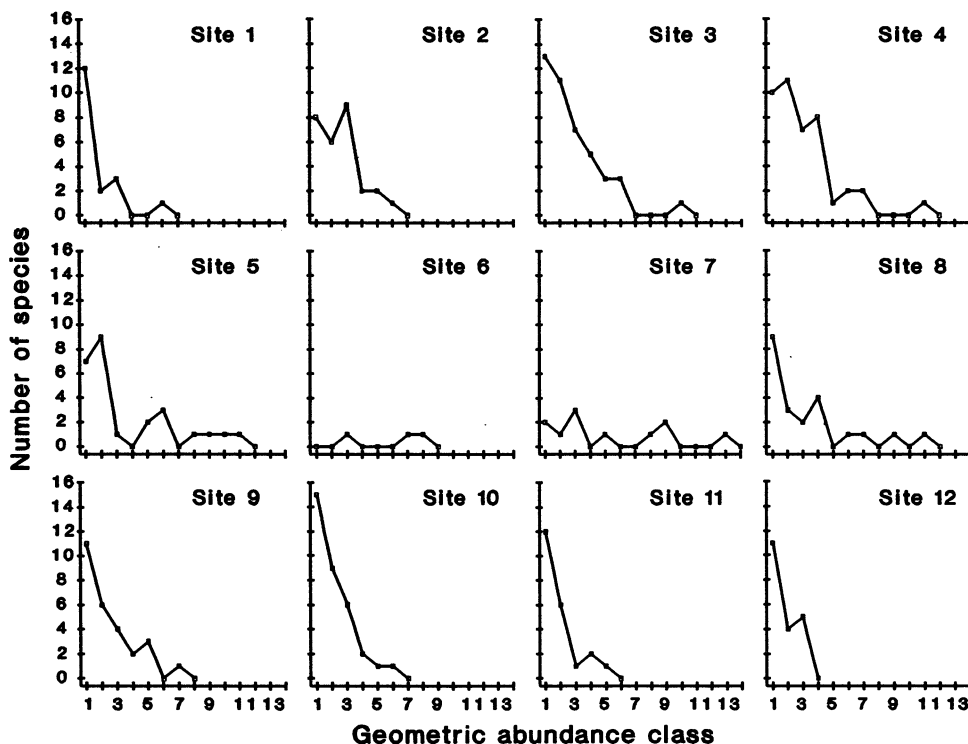


Fig. 1.6. Garroch Head macrofauna (G). Plots of number of species against number of individuals per species in $\times 2$ geometric classes, for the 12 sampling sites of Fig. 1.5.

Table 1.5. Multivariate techniques. Summary of analyses for the four stages.

| Stages | Multivariate examples | | |
|------------------------------------|--|---|--|
| | Hierarchical clustering (Ch 2, 3) | MDS ordination (Ch 5) | PCA ordination (Ch 4) |
| 1) Representing communities | Dendrogram of samples | Configuration plot of samples (often 2-dimensional) | |
| 2) Discriminating sites/conditions | ANOSIM on sample similarity matrix (Ch 6) Similarity percentage breakdown (Ch 7) gives species responsible | | ANOSIM on Euclidean distances (or multinormal tests, rarely valid) |
| 3) Determining stress levels | Recent suggestions: "meta-analyses", variability measures, breakdown of seriation (Ch 15) | | |
| 4) Linking to environment | Visual: superimposing environmental variables on biotic ordinations (Ch 11) Analytical: finding subset of environmental variables whose ordination "best matches" the biotic ordination (Ch 11) (Causality: see Ch 12) | | |

Table 1.6. Frierfjord macrofauna (F). Bray-Curtis similarities, after \sqrt{v} -transformation of counts, for every pair of replicate samples from sites A, B, C only (four replicates per site).

| | | | | | | | | | | | |
|----|----|----|----|----|----|----|----|----|----|----|----|
| A1 | A2 | A3 | A4 | B1 | B2 | B3 | B4 | C1 | C2 | C3 | C4 |
| A1 | - | | | | | | | | | | |
| A2 | 61 | - | | | | | | | | | |
| A3 | 69 | 60 | - | | | | | | | | |
| A4 | 65 | 61 | 66 | | | | | | | | |
| B1 | 37 | 28 | 37 | 35 | - | | | | | | |
| B2 | 42 | 34 | 31 | 32 | 55 | - | | | | | |
| B3 | 45 | 39 | 39 | 44 | 66 | 66 | - | | | | |
| B4 | 37 | 29 | 29 | 37 | 59 | 63 | 60 | - | | | |
| C1 | 35 | 31 | 27 | 25 | 28 | 56 | 40 | 34 | - | | |
| C2 | 40 | 34 | 26 | 29 | 48 | 69 | 62 | 56 | 56 | - | |
| C3 | 40 | 31 | 37 | 39 | 59 | 61 | 67 | 53 | 40 | 66 | - |
| C4 | 36 | 28 | 34 | 37 | 65 | 55 | 69 | 55 | 38 | 64 | 74 |

coefficient" which reflects the extent to which the two sets of ranks do not agree, the ordination gives a simple and compelling visual representation of "closeness" of the species composition for any two samples.

The PCA technique (Chapter 4) takes a different starting position, and makes rather different assumptions about the definition of (dis)similarity of two samples, but again ends up with an ordination plot, usually in two or three dimensions, which approximates the continuum of relationships between samples (e.g. Fig. 1.8). In fact, PCA is a rather unsatisfactory procedure for most species-by-samples matrices, for at least two reasons:

- it defines dissimilarity of samples in an inflexible way (Euclidean distance in the full-dimensional species space, Chapter 4), not well-suited to the rather special nature of species abundance data, with its predominance of zero values;
- it requires exclusion of the species which are less common, so that the number of species retained is comparable with the number of samples.

However, a description of its operation is included in this manual because it is an historically important technique, the first ordination method to be devised and one which is still commonly encountered¹⁰, and because it comes into its own in the analysis of environmental samples. Abiotic variables (sediment grain size, salinity, contaminant levels etc.) are usually relatively few in number, are continuously scaled, and their distributions can be transformed so that standard correlation coefficients (and Euclidean distances) are appropriate ways of describing their inter-relationships. PCA is then a fully satisfactory means of producing a low-dimensional summary, and even has some advantages over MDS in providing an interpretation of the main axes of the plot.

10. In fact, rather a bewildering array of ordination techniques are in common use (e.g. Principal Co-ordinates Analysis, Correspondence Analysis, Detrended Correspondence Analysis, etc). Chapter 5 has some brief remarks on their relation to PCA and MDS but this manual concentrates only on the two ordination methods available in PRIMER.

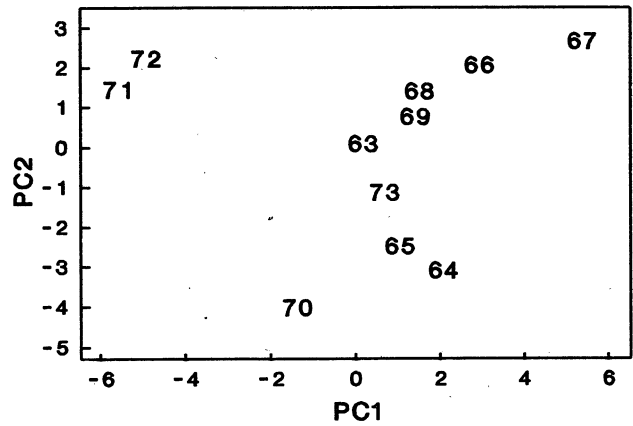
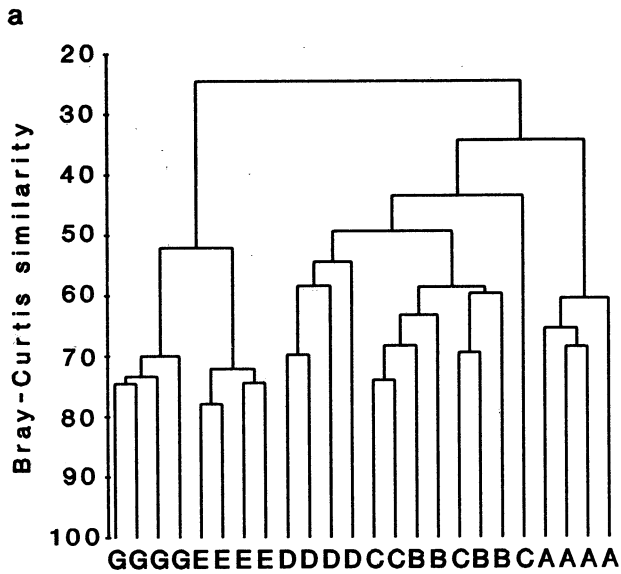


Fig. 1.8. Loch Linnhe macrofauna (L). Two-dimensional principal component analysis (PCA) ordination of the \sqrt{x} -transformed abundances from the 11 years 1963-1973, omitting the less-common species.

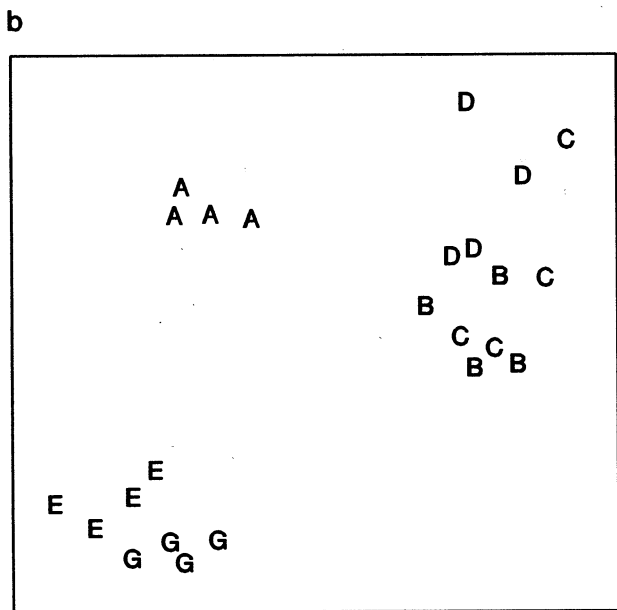


Fig. 1.7. Frierfjord macrofauna (F). a) Dendrogram for hierarchical clustering (group-average linking); b) non-metric multi-dimensional scaling (MDS) ordination in two dimensions; both computed for the four replicates from each of the six sites (A-E, G), using the similarity matrix partially shown in Table 1.4.

Discriminating sites/conditions from a multivariate analysis requires non-“classical” hypothesis testing ideas, since it is totally invalid to make the standard assumptions of normality (which in this case would need to be multivariate normality of the 100+ dimensions of the different species!). Instead, Chapter 6 describes a simple permutation or randomisation test (of the type first studied by Mantel, 1967), that makes very few assumptions about the data and is therefore widely applicable. In Fig. 1.7b for example, it

is clear without further testing that site A has a different community composition across its replicates than the groups (E, G) or (B, C, D); much less clear is whether there is any statistical evidence of a distinction between the B, C and D sites. A non-parametric test of the null hypothesis of “no site differences between B, C and D” can be constructed by defining a statistic which contrasts between-site and within-site “distances”, then recomputing it for all possible permutations of the 12 labels (4 Bs, 4 Cs and 4 Ds) between the 12 locations on the MDS. If these arbitrary site relabellings generate values of the statistic which are similar to the value for the real labelling, then there can clearly be little evidence that the sites are biologically distinguishable. This idea is formalised and extended to more complex sample designs in Chapter 6; for reasons which are described there it is preferable to compute a “between versus within site” summary statistic directly from the (rank) similarity matrix rather than the distances on the MDS plot. This, and the analogy with ANOVA, suggests the term ANOSIM for the test (Analysis of Similarities, Clarke and Green, 1988; Clarke, 1993).¹¹ It is possible to employ the same test in connection with PCA, using an underlying dissimilarity matrix of Euclidean distances, though when the ordination is of a relatively small number of environmental variables, which can be transformed into approximate multivariate normality, then abiotic differences between sites can be

11. PRIMER performs tests for one- and two-way crossed and nested designs in the program ANOSIM, with a special type of two-way crossed design tested in ANOSIM2.

tested by a standard multivariate equivalent of ANOVA (MANOVA, e.g. Mardia *et al.*, 1979).

Part of the process of discriminating sites, times, treatments etc., where successful, is the ability to identify the species that are principally responsible for these distinctions: it is all too easy to lose sight of the basic data matrix in a welter of sophisticated multivariate analyses! Similarly, one might as a result of a cluster analysis determine certain sites/times that group together, and again wish to identify which species are mainly responsible for the observed clustering. Note the distinction here between *a priori* groups, identified before examination of the data, and *a posteriori* groups, identified as a result of the data analysis (the ANOSIM tests are *only* applicable to *a priori* hypotheses). These ideas are pursued in Chapter 7, both through rearrangement of the data matrix and through a possible partition of the average Bray–Curtis dissimilarity between groups of samples, into components from different species (similarity percentage breakdown, SIMPER, Clarke, 1993).

In the *determination of stress levels*, whilst the multivariate techniques are sensitive (Chapter 14) and well-suited to establishing community differences associated with different sites/times/treatments etc., their species-specific basis would appear to make them unsuitable for drawing general inferences about the “pollution status” of an isolated group of samples. Even in comparative studies, on the face of it there is not a clear sense of “directionality” of change (e.g. deleteriousness), when it is established that communities at putatively impacted sites differ from those at control sites. Nonetheless, there are a number of ways in which such directionality *has* been ascribed in recent studies, whilst retaining an essentially multivariate form of analysis (Chapter 15):

- a) a “meta-analysis” – a combined ordination of data from NE Atlantic shelf waters, at a coarse level of taxonomic discrimination¹² – suggests a common directional change in the balance of taxa under a variety of types of pollution/disturbance (Warwick and Clarke, 1993a);
- b) a number of studies demonstrate increased “multivariate dispersion” among replicates under impacted conditions, in comparison to controls (Warwick and Clarke, 1993b);

12. The effect of carrying out the various graphical and multivariate analyses at taxonomic levels higher than species is the subject of Chapter 10.

- c) another feature of disturbance, demonstrated in a particular coral community study, but with the potential for wider applicability, is a loss of smooth “seriation” patterns along transects (e.g. of increasing depth), again in comparison to controls in time or space (Clarke *et al.*, 1993).

Two methods of *linking multivariate biotic patterns to environmental variables* are explored in Chapter 11; these are illustrated here by the Garroch Head dump–ground study described earlier (Fig. 1.5). The MDS of the macrofaunal communities from the 12 sites is shown in Fig. 1.9a; this is based on Bray–Curtis similarities computed from (transformed) species biomass values.¹³ A steady change in the community is apparent as the dump centre (site 6) is approached along the West arm of the transect (sites 1 to 6), with a mirrored structure along the East arm (sites 6 to 12), so that the samples from the two ends of the transect have similar species composition. That this biotic pattern correlates with the organic loading of the sediments can best be seen by superimposing the values for a single environmental variable, such as Carbon concentration, on the MDS configuration. Fig. 1.9b represents C values by circles of differing diameter, placed at the corresponding site locations on the MDS, and the pattern across sites of the 11 available environmental variables (sediment concentrations of C, N, Cu, Cd, Zn, Ni, etc.) can be viewed in this way (Chapter 11).¹⁴

A different approach is required in order to answer questions about *combinations* of environmental variables, for example to what extent the biotic pattern can be “explained” by knowledge of the full set, or a

13. Chapter 13, and the meta-analysis section in Chapter 15, discuss the relative merits and drawbacks of using species abundance or species biomass when both are available; in fact, Chapter 13 is a wider discussion of the relative advantages of sampling particular components of the biota, for a study on the effects of pollutants.

14. The flexibility is clearly needed to plot an MDS configuration several times, superimposing different environmental variables. Such situations are the main motivation for the modular construction of the PRIMER package, with its stand-alone routines that exchange information via files. Thus, a similarity matrix is output by CLUSTER and input to MDS (and BIOENV, ANOSIM etc.), and configuration co-ordinates are output by MDS (and PCA) and input to the plotting routine CONPLOT. This can then be run repeatedly with differing conversion files of site designations, or different columns of an environmental file, without the need to re-run the similarity or MDS computations.

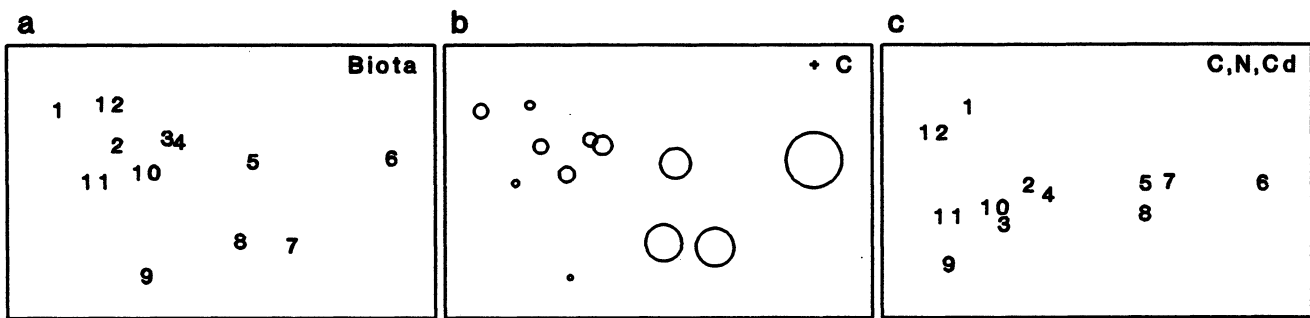


Fig. 1.9. Garroch Head macrofauna (G). a) MDS ordination of Bray–Curtis similarities from $\sqrt{\cdot}$ -transformed species biomass data for the sites shown in Fig. 1.5; b) the same MDS but with superimposed circles of increasing size, representing increasing carbon concentrations in matched sediment samples; c) ordination of (log-transformed) carbon, nitrogen and cadmium concentrations in the sediments at the 12 sites.

subset, of the abiotic variables. Though there is clearly one strong underlying gradient in Fig. 1.9a (horizontal axis), corresponding to an increasing level of organic enrichment, there are nonetheless secondary community differences (e.g. on the vertical axis) which may be amenable to explanation by metal concentrations, for example. The heuristic approach adopted here is to display the *multivariate* pattern of the environmental data, ask to what extent it matches the between-site relationships observed in the biota, and then maximise some *matching coefficient* between the two, by examining all possible subsets of the abiotic variables (the BIO-ENV procedure, Chapter 11)¹⁵.

Fig. 1.9c is based on this optimal subset for the Garroch Head sediment variables, namely (C, N, Cd). It is an MDS plot, using Euclidean distance for its dissimilarities,¹⁶ and is seen to replicate the pattern in Fig. 1.9a rather closely. In fact, the optimal match is determined by correlating the underlying dissimilarity matrices rather than the ordinations themselves, in parallel with the reasoning behind the ANOSIM tests, discussed earlier.

The suggestion is therefore that the biotic pattern of the Garroch Head sites is associated not just with an organic enrichment gradient but also with a particular heavy metal. It is important, however, to realise the

limitations of such an “explanation”. Firstly, there are usually other combinations of abiotic variables which will correlate nearly as well with the biotic pattern, particularly as here when the environmental variables are strongly inter-correlated amongst themselves. Secondly, there can be no direct implication of *causality* of the link between these abiotic variables and the community structure, based solely on field survey data: the real driving factors could be unmeasured but happen to correlate highly with the variables identified as producing the optimal match. This is a general feature of inference from purely observational studies and can only be avoided formally by “randomising out” effects of unmeasured variables; this requires random allocation of treatments to observational units for field or laboratory-based community *experiments* (Chapter 12).

EXAMPLE: Nutrient enrichment experiment, Solbergstrand

An example is given in Table 1.7 of meiofaunal community data from Solbergstrand, Norway (N), in which 12 undisturbed box cores of sediment were transferred into a mesocosm facility and separately “dosed” with two levels of increased nutrients (low, L, and high, H), with some boxes remaining undosed (control, C). Fig. 1.10 shows the MDS plots of the four replicate boxes from each treatment, separately for the copepod and nematode components of the meiofaunal communities (see also Chapter 12). For the copepods, there is a clear imputation of a (causal) response to the treatment, though this is less apparent for the nematodes: it is to test this type of null hypothesis (“no evidence of treatment effect”) that the ANOSIM tests of Chapter 6 are principally designed.

15. The BIOENV program in PRIMER optimises the match over all combinations of abiotic variables or, in cases where this is not computationally feasible, over a specified subset of them (e.g. all two- or three-variable combinations).

16. It is, though, virtually indistinguishable in this case from a PCA, because of the small number of variables and the implicit use of the same dissimilarity matrix for both techniques.

Table 1.7. Nutrient enrichment experiment, Solbergstrand mesocosm, Norway (N). Meiofaunal abundances (shown for copepods only) from four replicate boxes for each of three treatments (Control, Low and High levels of added nutrients).

| Species | Control | | | | Low dose | | | | High dose | | | |
|-------------------------------------|---------|----|----|----|----------|-----|-----|-----|-----------|----|-----|----|
| | C1 | C2 | C3 | C4 | L1 | L2 | L3 | L4 | H1 | H2 | H3 | H4 |
| <i>Halectinosoma gothiceps</i> | 0 | 0 | 1 | 1 | 16 | 23 | 8 | 16 | 0 | 1 | 0 | 0 |
| <i>Danielssania fusiformis</i> | 1 | 1 | 1 | 1 | 1 | 3 | 8 | 5 | 1 | 0 | 0 | 3 |
| <i>Tisbe sp. 1 (gracilis group)</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 27 | 119 | 31 |
| <i>Tisbe sp. 2</i> | 0 | 0 | 0 | 0 | 45 | 22 | 39 | 25 | 6 | 0 | 3 | 32 |
| <i>Tisbe sp. 3</i> | 0 | 0 | 0 | 0 | 86 | 83 | 88 | 0 | 5 | 29 | 0 | 20 |
| <i>Tisbe sp. 4</i> | 0 | 0 | 0 | 0 | 151 | 249 | 264 | 87 | 8 | 0 | 0 | 34 |
| <i>Tisbe sp. 5</i> | 0 | 0 | 0 | 0 | 129 | 0 | 0 | 115 | 4 | 0 | 1 | 40 |
| <i>Typhlamphiascus typhlops</i> | 4 | 2 | 2 | 4 | 5 | 8 | 4 | 3 | 0 | 0 | 0 | 0 |
| <i>Bulbamphiascus imus</i> | 1 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Stenhelia reflexa</i> | 3 | 1 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Amphiascus tenuiremis</i> | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 6 | 0 | 0 | 0 | 0 |
| <i>Ameira parvula</i> | 0 | 0 | 0 | 0 | 4 | 2 | 3 | 2 | 2 | 0 | 1 | 2 |
| <i>Proameira simplex</i> | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 5 | 0 | 0 | 0 | 0 |
| <i>Leptosyllus paratypicus</i> | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Enhydrosoma longifurcatum</i> | 2 | 2 | 1 | 2 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Laophontidae indet. | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| <i>Ancorabolis mirabilis</i> | 3 | 0 | 4 | 4 | 2 | 18 | 3 | 3 | 27 | 3 | 1 | 0 |
| Unidentified Copepodites | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 3 | 0 | 1 | 0 | 0 |
| | | | | | | | | | | | | |

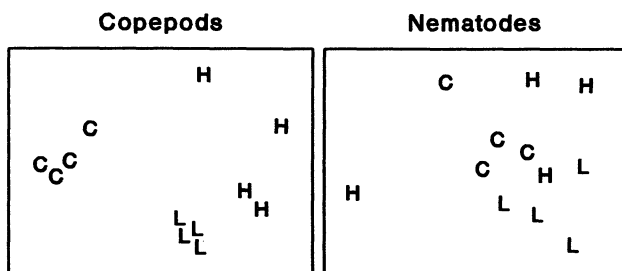


Fig. 1.10. Nutrient enrichment experiment (N). Separate MDS ordinations of $\sqrt{\sqrt{\cdot}}$ -transformed abundances for copepod and nematode species, in four replicate boxes from each of three treatments (C, L, H).

SUMMARY

A framework has been outlined of three categories of technique (*univariate, graphical/distributional and multivariate*) and four analysis stages (*representing communities, discriminating sites/conditions, determining levels of stress and linking to environmental variables*). The least familiar tools, and the most powerful, are in the multivariate category, and those that underlie the PRIMER programs in particular are now examined from first principles.

CHAPTER 2: MEASURES OF SIMILARITY OF SPECIES ABUNDANCE / BIOMASS BETWEEN SAMPLES

SIMILARITY FOR QUANTITATIVE DATA MATRICES

Data matrix

The available biological data is assumed to consist of an array with p rows (species) and n columns (samples), whose entries are counts of each species for each sample, or the total biomass of all individuals of each species in each sample. For the moment nothing further is assumed about the structure of the samples. They might consist of one or more replicates (repeated samples) from a number of different sites, times or experimental "treatments" but this information is *not* used in the initial analysis. The strategy outlined in Chapter 1 is to *observe* any pattern of similarities and differences across the samples (i.e. let the biology "tell its own story") and, only later, compare this with known or hypothesised inter-relations between the samples based on environmental or experimental factors.

Similarity coefficient

The starting point for many of the analyses that follow is the concept of *similarity* (S) between any pair of samples, in terms of the biological communities they contain. Inevitably, because the information for each sample is multivariate (many species), there are many ways of defining similarity, each giving different weight to different aspects of the community. For example, some definitions might concentrate on the similarity in abundance of the few commonest species whereas others pay more attention to concurrence of rare species.

The data matrix itself may first be modified; there are three main possibilities.

- The absolute numbers (or biomass), i.e. the fully quantitative data observed for each species, are most commonly used. In this case, two samples are considered perfectly similar only if they contain the same species in *exactly* the same abundance.
- The relative numbers (or biomass) are sometimes used, i.e. the data is *standardised* to give the percentage of total abundance/biomass (over all species) that is accounted for by each species. Thus each matrix entry is divided by its column total (and

multiplied by 100) to form the new array. Such standardisation will be essential if, for example, differing *and unknown* volumes of sediment or water are sampled, so that absolute numbers of individuals are not comparable between samples. Even if sample volumes are the same (or, if different, abundances are adjusted to a unit sample volume), it may still sometimes be biologically more relevant to define two samples as being perfectly similar when they have the same % *composition* of species, fluctuations in total abundance (or biomass) being of no interest.

- A reduction to simple presence or absence of each species may be all that is justifiable. For example, sampling artefacts may make quantitative counts totally unreliable, or concepts of abundance may be difficult to define for some important faunal components.

A similarity coefficient S is conventionally defined to take values in the range (0,100%), or less commonly (0,1), with the ends of the range representing the extreme possibilities:

$S = 100\%$ (or 1) if two samples are totally similar;

$S = 0$ if two samples are totally dissimilar.

What constitutes total similarity, and particularly total dissimilarity, of two samples depends on the specific similarity coefficient adopted but there are clearly some properties that it would be desirable for a coefficient to possess. For example, S should equal zero when two samples have no species in common and S must equal 100% if two samples have identical entries (after data reduction, in cases b and c above).

Similarity matrix

Similarities are calculated between every pair of samples and it is conventional to set these $n(n-1)/2$ values out in a lower triangular matrix. This is a square array, with row and column labels being the sample numbers 1 to n , but it is not necessary to fill in either the diagonals (similarity of sample j with itself is always 100%!) or the upper right triangle (the similarity of sample j to sample k is the same as the similarity of sample k to sample j , of course).

Similarity matrices are the basis (explicitly or implicitly) of many multivariate methods, both in the representation given by a clustering or ordination

analysis and in some associated statistical tests. A similarity matrix can be used to:

- a) discriminate sites (or times) from each other, by noting that similarities between replicates within a site are consistently higher than similarities between replicates at different sites (ANOSIM test, Chapter 6);
- b) cluster sites into groups that have similar communities, so that similarities within each group of sites are usually higher than those between groups (Clustering, Chapter 3);
- c) allow a gradation of sites to be represented graphically, in the case where site A has some similarity with site B, B with C, C with D but A and C are less similar, A and D even less so etc. (Ordination, Chapter 4).

Species similarity matrix

In a complementary way, the original data matrix can be thought of as describing the pattern of occurrences of each species across the given set of samples, and a matching triangular array of similarities can be constructed between every pair of species. Two species are "similar" (S' near one) if they have significant representation at the same set of sites, and totally "dissimilar" ($S' = 0$) if they never co-occur. Species similarities are discussed later in this chapter, and the resulting clustering and ordination diagrams in Chapter 7, but for the bulk of this manual "similarity" refers to *between-sample* similarity.

Bray-Curtis coefficient

Of the numerous similarity coefficients that have been suggested over the years, one has become particularly common in ecological work, resulting from a terrestrial application (Bray and Curtis, 1957). It is also sometimes referred to as the Czekanowski coefficient. The similarity between the j th and k th samples, S_{jk} , has two definitions (they are entirely equivalent, as can be seen from some simple algebra or by calculating a few examples):

$$S_{jk} = 100 \left(1 - \frac{\sum_{i=1}^p |y_{ij} - y_{ik}|}{\sum_{i=1}^p (y_{ij} + y_{ik})} \right) \tag{2.1}$$

$$= 100 \frac{\sum_{i=1}^p 2 \min(y_{ij}, y_{ik})}{\sum_{i=1}^p (y_{ij} + y_{ik})}$$

Here y_{ij} represents the entry in the i th row and j th column of the data matrix, i.e. the abundance (or biomass) for the i th species in the j th sample ($i = 1, 2, \dots, p; j = 1, 2, \dots, n$). Similarly, y_{ik} is the count for the i th species in the k th sample. $|...|$ represents the absolute value of the difference (the sign is ignored) and $\min(...)$ the minimum of the two counts; the separate sums in the numerator and denominator are both over all rows (species) in the matrix.

EXAMPLE: Loch Linnhe macrofauna

A trivial example, used in this and the following chapter to illustrate simple manual computation of similarities and hierarchical clusters, is provided by extracting six species and four years from the Loch Linnhe macrofauna data $\{L\}$ of Pearson (1975), seen already in Fig. 1.3 and Table 1.4. (Of course, arbitrary extraction of "interesting" species and years is not a legitimate procedure in a real application; it is done here simply as a means of showing the computational steps).

Table 2.1. Loch Linnhe macrofauna $\{L\}$ subset. (a) Abundance (untransformed) for some selected species and years. (b) The resulting Bray-Curtis similarities between every pair of samples.

| (a) Year: | 64 | 68 | 71 | 73 | (b) | | | | |
|------------------|----|-----|-----|----|----------|----|----|---|---|
| (Sample: | 1 | 2 | 3 | 4) | Sample 1 | 2 | 3 | 4 | |
| Species | | | | | 1 | - | | | |
| <i>Echinoca.</i> | 9 | 0 | 0 | 0 | 2 | 8 | - | | |
| <i>Myrioche.</i> | 19 | 0 | 0 | 3 | 3 | 0 | 42 | - | |
| <i>Labidopl.</i> | 9 | 37 | 0 | 10 | 4 | 39 | 21 | 4 | - |
| <i>Amaeana</i> | 0 | 12 | 144 | 9 | | | | | |
| <i>Capitella</i> | 0 | 128 | 344 | 2 | | | | | |
| <i>Mytilus</i> | 0 | 0 | 0 | 0 | | | | | |

Table 2.1a shows the data matrix of counts and Table 2.1b the resulting lower triangular matrix of Bray-Curtis similarity coefficients. For example, using the first form of equation (2.1), the similarity between samples 1 and 4 (years 1964 and 1973) is:

$$S_{14} = 100 \left(1 - \frac{9+16+1+9+2+0}{9+22+19+9+2+0} \right) = 39.3$$

The second form of equation (2.1) can be seen to give the same result:

$$S_{14} = 100 \left(\frac{2[0+3+9+0+0+0]}{9+22+19+9+2+0} \right) = 39.3$$

Computation is therefore simple and it is easy to verify that the coefficient possesses the following desirable properties.

- a) $S = 0$ if the two samples have no species in common since $\min(y_{ij}, y_{ik}) = 0$ for all i (e.g. samples 1 and 3 of

Table 2.1a). Of course, $S = 100$ if two samples are identical, since $|y_{ij} - y_{ik}| = 0$ for all i .

- b) A scale change in the measurements does not change S . For example, biomass could be expressed in g rather than mg or abundance changed from numbers per cm² of sediment surface to numbers per m²; all y values are simply multiplied by the same constant and this cancels in the numerator and denominator terms of equation (2.1).
- c) "Joint absences" also have no effect on S . In Table 2.1a the last species is absent in all samples; omitting this species clearly makes no difference to the two summations in equation (2.1). That similarity should depend on species which are present in one or other (or both) samples, and *not* on species which are absent from both, is usually a desirable property. As Field *et al.* (1982) put it: "Taking account of joint absences has the effect of saying that estuarine and abyssal samples are similar because both lack outer-shelf species". Nonetheless, independence of joint absences is a property not shared by all similarity coefficients.

Transformation of raw data

In one or two ways, the similarities of Table 2.1b are not a good reflection of the overall match between the samples, taking all species into account. To start with, the similarities all appear too low; samples 2 and 3 would seem to deserve a similarity rating higher than 50%. As will be seen later, this is not an important consideration since the most useful multivariate methods depend on the relative order (*ranking*) of the similarities in the triangular matrix, rather than their absolute values. More importantly, the similarities of Table 2.1b are unduly dominated by the counts for the two most abundant species (4 and 5), as can be seen from studying the form of equation (2.1): terms involving species 4 and 5 dominate the sums in both numerator and denominator. Yet the larger abundances in the original data matrix will often be extremely variable in replicate samples (in statistical terms, variance is often found to increase with the square of the mean) and it is quite undesirable to base an assessment of similarity of two communities only on the counts for a handful of very abundant species.

The answer is to transform the original y values (counts or biomass) *before* computing the Bray-Curtis similarities. Two useful transformations are the *log transform*, $\log(1 + y)$, and the *double root* (or *4th root*) transform $\sqrt[4]{y}$. There is more on the effects of transformation later in the manual; for now it is only necessary to note that the $\log(1 + y)$ and $\sqrt[4]{y}$ transforms

have an approximately similar and fairly severe effect in down-weighting the importance of the very abundant species so that the less dominant, and even the rare species, play some role in determining similarity of two samples. The result of the $\sqrt[4]{y}$ transform for the previous example is shown in Table 2.2a and the Bray-Curtis similarities computed from these transformed abundances, using equation (2.1), are given in Table 2.2b.¹

Table 2.2. Loch Linnhe macrofauna {L} subset. (a) $\sqrt[4]{y}$ -transformed abundance for the four years and six species of Table 2.1. (b) Resulting Bray-Curtis similarity matrix.

| (a) Year: | 64 | 68 | 71 | 73 | (b) | | | | |
|-----------|-----|-----|-----|-----|--------|----|----|----|---|
| (Sample: | 1 | 2 | 3 | 4) | Sample | 1 | 2 | 3 | 4 |
| Species | | | | | 1 | - | | | |
| Echinoca. | 1.7 | 0 | 0 | 0 | 2 | 26 | - | | |
| Myrioche. | 2.1 | 0 | 0 | 1.3 | 3 | 0 | 68 | - | |
| Labidopl. | 1.7 | 2.5 | 0 | 1.8 | 4 | 52 | 68 | 42 | - |
| Amaeana | 0 | 1.9 | 3.5 | 1.7 | | | | | |
| Capitella | 0 | 3.4 | 4.3 | 1.2 | | | | | |
| Mytilus | 0 | 0 | 0 | 0 | | | | | |

There is a general increase in similarity levels but, of more importance, the rank order of similarities is no longer the same as in Table 2.1b (eg $S_{24} > S_{14}$ and $S_{34} > S_{12}$ now), showing that transformations *can* have a significant effect on the final ordination or clustering display. In fact, for very variable data, choice of transformation can sometimes be more critical than choice of similarity coefficient or ordination technique, and the subject therefore merits a chapter to itself (Chapter 9).

Canberra coefficient

An alternative to transformation is to select a similarity coefficient that *automatically* adjusts the weighting given to each species when computed on original counts (or biomass). One such possibility given by Lance and Williams (1967) and referred to as the *Canberra coefficient*, defines similarity between sample j and sample k as:

$$S_{jk} = 100 \left(1 - p^{-1} \sum_{i=1}^p \frac{|y_{ij} - y_{ik}|}{(y_{ij} + y_{ik})} \right) \quad (2.2)$$

Clearly, this has a strong likeness to the Bray-Curtis coefficient, but the absolute differences in counts for each species are *separately* scaled, i.e. the denominator

1. Bray-Curtis is the main coefficient calculated by the PRIMER CLUSTER program, which also allows a range of transformations of the data.

scaling term is *inside* not outside the summation over species. For example, from Table 2.1a, the Canberra similarity between samples 1 and 4 is:

$$S_{14} = 100(1 - \frac{1}{5}(\frac{9}{9} + \frac{16}{22} + \frac{1}{19} + \frac{9}{9} + \frac{2}{2})) = 24.4$$

Note that joint absences have no effect here because they are deliberately excluded and p is redefined as the number of species that are present in one or other sample.

The separate scaling constrains each species to contribute equally (potentially) to the similarity between two samples. However abundant a species is, its contribution to S can never be more than $100/p$, and a rare species with a single individual in each of the two samples contributes the same as a common species with 1000 individuals in each. Whilst there may be circumstances in which this is desirable, more often it leads to *overdomination* of the pattern by a large number of rare species, of no real significance. (Often the sampling strategy is incapable of adequately quantifying the rarer species so that they are distributed arbitrarily, to some degree, across the samples).

Correlation coefficient

A common statistical means of assessing the relationship between two columns of data (samples j and k here) is the standard *product moment*, or *Pearson*, correlation coefficient

$$r_{jk} = \frac{\sum_i (y_{ij} - \bar{y}_j)(y_{ik} - \bar{y}_k)}{\sqrt{[\sum_i (y_{ij} - \bar{y}_j)^2 \cdot \sum_i (y_{ik} - \bar{y}_k)^2]}} \quad (2.3)$$

where \bar{y}_j is defined as the mean count (or biomass) over all species for the j th sample. In this form it is not a similarity coefficient, since it takes values in the range $(-1, 1)$ not $(0, 100)$, with positive correlation (r near $+1$) if high counts in one sample match high counts in the other, and negative correlation ($r < 0$) if high counts match absences. There are a number of ways of converting r to a similarity coefficient, the most obvious for community data being:

$$S_{jk} = 50(1 + r_{jk}) \quad (2.4)$$

Whilst correlation is sometimes used as a similarity coefficient explicitly in this form, and more often implicitly as the similarity measure underlying certain ordination techniques (eg Principal Components Analysis, Chapter 4), it is not particularly suitable for much biological community data, with its plethora of

zero values. For example, it violates the criterion that S should not depend on joint absences; here two columns are more highly positively correlated (and give S nearer 100) if species are added which have zero counts for both samples. If correlation is to be used as a measure of similarity, it makes good sense to transform the data initially, exactly as for the Bray-Curtis computation, so that large counts or biomass do not totally dominate the coefficient.

PRESENCE/ABSENCE DATA

As discussed at the beginning of this chapter, quantitative uncertainty may make it desirable to reduce the data simply to presence or absence of each species in each sample, or this may be the only feasible or cost-effective option for data collection in the first place. Alternatively, reduction to presence/absence may be thought of as the ultimate in severe transformation of counts; the data matrix (e.g. in Table 2.1a) is replaced by 1 (presence) or 0 (absence) and Bray-Curtis similarity (say) computed. This will have the effect of giving equal weight to all species, whether rare or abundant (and will thus have somewhat similar effect to the Canberra coefficient).

Many similarity coefficients have been proposed based on $(0, 1)$ data arrays; see, for example, Sneath and Sokal (1973). When computing similarity between samples j and k , the two columns of data can be reduced to the following four summary statistics without any loss of relevant information.

a = the number of species which are present in both samples

b = the number of species present in sample j but absent from sample k

c = the number of species present in sample k but absent from sample j

d = the number of species absent from both samples

For example, when comparing samples 1 and 4 from Table 2.1a, these frequencies are:

| | | | | |
|-----------|---|-----------|---------|---------|
| | | Sample 4: | 1 | 0 |
| Sample 1: | 1 | | $a = 2$ | $b = 1$ |
| | 0 | | $c = 2$ | $d = 1$ |

In fact, because of the symmetry no distinction should be drawn between b and c ; sensible coefficients will depend only on $b + c$. Similarly, similarity measures that are not affected by joint absences will not contain d . The following are some of the more commonly advocated coefficients.

The "simple matching" similarity between samples j and k is defined as:

$$S_{jk} = 100.(a+d)/(a+b+c+d) \quad (2.5)$$

so called because it represents the probability ($\times 100$) of a single species picked at random (from the full species list) being present in both samples or absent in both samples. Note that S is a function of d here, and thus depends on joint absences.

If the "simple matching" coefficient is adjusted, by first removing all species which are jointly absent from samples j and k , one obtains the *Jaccard coefficient*:

$$S_{jk} = 100.a/(a+b+c) \quad (2.6)$$

i.e. S is the probability ($\times 100$) that a single species picked at random (from the reduced species list) will be present in both samples.

A popular coefficient found under several names, commonly *Sorenson* or *Dice*, is:

$$S_{jk} = 100.2a/(2a+b+c) \quad (2.7)$$

Note that this is identical to the Bray-Curtis coefficient when the latter is calculated on (0, 1) presence/absence data, as can be seen most clearly from the second form of equation (2.1).² For example, reducing Table 2.1a to (0,1) data, and comparing samples 1 and 4 as previously, equation (2.1) gives:

$$S_{14} = 100 \left\{ \frac{2[0+1+1+0+0+0]}{1+2+2+1+1+0} \right\} = 57.1$$

This is clearly the same construction as substituting $a = 2$, $b = 1$, $c = 2$ into equation (2.7).

Among the many other coefficients that have been proposed, one that can be found occasionally in marine ecological studies is that of *McConnaughey* (1964):

$$S_{jk} = 100[a(2a+b+c)]/[2(a+b)(a+c)] \quad (2.8)$$

RECOMMENDATIONS

1) In most ecological studies, it seems to make sense to use a coefficient which does not depend on the number of species which are jointly absent from both samples.

2. Thus the Sorenson coefficient can be obtained in the PRIMER CLUSTER program by "transforming" the data to presence/absence and selecting Bray-Curtis similarity.

2) Similarities calculated on original abundance (or biomass) values can often be over-dominated by a small number of highly abundant (or large-bodied) species, so that they fail to reflect similarity of overall community composition.

3) Some coefficients (such as the Canberra) which separately scale the contribution of each species to adjust for this, have a tendency to over-compensate, i.e. rare species, which may be arbitrarily distributed across the samples, are given equal weight to very common ones. The same criticism applies to reduction of the original matrix to simple presence/absence of each species. In addition, the latter loses potentially valuable information about the approximate prevalence of a species (absent, rare, present in modest numbers, common, very abundant etc).

4) A balanced compromise is often to apply a similarity coefficient such as Bray-Curtis to counts or biomass values which have been moderately (\sqrt{y}) or fairly severely transformed ($\log(1+y)$ or $\sqrt[3]{y}$). All species then contribute something to the definition of similarity whilst the retention of some information on the prevalence of a species ensures that the commoner species are generally given greater weight than the rare ones.

5) Initial standardisation is occasionally desirable, dividing each count by the total abundance of all species in that sample; this is essential when non-comparable, *unknown* sample volumes have been taken. Without this column standardisation, the Bray-Curtis coefficient will reflect differences between two samples due both to differing community composition and/or differing *total* abundance. The standardisation removes any effect of the latter; whether this is desirable is a biological rather than statistical question. (Experience with benthic communities suggests that the standardisation should usually be avoided, valuable biological information being contained in the abundance or biomass totals). Note, however, that column standardisation does not remove the need subsequently to transform the data matrix, if the similarities are to take account of more than just the few commonest species.³

3. In CLUSTER, standardisation is not the default option for sample similarities but, if selected, it is therefore carried out before any transformation.

SPECIES SIMILARITIES

Starting with the original data matrix of counts or biomass, the similarity between any pair of *species* can be defined in an analogous way to that for samples, but this time involving comparison of the *i*th and *l*th row (*species*) across all $j = 1, \dots, n$ columns (*samples*).

Bray-Curtis coefficient

The Bray-Curtis similarity between species *i* and *l* is:

$$S'_{il} = 100 \left(1 - \frac{\sum_{j=1}^n |y_{ij} - y_{lj}|}{\sum_{j=1}^n (y_{ij} + y_{lj})} \right) \quad (2.9)$$

The extreme values are (0, 100) as previously:

$S' = 0$ if two species have no samples in common (*i.e.* are never found at the same sites),

$S' = 100$ if the *y* values for two species are the same at all sites.

However, different initial treatment of the data is required, in two respects.

1) Similarities between rare species have little meaning; very often such species have single occurrences, distributed more or less arbitrarily across the sites, so that S' is usually zero (or occasionally 100). If these values are left in the similarity matrix they will tend to confuse and disrupt the patterns in any subsequent clustering or ordination analysis; the rarer species should thus be omitted from the data matrix before computing species similarities.

2) A different form of standardisation of the data matrix is appropriate and (in contrast to the samples analysis) it usually makes sense to carry this out routinely in place of a transformation. Two species could have quite different mean levels of abundance yet be "perfectly similar" in the sense that their counts are in strict ratio to each other across the samples. One species might be of much larger body size, and thus tend to have smaller counts, for example; or there might be a direct host-parasite relationship between the two species. It is therefore appropriate to standardise the original data by dividing each entry by its *row* (*species*) total (and multiplying by 100):

$$y_{ij}^* = 100 y_{ij} / (\sum_{k=1}^n y_{ik}) \quad (2.10)$$

before computing the similarities (S'). The effect of this can be seen in the following artificial example, for three species and five samples.

| Counts | | | | | | Similarities | | | |
|---------|----|---|---|----|----|--------------|----|---|---|
| Sample: | 1 | 2 | 3 | 4 | 5 | Species | 1 | 2 | 3 |
| Species | | | | | | 1 | - | | |
| 1 | 2 | 0 | 0 | 4 | 4 | 2 | 33 | - | |
| 2 | 10 | 0 | 0 | 20 | 20 | 3 | 20 | 7 | - |
| 3 | 0 | 4 | 4 | 1 | 1 | | | | |

↓ Standardise

| Sample: | 1 | 2 | 3 | 4 | 5 | Species | 1 | 2 | 3 |
|---------|----|----|----|----|----|---------|-----|----|---|
| Species | | | | | | 1 | - | | |
| 1 | 20 | 0 | 0 | 40 | 40 | 2 | 100 | - | |
| 2 | 20 | 0 | 0 | 40 | 40 | 3 | 20 | 20 | - |
| 3 | 0 | 40 | 40 | 10 | 10 | | | | |

From the original matrix, the Bray-Curtis similarity between species 1 and 2, for example, is only $S' = 33\%$ but the two species are found in strict proportion to each other across the samples so that, after row standardisation, they have a more realistic similarity of $S' = 100\%$. Note that it is not clear that a transformation now serves any useful purpose. Its role in the samples analysis was to reduce (though not totally remove) the large disparities in counts or biomass between species; the standardisation by row total has here removed such differences.

Correlation coefficient

The standard product moment correlation coefficient defined in equation (2.3), and modified to a similarity in equation (2.4), is perhaps more appropriate for defining species similarities than it was for samples, in that it automatically incorporates a type of row standardisation. In fact, this is a full *normalisation* (subtracting the row mean from each count and dividing by the row standard deviation) and it is less appropriate than the simple row standardisation above. In addition, the previous argument about the effect of joint absences is equally appropriate to species similarities: an intertidal species is no more similar to a deep-sea species because neither is found in shelf samples. A correlation will again be a function of joint absences; the Bray-Curtis coefficient will not.

RECOMMENDATION

For species similarities, a coefficient such as Bray-Curtis calculated on row-standardised and untransformed data seems most appropriate. The rarer species (usually at least half of the species set) should first be removed from the matrix, to have any chance of an interpretable clustering or ordination analysis. There are several ways of doing this, all of them

arbitrary to some degree. Field *et al.* (1982) suggest removal of all species that never constitute more than $p\%$ of the total abundance (/biomass) of any sample, where p is (arbitrarily) chosen to leave in around 50 or 60 species (typically $p = 3\%$ or so). This is preferable to simply retaining the 50 or 60 species with the highest total abundance across all samples, since the latter strategy may result in omitting several species which are key constituents of a site which is characterised by a low total number of individuals.⁴ It is important to note, however, that this inevitably arbitrary process of omitting species is *not necessary* for the more usual between-sample similarity calculations. There the computation of the Bray-Curtis coefficient down-weights the contributions of the less common species in an entirely natural and continuous fashion (the rarer the species the less it contributes, on average), and all species should be retained in these calculations.

DISSIMILARITY COEFFICIENTS

The converse concept to similarity is that of *dissimilarity*, the degree to which two samples are unlike each other. Though similarity and dissimilarity are just opposite sides of the same coin, the latter is a more natural starting point in constructing ordinations, in which *dissimilarities* (δ) between pairs of samples are turned into *distances* (d) between sample locations on a "map". Thus large dissimilarity implies that samples should be located at a large distance from each other, and dissimilarities near 0 imply nearby location; δ must therefore always be positive, of course.

Similarities can easily be turned into dissimilarities, by:

$$\delta = 100 - S \quad (2.11)$$

For example, for the Bray-Curtis coefficient this gives:

$$\delta_{jk} = 100 \cdot \frac{\sum_{i=1}^p |y_{ij} - y_{ik}|}{\sum_{i=1}^p (y_{ij} + y_{ik})} \quad (2.12)$$

which has limits $\delta = 0$ (no dissimilarity) and $\delta = 100$ (total dissimilarity).

4. The PRIMER CLUSTER program will compute Bray-Curtis species similarities, with or without row standardisation and transformation (though the default is as recommended here), and allowing prior reduction by the $p\%$ criterion, either by specifying p or the number N of retained species.

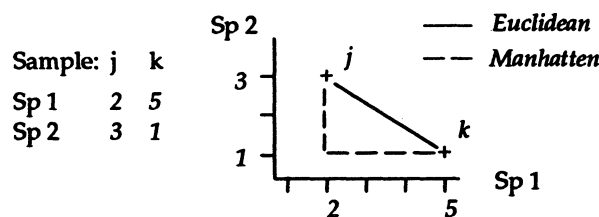
However, rather than conversion from similarities, other important dissimilarity measures arise in the first place as distances. Their role as implicit dissimilarity matrices underlying particular ordination techniques will be seen more clearly later (e.g. in Principal Components Analysis, Chapter 4).

Euclidean distance

The natural distance between any two points in space is referred to as *Euclidean* distance (from classical or Euclidean geometry). In the context of a species abundance matrix, the Euclidean distance between samples j and k is defined algebraically as:

$$d_{jk} = \sqrt{\sum_{i=1}^p (y_{ij} - y_{ik})^2} \quad (2.13)$$

This can best be understood, geometrically, by taking the special case where there are only two species so that samples can be represented by points in 2-dimensional space, namely their position on the two axes of Species 1 and Species 2 counts. This is illustrated below for a specific two samples by two species abundance matrix. The co-ordinate points (2, 3) and (5, 1) on the (Sp. 1, Sp. 2) axes are the two samples j and k . The direct distance d_{jk} between them of $\sqrt{[(2-5)^2 + (3-1)^2]}$ (Pythagoras) clearly corresponds to equation (2.13).



It is easy to envisage the extension of this to a matrix with three species; the two points are now simply located on 3-dimensional species axes and their straight line distance apart is a natural geometric concept. Algebraically, it is the root of the sums of squared distances apart along the three axes, equation (2.13). Extension to four and higher numbers of species (dimensions) is harder to envisage geometrically (in our 3-dimensional world) but the concept remains unchanged and the algebra is no more difficult to understand in higher dimensions than three: additional squared distances apart on each new species axis are added to the summation under the square root in (2.13). In fact, this concept of representing a species-by-samples matrix as points in high-dimensional species space is a very fundamental and important one and will be met again in Chapter 4,

where it is crucial to an understanding of Principal Components Analysis.

Manhattan (or city-block) distance

Euclidean distance is not the only way of defining distance apart of two samples in species space; an alternative is to sum the distances along each species axis:

$$d_{jk} = \sum_{i=1}^p |y_{ij} - y_{ik}| \quad (2.14)$$

This is often referred to as *Manhattan* (or *city-block*) distance because in two dimensions it corresponds to the distance you would have to travel to get between any two locations in a city whose streets are laid out in a rectangular grid. It is illustrated for the simple example above by the dashed lines. Manhattan distance is of interest here because of its obvious close affinity to Bray-Curtis dissimilarity, equation (2.12). In fact, when a data matrix has initially been column standardised (but not transformed), Bray-Curtis dissimilarity is just (half) the Manhattan distance, since the summation in the bottom line of (2.12) then always takes the value 200.

[It is worth noting a point of terminology in passing, though not one of any great practical consequence. Euclidean and Manhattan measures, (2.13) and (2.14), are called distances or *metrics* because they obey the triangle inequality, i.e. for any three samples j, k, r :

$$d_{jk} + d_{kr} \geq d_{jr} \quad (2.15)$$

Bray-Curtis dissimilarity does not, in general, satisfy the triangle inequality, so should not be called a metric. However, many other useful dissimilarity coefficients are also not metrics. For example, the square of

Euclidean distance (i.e. equation (2.13) without the $\sqrt{\quad}$ sign) is another natural definition of "distance" which is not a metric, yet dissimilarities from this would have the same rank order as those from Euclidean distance and therefore give rise, for example, to identical MDS ordinations (see Chapter 5). It follows that whether a similarity coefficient is, or is not, a metric is likely to be of limited practical significance for the strategy this manual advocates.]

RECOMMENDATION

There are thus a variety of means of generating a similarity or dissimilarity (/distance) matrix to input to the next stage of a multivariate analysis, which might be either a clustering or ordination of samples, Fig. 2.1. For comparative purposes it may sometimes be of interest to use Euclidean distance in the species space as input to a cluster analysis⁵ (an example is given later in Fig. 5.5) but, in general, the recommendation remains unchanged: Bray-Curtis similarity/dissimilarity, computed after suitable transformation, will often be a satisfactory coefficient for *biological* data on community structure. Background physical or chemical data is a different matter since it is usually of a rather different type, and Chapter 11 shows the usefulness of the concept of Euclidean distance in the (normalised) *environmental variable space*. Initially, though, concentration is on analysing the biological data in isolation, and the next stage will often be to perform a cluster analysis (Fig. 2.1).

5. The PRIMER CLUSTER program can generate Euclidean distances (normalised or not, see page 4-6), on either biotic or environmental input matrices.

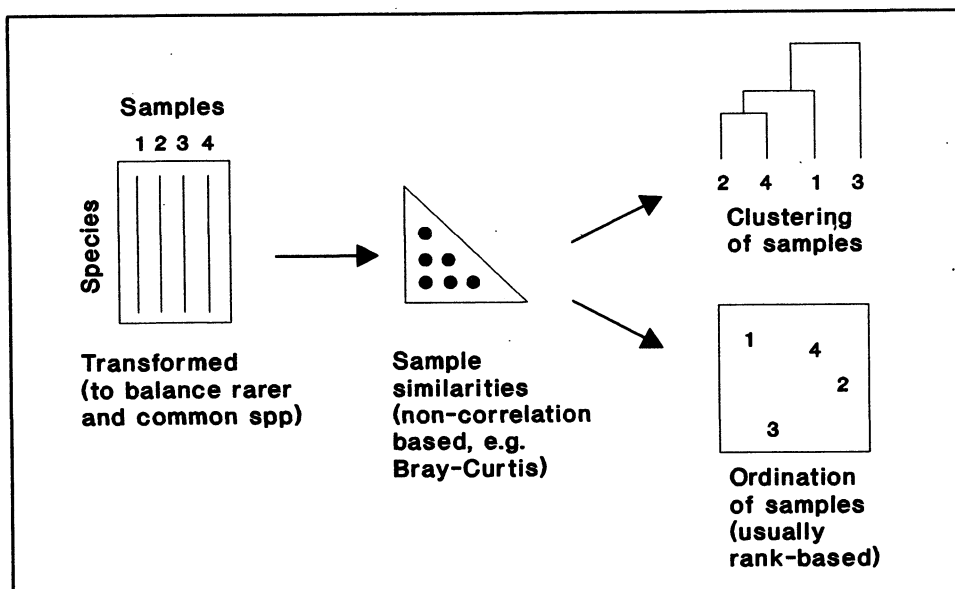


Fig. 2.1. Stages in a multivariate analysis based on similarity coefficients.

CHAPTER 3: HIERARCHICAL CLUSTERING

CLUSTER ANALYSIS

The previous chapter has shown how to replace the original data matrix with pairwise similarities, chosen to reflect the particular aspect of similarity in community structure (similarity in counts of abundant species, similarity in general disposition of rare species etc) which the biologist requires to emphasise for the study in question. Typically, the number of pairwise similarities is large, $n(n-1)/2$ for n samples, and it can often be no easier to detect a pattern in the resulting lower triangular similarity matrix than it is in the original data. Table 3.1 illustrates this for just a portion (roughly a quarter) of the similarity matrix for the Frierfjord macrofauna data {F}. Close examination shows that the four replicates within site A generally have higher within-site similarities than do pairs of replicates within sites B and C, or replicates *between* sites, but the pattern is far from clear. What is needed is a graphical display linking samples that have mutually high levels of similarity.

Table 3.1. Frierfjord macrofauna counts {F}. Bray-Curtis similarities, after $\sqrt{\cdot}$ transformation of counts, for every pair of replicate samples from sites A, B, C only (four replicate samples per site).

| | A1 | A2 | A3 | A4 | B1 | B2 | B3 | B4 | C1 | C2 | C3 | C4 |
|----|----|----|----|----|----|----|----|----|----|----|----|----|
| A1 | - | | | | | | | | | | | |
| A2 | 61 | - | | | | | | | | | | |
| A3 | 69 | 60 | - | | | | | | | | | |
| A4 | 65 | 61 | 66 | - | | | | | | | | |
| B1 | 37 | 28 | 37 | 35 | - | | | | | | | |
| B2 | 42 | 34 | 31 | 32 | 55 | - | | | | | | |
| B3 | 45 | 39 | 39 | 44 | 66 | 66 | - | | | | | |
| B4 | 37 | 29 | 29 | 37 | 59 | 63 | 60 | - | | | | |
| C1 | 35 | 31 | 27 | 25 | 28 | 56 | 40 | 34 | - | | | |
| C2 | 40 | 34 | 26 | 29 | 48 | 69 | 62 | 56 | 56 | - | | |
| C3 | 40 | 31 | 37 | 39 | 59 | 61 | 67 | 53 | 40 | 66 | - | |
| C4 | 36 | 28 | 34 | 37 | 65 | 55 | 69 | 55 | 38 | 64 | 74 | - |

Cluster analysis (or **classification**) aims to find "natural groupings" of samples such that samples within a group are more similar to each other, generally, than samples in different groups. Cluster analysis is used in the present context in the following ways.

a) Different sites (or different times at the same site) can be seen to have differing community composi-

tions by noting that replicate samples within a site form a cluster that is distinct from replicates within other sites. This can be an important hurdle to overcome in any analysis; if replicates for a site are clustered more or less randomly with replicates from every other site then further interpretation is likely to be dangerous. (A more formal statistical test for distinguishing sites is the subject of Chapter 6).

- b) When it is established that sites can be distinguished from one another (or, when replicates are not taken, it is assumed that a single sample is representative of that site or time), sites or times can be partitioned into groups with similar community structure.
- c) Cluster analysis of the *species* similarity matrix can be used to define species assemblages, ie groups of species that tend to co-occur in a parallel manner across sites.

Range of methods

Literally hundreds of clustering methods exist, some of them operating on similarity/dissimilarity matrices whilst others are based on the original data. Everitt (1980) and Cormack (1971) give excellent and readable reviews. Clifford and Stephenson (1975) is another well-established text on classification methods, from an ecological viewpoint.

Five classes of clustering methods can be distinguished, following the categories of Cormack (1971).

- 1) **Hierarchical methods.** Samples are grouped and the groups themselves form clusters at lower levels of similarity.
- 2) **Optimising techniques.** A single set of mutually exclusive groups (usually a pre-specified number) is formed by optimising some clustering criterion, for example minimising a within-cluster distance measure in the species space.
- 3) **Mode-seeking methods.** These are based on considerations of *density* of samples in the neighbourhood of other samples, again in the species space.
- 4) **Clumping techniques.** The term "clumping" is reserved for methods in which samples can be placed in more than one cluster.
- 5) **Miscellaneous techniques.**

Cormack (1971) also warned against the indiscriminate use of cluster analysis: "availability of ... classification techniques has led to the waste of more valuable scientific time than any other 'statistical' innovation". The ever larger number of techniques and their increasing accessibility on modern computer systems makes this warning no less pertinent today. The policy adopted here is to concentrate on a single technique that has been found to be of widespread utility in ecological studies, whilst emphasising the potential arbitrariness in all classification methods and stressing the need to perform a cluster analysis in conjunction with a range of other techniques (eg ordination, statistical testing) to obtain balanced and reliable conclusions.

HIERARCHICAL AGGLOMERATIVE CLUSTERING

The most commonly used clustering techniques are the *hierarchical agglomerative* methods. These usually take a similarity matrix as their starting point and successively *fuse* the samples into groups and the groups into larger clusters, starting with the highest mutual similarities then gradually lowering the similarity level at which groups are formed.¹ The process ends with a single cluster containing all samples. Hierarchical *divisive* methods perform the opposite sequence, starting with a single cluster and *splitting* it to form successively smaller groups.

The result of a hierarchical clustering is represented by a tree diagram or *dendrogram*, with the *x* axis representing the full set of samples and the *y* axis defining a similarity level at which two samples or groups are considered to have fused. Note that there is no firm convention for which way up the dendrogram should be portrayed (increasing or decreasing *y* axis values) or even whether the tree can be placed on its side; all three possibilities can be found in this manual!

Fig. 3.1 shows a dendrogram for the similarity matrix from the Frierfjord macrofaunal abundances, a subset of which is shown in Table 3.1. It can be seen that all four replicates from sites A, D, E and G fuse with each other to form distinct site groups *before* they amalgamate with samples from any other site; that, conversely, site B and C replicates are not distin-

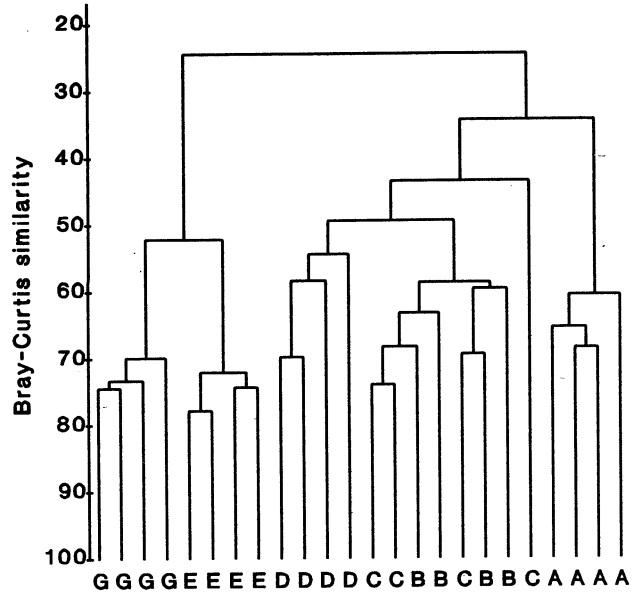


Fig. 3.1. Frierfjord macrofauna counts (F). Dendrogram for hierarchical clustering (using group-average linking) of four replicate samples from each of sites A-E, G, based on the Bray-Curtis similarity matrix shown (in part) in Table 3.1.

guished, and that A, E and G do not link to B, C and D until quite low levels of between-group similarities are reached.

The mechanism by which Fig. 3.1 is extracted from the similarity matrix, including the various options for defining what is meant by the similarity of two groups of samples, is best described for a simpler example.

Construction of dendrogram

Table 3.2 shows the steps in the successive fusing of samples, for the subset of Loch Linnhe macrofaunal abundances used as an example in the previous chapter. The data matrix has been $\sqrt{}$ -transformed, and the first triangular array is the Bray-Curtis similarity of Table 2.2.

Samples 2 and 4 are seen to have the highest similarity (underlined) so they are combined, at similarity level 68.1%. (Above this level there are considered to be four clusters, simply the four separate samples.) A new similarity matrix is then computed, now containing three clusters: "1", "2&4" and "3". The similarity between cluster "1" and cluster "3" is unchanged at 0.0 of course but what is an appropriate definition of similarity $S(1, 2\&4)$ between clusters "1" and "2&4", for example? This will be some function of the similarities $S(1,2)$, between samples 1 and 2, and $S(1,4)$, between 1 and 4; there are three main possibilities here.

1. The PRIMER program CLUSTER displays the dendrogram from hierarchical agglomerative clustering, allowing a choice from the three linkage possibilities described below and various options for axis labelling, orientation etc.

Table 3.2. Loch Linnhe macrofauna [L] subset. Abundance array after $\sqrt{\cdot}$ -transform, the resulting Bray-Curtis similarity matrix and the successively fused similarity matrices from a hierarchical clustering, using group average linking.

| Year: | 64 | 68 | 71 | 73 | | Sample | 1 | 2 | 3 | 4 | | Sample | 1 | 2&4 | 3 | | Sample | 1 | 2&3&4 |
|-----------|-----|-----|-----|-----|---|--------|------|------|------|---|---|--------|------|------|---|---|--------|------|-------|
| Species | | | | | → | 1 | - | | | | → | 1 | - | | | → | 1 | - | |
| Echinoca. | 1.7 | 0 | 0 | 0 | | 2 | 25.6 | - | | | | 2&4 | 38.9 | - | | | 2&3&4 | 25.9 | - |
| Myrioche. | 2.1 | 0 | 0 | 1.3 | | 3 | 0.0 | 67.9 | - | | | 3 | 0.0 | 55.0 | - | | | | |
| Labidopl. | 1.7 | 2.5 | 0 | 1.8 | | 4 | 52.2 | 68.1 | 42.0 | - | | | | | | | | | |
| Amaeana | 0 | 1.9 | 3.5 | 1.7 | | | | | | | | | | | | | | | |
| Capitella | 0 | 3.4 | 4.3 | 1.2 | | | | | | | | | | | | | | | |
| Mytilus | 0 | 0 | 0 | 0 | | | | | | | | | | | | | | | |

- a) **Single linkage.** $S(1, 2\&4)$ is the maximum of $S(1, 2)$ and $S(1, 4)$, i.e. 52.2%.
- b) **Complete linkage.** $S(1, 2\&4)$ is the minimum of $S(1, 2)$ and $S(1, 4)$, i.e. 25.6%.
- c) **Group-average link.** $S(1, 2\&4)$ is the average of $S(1, 2)$ and $S(1, 4)$, i.e. 38.9%.

Table 3.2 adopts group-average linking, hence

$$S(2\&4, 3) = [S(2, 3) + S(4, 3)]/2 = 55.0$$

The new matrix is again examined for the highest similarity, defining the next fusing; here this is between "2&4" and "3", at similarity level 55.0%. The matrix is again reformed for the two new clusters "1" and "2&3&4" and there is only a single similarity, $S(1, 2\&3\&4)$, to define. For group-average linking, this is the mean of $S(1, 2\&4)$ and $S(1, 3)$ but it must be a weighted mean, allowing for the fact that there are twice as many samples in cluster "2&4" as in cluster "3". Here:

$$S(1, 2\&3\&4) = [2 \times S(1, 2\&4) + 1 \times S(1, 3)]/3$$

$$= (2 \times 38.9 + 1 \times 0)/3 = 25.9$$

Though it is computationally efficient to form each successive similarity matrix by taking weighted averages of the similarities in the previous matrix, an alternative which is entirely equivalent (and perhaps conceptually simpler) is to define the similarity between two groups as the simple (unweighted) average of all between-group similarities in the initial triangular matrix. Thus:

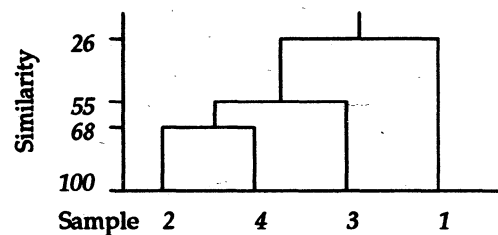
$$S(1, 2\&3\&4) = [S(1, 2) + S(1, 3) + S(1, 4)]/3$$

$$= (25.6 + 0.0 + 52.2)/3 = 25.9,$$

the same answer as above.

The final merge of all samples into a single group therefore takes place at similarity level 25.9%, and the clustering process for the group-average linking

shown in Table 3.2 can be displayed in the following dendrogram.



Dendrogram features

This example raises a number of more general points about the use and appearance of dendrograms.

- 1) Samples need to re-ordered along the x axis, for clear presentation of the dendrogram; it is always possible to arrange samples in such an order that none of the dendrogram branches cross each other.
- 2) The resulting order of samples on the x axis is not unique. A simple analogy is with a child's "mobile"; the vertical lines are strings and the horizontal lines rigid bars. When the whole structure is suspended by the top string, the bars can rotate freely, generating many possible re-arrangements of samples on the x axis. For example, in the above figure, samples 2 and 4 could switch places (sequence 4, 2, 3, 1) or sample 1 move to the opposite side of the diagram (sequence 1, 2, 4, 3), but a sequence such as 1, 2, 3, 4 is not possible. It follows that to use the x axis sequence as an ordering of samples is misleading.
- 3) Cluster analysis attempts to group samples into discrete clusters not display their inter-relationship on a continuous scale; the latter is the province of ordination and this would be preferable for the simple example above. Clustering imposes a rather arbitrary grouping on what appears to be a continuum of change from an unpolluted year (1964), through steadily increasing impact (loss of some species, increase in abundance of "opportu-

nists" like *Capitella*), to the start of a reversion to an improved condition in 1973. Of course it is unnecessary and undesirable to attempt serious interpretation of such a small subset of data but, even so, the equivalent MDS ordination for this subset (met in Chapter 5) contrasts well with the relatively unhelpful information in the above dendrogram. (A PCA ordination of the full data set can be seen in Fig. 4.1.)

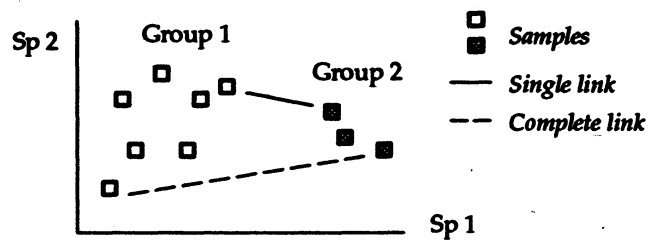
- 4) The hierarchical nature of this clustering procedure dictates that, once a sample is grouped with others, it will never be separated from them in a later stage of the process. Thus, early borderline decisions which may be somewhat arbitrary are perpetuated through the analysis and may sometimes have a significant effect on the shape of the final dendrogram. For example, similarities $S(2, 3)$ and $S(2, 4)$ above are very nearly equal. Had $S(2, 3)$ been just greater than $S(2, 4)$, rather than the other way round, the final picture would have been a little different. In fact, the reader can verify that had $S(1,4)$ been around 56% (say), the same marginal shift in the values of $S(2, 4)$ and $S(2, 3)$ would have had radical consequences, the final dendrogram now grouping 2 with 3 and 1 with 4 before these two groups come together in a single cluster. From being the first to be joined, samples 2 and 4 now only link up at the final step. Such situations are certain to arise if, as here, one is trying to force what is essentially a steadily changing pattern into discrete clusters.

Dissimilarities

Exactly the converse operations are needed when clustering from a dissimilarity rather than a similarity matrix. The two samples or groups with the *lowest* dissimilarity at each stage are fused. The single linkage definition of dissimilarity of two groups is the *minimum* dissimilarity over all pairs of samples between groups; complete linkage selects the *maximum* dissimilarity and group-average linking involves just an unweighted mean dissimilarity.

Linkage options

The differing consequences of the three linkage options, single, complete and group-average, are most easily seen for the special case used in Chapter 2, where there are only two species (rows) in the original data matrix. Samples are then points in the *species space*, with the (x,y) axes representing abundances of (Sp. 1, Sp. 2) respectively. Take also the case where dissimilarity between two samples is defined simply as their (Euclidean) distance apart in this plot.



In the above diagram, the single link dissimilarity between Groups 1 and 2 is then simply the minimum distance apart of the two groups, giving rise to an alternative name for single linkage, namely *nearest neighbour* clustering. Complete linkage dissimilarity is clearly the maximum distance apart of any two samples in the different groups, namely *furthest neighbour* clustering. (Group-average dissimilarity is just the average distance apart of the two groups).

Single and complete linkage have some attractive theoretical properties. For example, they are effectively *non-metric*. Suppose that the Bray-Curtis (say) similarities in the original triangular matrix are replaced by their *ranks*, ie the highest similarity is given the value 1, the next highest 2, down to the lowest similarity with rank $n(n - 1)/2$ for n samples. Then a single (or complete) link clustering of the ranked matrix will have exactly the same *structure* as that based on the original similarities (though the y axis similarity scale in the dendrogram will be transformed in some non-linear way). This is a desirable feature since the precise similarity values will not often have any direct significance; what matters is their relationship to each other and any non-linear (monotonic) rescaling of the similarities would ideally not affect the analysis. This is also the stance taken for the preferred ordination technique in this manual's strategy, the method of non-metric multi-dimensional scaling (MDS, Chapter 5).

However, in practice, single link clustering has a tendency to produce chains of linked samples, with each successive stage just adding another single sample onto a large group. Complete linkage will tend to have the opposite effect, with an emphasis on small clusters at the early stages. (These characteristics can be reproduced by experimenting with the special case above, generating nearest and furthest neighbours in a 2-dimensional species space). Group-averaging, on the other hand, is often found empirically to strike a balance in which a moderate number of medium-sized clusters are produced, and only grouped together at a later stage.

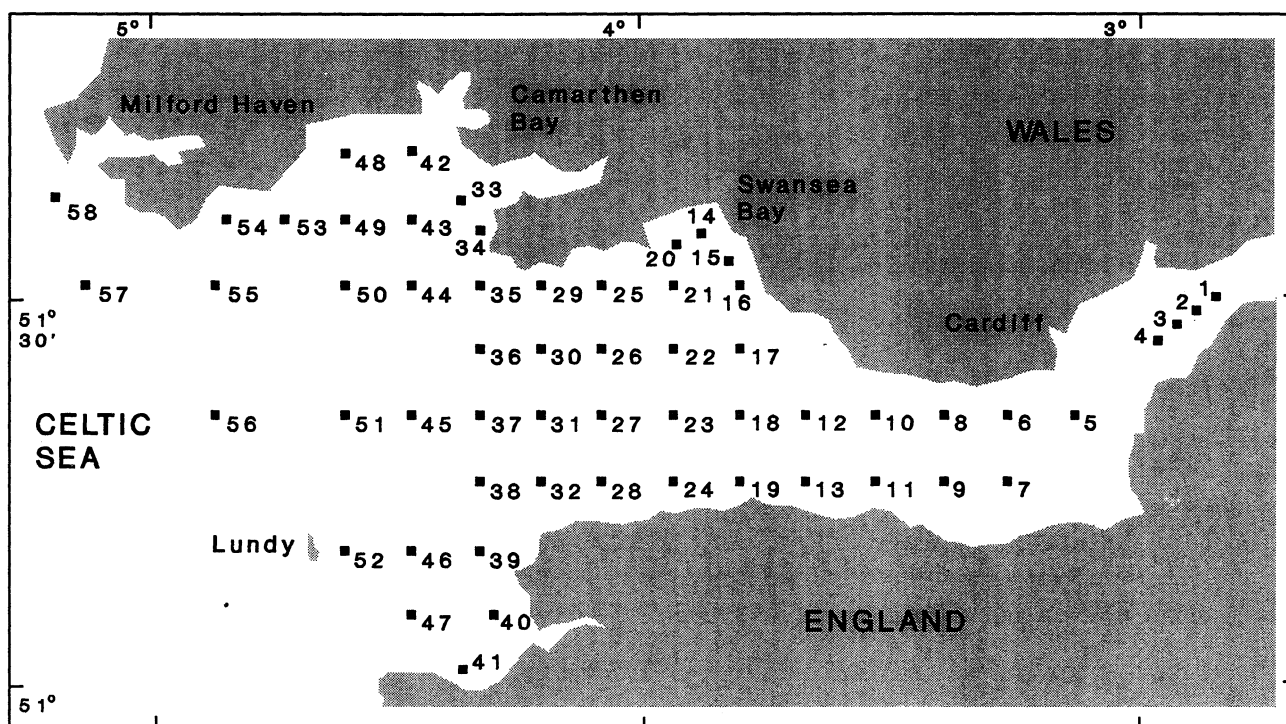


Fig. 3.2 Bristol Channel zooplankton {B}. Sampling sites.

EXAMPLE: Bristol Channel zooplankton

Collins and Williams (1982) perform hierarchical cluster analyses of zooplankton samples, collected by double oblique hauls at 57 sites in the Bristol Channel UK, for three different seasons in 1974 {B}. This is not a pollution study but a baseline survey carried out by the Plymouth laboratory, as part of a major programme to understand and model the ecosystem of the estuary. Fig. 3.2 is a map of the sample locations, sites 1-58 (site 30 not sampled).

Fig. 3.3 shows the results of a hierarchical clustering using group-average linking on data sampled during April 1974. The raw data were expressed as numbers per cubic metre for each of 24 holozooplankton species, and Bray-Curtis similarities calculated on \sqrt{v} -transformed abundances. From the resulting dendrogram, Collins and Williams select the four groups determined at a 55% similarity level and characterise these as *true estuarine* (sites 1-8, 10, 12), *estuarine and marine* (9, 11, 13-27, 29), *euryhaline marine* (28, 31, 33-35, 42-44, 47-50, 53-55) and *stenohaline marine* (32, 36-41, 45, 46, 51, 52, 56-58). A corresponding clustering of *species* and a re-ordering of the rows and columns of the original data matrix allows the identification of a number of species groups characterising these main site clusters, as is seen later (Chapter 7).

The dendrogram provides a sequence of fairly convincing groups; once each of the four main groups has formed it remains separate from other groups over a relatively large drop in similarity. Even so, a cluster analysis gives an incomplete and disjointed picture of the sample pattern. Remembering the analogy of the "mobile", it is not clear from the dendrogram alone whether there is any natural sequence of community change across the four main clusters (implicit in the designations *true estuarine*, *estuarine and marine*, *euryhaline marine*, *stenohaline marine*). For example, the *stenohaline marine* group could just as correctly have been rotated to lie between the *estuarine and marine* and *euryhaline marine* groups. In fact, there is a strong (and more-or-less continuous) gradient of community change across the region, associated with the changing salinity levels. This is best seen in an ordination of the 57 samples on which are superimposed the salinity levels at each site; this example is therefore returned to in Chapter 11.

RECOMMENDATIONS

- 1) Hierarchical clustering with group-average linking, based on sample similarity or dissimilarity matrices such as Bray-Curtis, has proved a useful technique in a number of ecological studies of the last two decades. It is appropriate for delineating groups of sites with distinct community structure

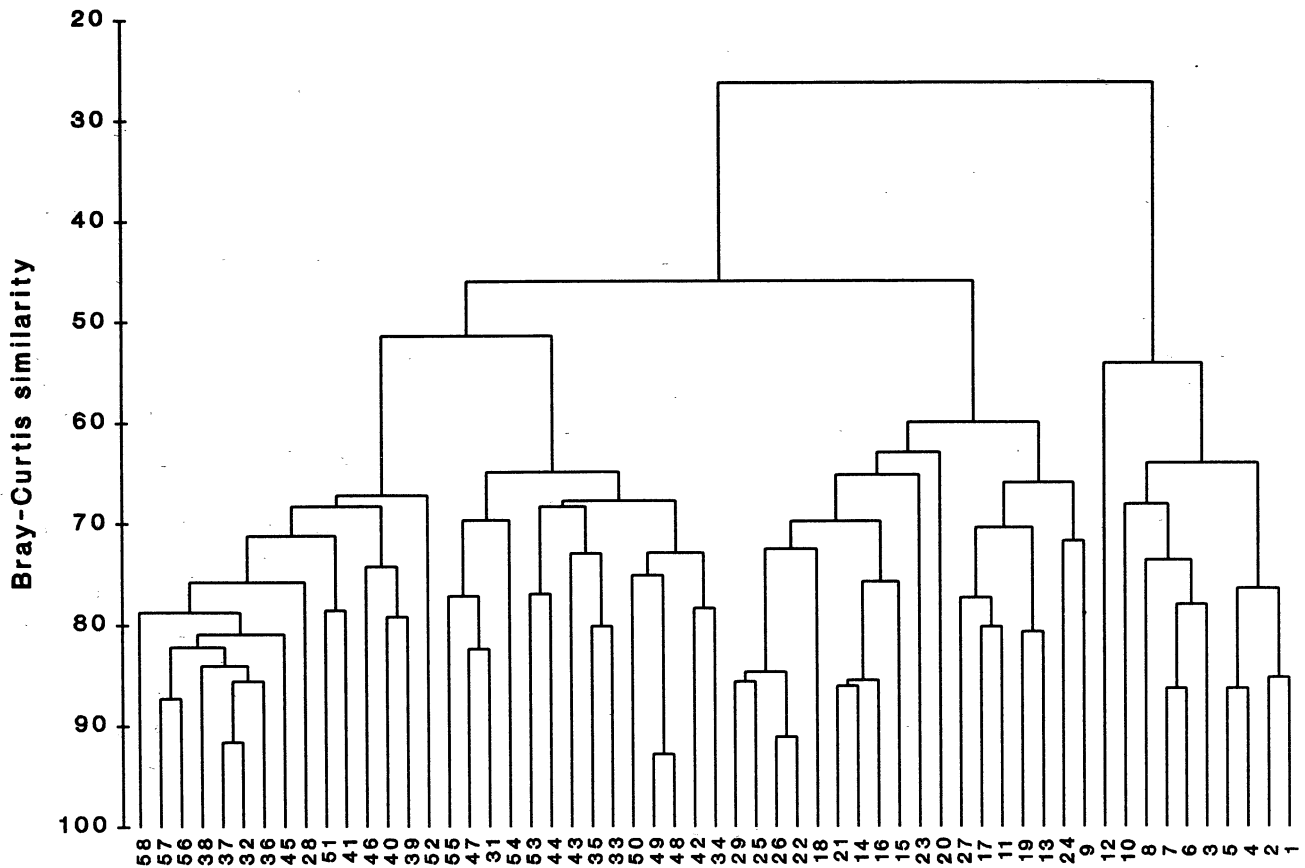


Fig. 3.3. Bristol Channel zooplankton (B). Dendrogram for hierarchical clustering of the 57 sites, using group-average linking of Bray-Curtis similarities calculated on $\sqrt{\sqrt{\cdot}}$ -transformed abundance data.

(this is not to imply that groups have no species in common, of course, but that different characteristic patterns of abundance are found consistently in different groups).

- 2) Clustering is less useful (and can be misleading) where there is a steady gradation in community structure across sites, perhaps in response to strong environmental forcing (eg large range of salinity, sediment grain size, depth of water column etc). Ordination is preferable in these situations.
- 3) Even for samples which are strongly grouped, cluster analysis is often best used in conjunction with ordination. Superimposition of the clusters (at various levels of similarity) on an ordination plot will allow any relationship between the groups to be more informatively displayed, and it will be seen later (Chapter 5) that agreement between the two representations strengthens belief in the adequacy of both.

CHAPTER 4: ORDINATION OF SAMPLES BY PRINCIPAL COMPONENT ANALYSIS (PCA)

ORDINATIONS

An *ordination* is a map of the samples, usually in two or three dimensions, in which the placement of samples, rather than representing their simple geographical location, reflects the similarity of their biological communities. To be more precise, *distances* between samples on the ordination attempt to match the corresponding *dissimilarities* in community structure: nearby points have very similar communities, samples which are far apart have few species in common or the same species at very different levels of abundance (or biomass). The word "attempt" is important here since there is no uniquely defined way in which this can be achieved. (Indeed, when a large number of species fluctuate in abundance in response to a wide variety of environmental variables, each species being affected in a different way, the community structure is essentially *high-dimensional* and it may be impossible to obtain a useful two or three-dimensional representation).

So, as with cluster analysis, several methods have been proposed, each using different forms of the original data and varying in their technique for approximating high-dimensional information in low-dimensional plots. They include:

- a) *Principal Components Analysis*, PCA (see, for example, Chatfield and Collins, 1980);
- b) *Principal Co-ordinates Analysis*, PCoA (Gower, 1966);
- c) *Correspondence Analysis* and *Detrended Correspondence Analysis*, DECORANA (Hill and Gauch, 1980);
- d) *Multi-Dimensional Scaling*, MDS; in particular *non-metric MDS* (see, for example, Kruskal and Wish, 1978).

A comprehensive survey of ordination methods is outside the scope of this volume. As with clustering methods, detailed explanation is given only of the techniques required for the analysis strategy adopted throughout the manual. This is not to deny the validity of other methods but simply to affirm the importance of applying, with *understanding*, one or two techniques

of proven utility. The two ordination methods selected are therefore the simplest (arguably) of the various options, at least in concept.

- a) PCA is the longest-established method, though the relative inflexibility of its definition limits its practical usefulness more to multivariate analysis of environmental data rather than species abundances or biomass; nonetheless it is still widely encountered and is of fundamental importance.
- b) Non-metric MDS is a more recent development, whose complex algorithm could only have been contemplated in an era of advanced computational power; however, its rationale can be very simply described and understood, and many people would argue that the need to make few (if any) assumptions about the data make it the most widely applicable and effective method available.

PRINCIPAL COMPONENTS ANALYSIS

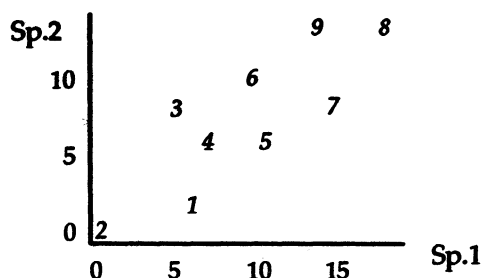
The starting point for a PCA is the original data matrix rather than a derived similarity matrix (though there is an *implicit* dissimilarity matrix underlying PCA, that of Euclidean distance). The data array is thought of as defining the positions of samples in relation to axes representing the full set of species, one axis for each species. This is the very important concept introduced in Chapter 2 (following equation (2.13)). Typically, there are many species so the samples are points in a very high-dimensional space.

A simple 2-dimensional example

It helps to visualise the process by again considering an (artificial) example in which there are only two species (and nine samples).

| | Sample | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|-----------|--------|---|---|---|---|----|----|----|----|----|
| Abundance | Sp. 1: | 6 | 0 | 5 | 7 | 11 | 10 | 15 | 18 | 14 |
| | Sp. 2: | 2 | 0 | 8 | 6 | 6 | 10 | 8 | 14 | 14 |

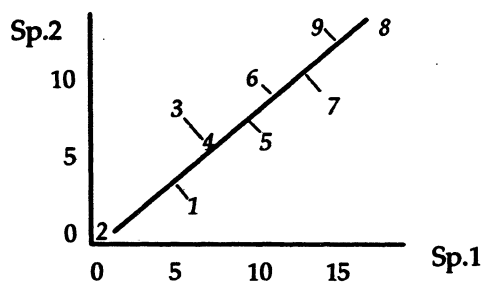
The nine samples are therefore points in two dimensions, and labelling these points with the sample number gives the following plot.



This is an ordination already, of 2-dimensional data on a 2-dimensional map, and it summarises pictorially all the relationships between the samples, without needing to discard any information at all. However, suppose for the sake of example that a 1-dimensional ordination is required, in which the original data is reduced to a genuine *ordering* of samples along a line. How do we best place the samples in order? One possibility (though a rather poor one!) is simply to ignore altogether the counts for one of the species, say Species 2. The Species 1 axis then automatically gives the 1-dimensional ordination (Sp.1 counts are again labelled by sample number):

| | | | | | | |
|--------|---|-----|----|----|----|-------------|
| Sample | 2 | 314 | 65 | 97 | 8 | |
| | 0 | 5 | 10 | 15 | 20 | Sp.1 counts |

(Think of this as projecting the points in the 2-dimensional space down onto the Sp.1 axis). Not surprisingly, this is a rather inaccurate 1-dimensional summary of the sample relationships in the full 2-dimensional data, eg samples 7 and 9 are rather too close together, certain samples seem to be in the "wrong order" (9 should be closer to 8 than 7 is, 1 should be closer to 2 than 3 is) etc. More intuitively obvious would be to choose the 1-dimensional picture as the (perpendicular) projection of points onto the line of "best fit" in the 2-dimensional plot.

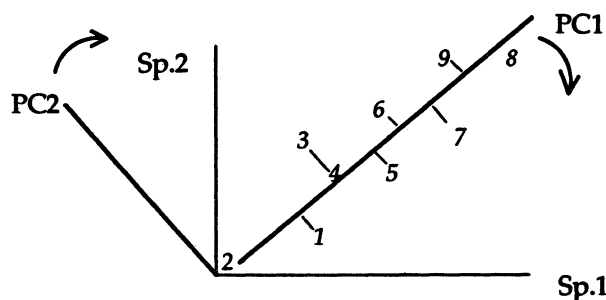


The 1-dimensional ordination, called the *first principal component axis (PC1)*, is then:

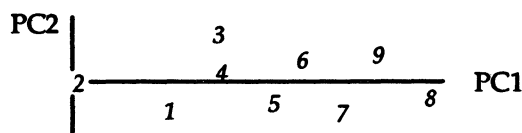
| | | | | | | | | | |
|--------|-------|---|----|---|---|---|---|---|-----|
| Sample | 2 | 1 | 34 | 5 | 6 | 7 | 9 | 8 | |
| | _____ | | | | | | | | PC1 |

and this picture is a much more realistic approximation to the 2-dimensional sample relationships (eg 1 is now closer to 2 than 3 is, 7, 9 and 8 are more equally spaced and in the "right" sequence etc).

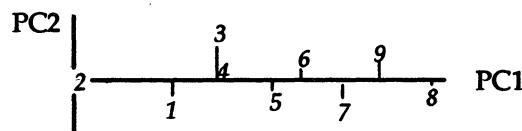
The *second principal component axis (PC2)* is defined as the axis *perpendicular* to PC1, and a full *principal component analysis* then consists simply of a *rotation* of the original 2-dimensional plot:



to give the following *principal component plot*.



Obviously the (PC1, PC2) plot contains exactly the same information as the original (Sp.1, Sp.2) graph. The whole point of the procedure though is that, as in the current example, we may be able to *dispense* with the second principal component (PC2): the points in the (PC1, PC2) space are projected onto the PC1 axis and relatively little information about the sample relationships is lost in this reduction of dimensionality.



Definition of PC1 axis

Up to now we have been rather vague about what is meant by the "best fitting" line through the sample points in 2-dimensional species space. There are two natural definitions. The first chooses the PC1 axis as the line which minimises the sum of squared

(perpendicular) distances of the points from the line.¹ The second approach comes from noting in the above example that the biggest differences between samples take place along the PC1 axis, with relatively small changes in the PC2 direction. The PC1 axis is therefore defined as that direction in which the *variance* of sample points projected perpendicularly onto the axis is *maximised*. In fact, these two separate definitions of the PC1 axis turn out to be *totally equivalent* and one can use whichever concept is easier to visualise.

Extension to 3-dimensional data

Suppose that the simple example above is extended to the following matrix of counts for *three* species.

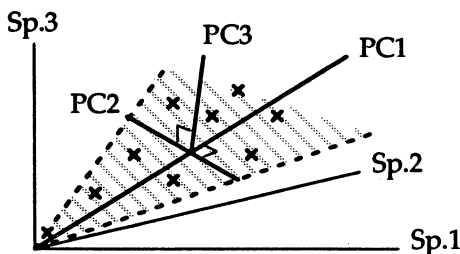
| | Sample | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|-----------|--------|---|---|---|---|----|----|----|----|----|
| Abundance | Sp. 1: | 6 | 0 | 5 | 7 | 11 | 10 | 15 | 18 | 14 |
| | Sp. 2: | 2 | 0 | 8 | 6 | 6 | 10 | 8 | 14 | 14 |
| | Sp. 3: | 3 | 1 | 6 | 6 | 9 | 11 | 10 | 16 | 15 |

Samples are now points in three dimensions (Sp.1, Sp.2 and Sp.3 axes) and there are therefore three principal component axes, again simply a rotation of the three species axes. The definition of the (PC1, PC2, PC3) axes generalises the 2-dimensional case in a natural way:

PC1 is the axis which *maximises* the *variance* of points projected perpendicularly onto it;

PC2 is constrained to be perpendicular to PC1, but is then again chosen as the direction in which the variance of points projected perpendicularly onto it is maximised;

PC3 is the axis perpendicular to both PC1 and PC2 (there is no choice remaining here).



1. This type of idea may be familiar from ordinary linear regression, except that the latter is formulated asymmetrically: the regression of *y* on *x* minimises the sum of squared vertical distances of points from the line.

An equivalent way of visualising this is again in terms of “best fit”: PC1 is the “best fitting” line to the sample points and, together, the PC1 and PC2 axes define a plane (stippled in the above diagram) which is the “best fitting” plane.

Algebraic definition

The above geometric formulation can be expressed algebraically. The three new variables (PCs) are just *linear combinations* of the old variables (species), such that PC1, PC2 and PC3 are *uncorrelated*. In the above example:

$$\begin{aligned}
 PC1 &= 0.62 \times Sp.1 + 0.52 \times Sp.2 + 0.58 \times Sp.3 \\
 PC2 &= -0.73 \times Sp.1 + 0.65 \times Sp.2 + 0.20 \times Sp.3 \quad (4.1) \\
 PC3 &= 0.28 \times Sp.1 + 0.55 \times Sp.2 - 0.79 \times Sp.3
 \end{aligned}$$

The principal components are therefore interpretable (in theory) in terms of the counts for each original species axis. Thus PC1 is a sum of roughly equal (and positive) contributions from each of the species; it is essentially ordering the samples from low to high total abundance. At a more subtle level, for samples with the same total abundance, PC2 then mainly distinguishes relatively high counts of Sp.2 (and low Sp.1) from low Sp.2 (and high Sp.1); Sp.3 values do not feature strongly in PC2 because the corresponding coefficient is small. Similarly the PC3 axis mainly contrasts Sp.3 and Sp.2 counts.

Variation explained by each PC

The definition of principal components given above is in terms of successively maximising the variance of sample points projected along each axis, with the variance therefore decreasing from PC1 to PC2 to PC3. It is thus natural to quote the values of these variances (in relation to their total) as a measure of the amount of “information” contained in each axis. Furthermore, it turns out that the total of the variances along all PC axes is equal to the total variance of points projected successively onto each of the original species axes. That is, letting *var*(PC_{*i*}) denote variance of samples on the *i*th PC axis and *var*(Sp._{*i*}) denote variance of points on the *i*th species axis (*i* = 1,2,3):

$$\sum_i var(PC_i) = \sum_i var(Sp_i) \quad (4.2)$$

Thus, the relative variation of points along the *i*th PC axis (as a percentage of the total), namely

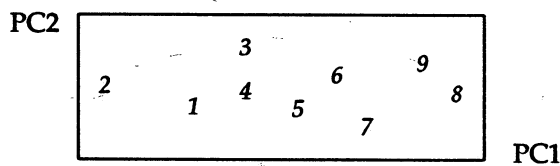
$$P_i = 100 \cdot \frac{var(PC_i)}{\sum_i var(PC_i)} = 100 \cdot \frac{var(PC_i)}{\sum_i var(Sp_i)} \quad (4.3)$$

has a useful interpretation as the % of the original total *variance explained* by the *i*th PC. For the simple

3-dimensional example above, PC1 explains 93%, PC2 explains 6% and PC3 only 1% of the variance in the original samples.

Ordination plane

This brings us back finally to the reason for rotating the original three species axes to three new principal component axes. The first two PCs represent a plane of "best fit", encompassing the maximum amount of variation in the sample points. The % variance explained by PC3 may be small and we can dispense with this third axis, projecting all points perpendicularly onto the (PC1, PC2) plane to give the 2-dimensional ordination plane that we have been seeking. For the above example this is:



and it is almost a perfect 2-dimensional summary of the 3-dimensional data, since PC1 and PC2 account for 99% of the total variation. In effect, the points lie on a plane (in fact, nearly on a line!) in the original species space, so it is no surprise to find that this PCA ordination differs negligibly from that for the initial 2-species example: the counts added for the third species were highly correlated with those for the first two species.

Higher-dimensional data

Of course there are many more species than three in a normal species by samples array, typically at least 30, but the approach to defining principal components and an ordination plane is the same. Samples are now points in (say) a 30-dimensional species space and the "best fitting" 2-dimensional plane is found and samples "projected" onto it to give the 2-dimensional PCA ordination. The PC axes are the "perpendicular directions" in this 30-dimensional space along which the variances of the points are (successively) maximised. The degree to which a 2-dimensional PCA succeeds in representing the full 30-dimensional information is seen in the percentage of total variance explained by the first two (of 30) principal components. Often PC1 and PC2 may not explain more than 40-50% of the total variation, and a 2-dimensional PCA ordination gives an inadequate and potentially misleading picture of the relationship between the samples. A 3-dimensional sample ordination, using the first three PC axes, may give a fuller picture or it

may be necessary to invoke PC4, PC5 etc before a reasonable percentage of the total variation is encompassed. Guidelines for an acceptable level of "% variance explained" are difficult to set, since they depend on the objectives of the study, the number of species and samples etc., but an empirical rule-of-thumb might be that a picture that accounts for as much as 70-75% of the original variation is likely to describe the overall structure rather well.

The geometric concepts of fitting planes and projecting points in (say) 30-dimensional space are not ones that most people are comfortable with (!) so it is important to realise that, *algebraically*, the central ideas are no more complex than in three dimensions. Equations like (4.1) simply extend to p ($= 30$) principal components, each a linear function of the p species counts. The "perpendicularity" (*orthogonality*) of the principal component axes is reflected in the zero values for all sums of cross-products of coefficients, e.g. for equation (4.1):

$$(0.62) \times (-0.73) + (0.52) \times (0.65) + (0.58) \times (0.20) = 0$$

$$(0.62) \times (0.28) + (0.52) \times (0.55) + (0.58) \times (-0.79) = 0$$

etc.,

the coefficients being scaled so that their sum of squares adds to one, e.g.

$$(0.62)^2 + (0.52)^2 + (0.58)^2 = 1$$

$$(-0.73)^2 + (0.65)^2 + (0.20)^2 = 1$$

etc.

There is clearly no difficulty in extending such relations to 4, 5 or any number of coefficients.

The algebraic construction of coefficients satisfying these conditions but also defining which "perpendicular directions" maximise variation of the samples in the species space, is outside the scope of this manual. It involves calculating eigenvalues and eigenvectors of a $p \times p$ matrix, see Chatfield and Collins (1980), for example. (Note that a knowledge of matrix algebra is essential to understanding this construction). The advice to the reader is to hang on to the geometric picture: all the essential ideas of PCA are present in visualising the construction of a 2-dimensional ordination plot from a 3-dimensional species space.

EXAMPLE: Loch Linnhe macrofauna

The various options available, and the limitations imposed when constructing an ordination using PCA, are best appreciated in the context of a real data set. A 2-dimensional PCA of the full Loch Linnhe macrofau-

nal abundance data $\{L\}$ is shown in Fig. 4.1. The original matrix contained a total of 115 species for the 11 samples, one for each year of the period 1963–1973. Pulp-mill effluent was first discharged to the loch in 1966 with an increased discharge in 1969/70 and a subsequent decrease in 1972/73.

Exclude less-common species

The retention of rarer species in a PCA ordination will have a strongly distorting effect, even supposing that the matrix operations to construct the ordination are possible. For the Loch Linnhe data there are 11 samples in 115-dimensional species space! An initial and drastic reduction in the number of species is necessary for the PCA algorithm to work. In fact, many of the species are represented only by a single individual in a single year and their omission will not be a serious loss to interpretation, but the necessity of making an (essentially arbitrary) decision about which species to exclude is one of the problems with applying PCA to biological community data. By contrast, the clustering methods of the last chapter were applied to a similarity matrix which could be constructed from *all* species, the rarer ones either being emphasised, as in reduction to presence/absence, or down-weighted automatically (though not ignored totally) by the choice of similarity coefficient and transformation. An ordination method based on this similarity matrix (for example, the MDS method of Chapter 5) clearly scores over PCA, in this respect.

In fact, Fig 4.1 is based on a data matrix of only 29 species, those making up more than 3% of the total abundance in at least one of the samples. (The rationale for this type of selection procedure was

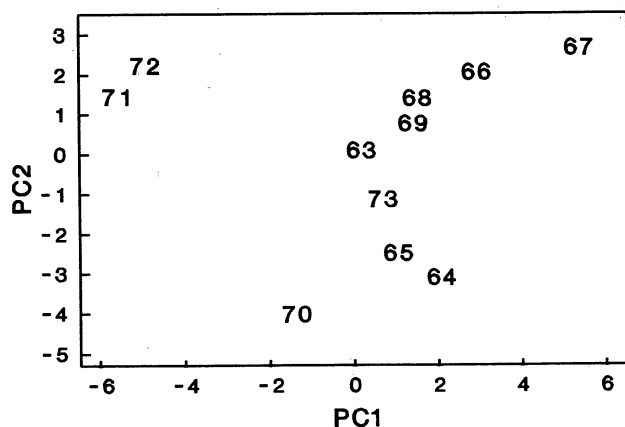


Fig. 4.1. Loch Linnhe macrofauna $\{L\}$. 2-dimensional PCA ordination of sample abundances ($\sqrt{\cdot}$ -transformed) from the 11 years 1963–1973. PC1 (x-axis) and PC2 (y-axis) together account for 57% of the total sample variability.

discussed in the section on species similarities in Chapter 2). Calculation of the principal components is now possible though, even so, the software package needs to handle its computations carefully. A total of 11 sample points will always fit perfectly into 10 dimensions (think of the lower-dimensional analogy again: 3 points in 3-dimensional space will always lie on a 2-dimensional plane). Thus, only 10 (at most) PC axes can be constructed, or to put it another way, all the sample variance can be explained by the first 10 PCs. In fact, the first two PCs in Fig. 4.1 explain 57% of the total variability so the 2-dimensional ordination does not give a fully satisfactory picture of the changing community pattern over the years. If this example were being pursued further, it would be advisable to look also at the third PC (at least), perhaps with some form of 3-dimensional perspective plot or by the three separate 2-dimensional plots of (PC1, PC2), (PC1, PC3) and (PC2, PC3). Nonetheless, one main feature is clear from Fig. 4.1: the relatively large change in community composition between 1970 and 1971, and the reversion in 1973 to a community which is more like the earlier years.

Transformation of abundance/biomass

In much the same way as was seen for the calculation of similarity coefficients in Chapter 2, it may be necessary to make an initial transformation of the abundance or biomass values to avoid over-domination of the resulting analysis by the very common species. For the Loch Linnhe data, *Capitella* numbers in a yearly sample range from 0 to over 4,000 individuals, whereas the bulk of the other species have counts in single or double figures. For untransformed data (and using a covariance-based analysis, as discussed below), the *Capitella* axis will clearly contain a substantial part of the overall variation of samples in the species space, so that the direction of the PC1 axis will tend to be dictated by that species alone. A more balanced picture will emerge after transformation: Fig. 4.1 is based on $\sqrt{\cdot}$ -transformed abundances.

Scale and location changes

The data matrix can also be *normalised* (after any transformation has taken place). For each species abundance, subtract the mean count and divide by the standard deviation over all samples for that species. This makes the variance of samples along all species axes the same (= 1) so all species are of potentially equal importance in determining the principal components. This normalised analysis is referred to as *correlation-based PCA* rather than the *covariance-based PCA* obtained when the data is not normalised

(the terminology comes from whether the algebraic extraction of eigenvalues and eigenvectors takes place on the correlation or covariance matrix between species).

When does one use normalisation and when transformation (or both)? In fact, the arguments are somewhat analogous to those seen in Chapter 2 for the computation of similarities. There, techniques which tended to weight all species equally (for example, the calculation of Canberra coefficients) were rejected in favour of methods that maintained a greater (though not overwhelmingly greater) importance for common species than for rarer ones. This was achieved through initial $\sqrt{\cdot}$, $\sqrt[3]{\cdot}$ or log transformation; the equivalent option here would be to take the same transformation and apply covariance-based PCA. It is true that, here, the set of species has first been drastically reduced so that all the rarer species are eliminated; nonetheless there seems no compelling reason why the remaining species should be given equal weight, as they would in a correlation-based PCA. Note that even if normalisation is used, it still makes sense to perform an initial transformation. This has the effect of reducing the inevitable *right-skewness* of the spread of sample counts along a species axis (i.e. abundances tend to bunch at smaller values with a "tail" of occasional large counts). Computation of variances and the resulting normalisation are really designed to cope with data which are, as the name implies, approximately *normally* distributed; transformation makes this more likely.

PCA OF ENVIRONMENTAL DATA

The conclusion above is that covariance-based PCA would probably be preferred to correlation-based PCA for species abundance matrices, though the summary at the end of this chapter makes it clear that neither is a very satisfactory ordination method for such data. There is one important situation, however, where PCA is a more useful tool and where normalisation is usually *essential*. This is in the multivariate analysis of environmental rather than species data. Conventionally, one has a matrix of p columns (variables) by n rows (samples)² with the variables perhaps being a mixture of physical parameters (grain size, salinity, depth of the water column) and other environmental or chemical measurements (nutrient levels, heavy metal, hydrocarbon or PCB concentrations etc). Patterns in the environmental data across samples can be displayed in an analogous way to species data, by a multivariate

ordination, and techniques for linking the biological and environmental summaries are discussed in Chapter 11.

PCA is more appropriate to environmental variables because of the form of the data (there are no large blocks of zero counts needing to be treated in a special way: it is no longer necessary to select a dissimilarity coefficient that ignores "joint absences" and some form of Euclidean distance measure makes more sense for environmental data). However, another crucial difference between species and environmental data is that the latter will usually have a complete mix of measurement scales (salinity in ‰, grain size in ϕ units, depth in m, metal concentrations in $\mu\text{g/g}$, PCBs in ng/g etc). In a multi-dimensional visualisation of the environmental data matrix, the samples are points referred to *environmental axes* rather than species axes, but what does it mean now to talk about (Euclidean) distance between two sample points in the *environmental variable space*? If the units on each axis differ, and have no natural connection with each other, then point A can be made to appear closer to point B than point C, or closer to point C than point B, simply by a change of scale on one of the axes (e.g. measuring PCBs in $\mu\text{g/g}$ not ng/g , or depth in fathoms rather than m). Obviously it would be entirely wrong for the PCA ordination to vary with such arbitrary scale changes. There is one natural solution to this: perform a *correlation-based* PCA, i.e. normalise all axes so that they have comparable (dimensionless) scales.

The problem does not arise for species data, of course, because though a scale change on the axes may be contemplated (e.g. changing counts from numbers of individuals per core to numbers per m^2 of sediment surface), the *same* scale change is made on each axis and the PCA ordination will be unaffected. The overall guideline would therefore be to use correlation-based PCA for environmental variables and covariance-based PCA for species data (if no alternative ordination method is available for the latter). In *both* cases, prior transformation is likely to be beneficial; different transformations may be desirable for

2. Note that this is the opposite convention to that used for abundance matrices, where the rows are the variables (species). Input to the PRIMER program PCA is expected to be an environmental data matrix (with rows as samples), so for a PCA on species data the matrix must first be transposed (using the program SWAP). PCA options include transformation of specified columns and normalisation (in that order); the output includes a plot file which is input to the plotting routine CONPLOT, to display the 2-d sample configuration.

different variables in the environmental analysis, e.g. contaminant concentrations will often be right-skewed (and require something like log transformation), salinity may be left-skewed (reverse log transformation) and sediment granulometry measures like "% mud" or "silt/clay" may need no transformation at all. These issues are returned to in Chapter 9.

PCA STRENGTHS

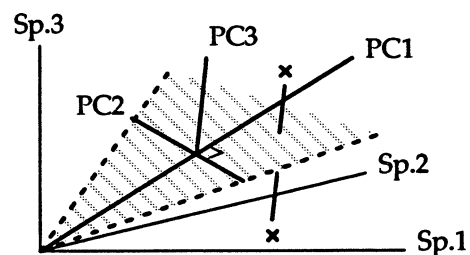
- 1) *PCA is conceptually simple.* Whilst the algebraic basis of the PCA algorithm requires a facility with matrix algebra for its understanding, the geometric concepts of a "best fitting" plane in the species space, and perpendicular projection of samples onto that plane, are relatively easily grasped. Some of the more recently proposed ordination methods, which either extend or supplant PCA (eg Principal Co-ordinates Analysis, Detrended Correspondence Analysis) can be very much harder to understand for practitioners without a mathematical background.
- 2) *It is computationally straightforward.* Again, this statement needs to be seen in relative terms. *Provided* the number of species is reduced, usually drastically, the required matrix operations pose no real problems to modern computing power and packages are widely available which carry out the necessary eigenvalue (*latent root*) extraction. That multivariate methods have only come to the fore as a practical data analysis tool in the last two decades should not be a surprise to anyone. Even the computationally simplest of techniques, PCA, could never be carried out manually in any realistic example. Nonetheless, PCA tends to take only seconds, rather than minutes or hours, of processing time on a personal computer. The constraints are mainly on the number of *species* handled, and large numbers of samples can usually be accommodated. This is in contrast to cluster and MDS analyses which tend to be more constrained by the number of *samples* they can handle; once the data is reduced to a similarity matrix between samples (the input form to both clustering and MDS) the number of species in the original matrix is irrelevant. PCA could therefore have a role, when there are large numbers of samples, in providing an initial picture which would suggest separation of the data into two (or more) distinct sets of samples, each of which is analysed by more accurate (but

more computationally-intensive) ordinations such as MDS.

- 3) *Ordination axes are interpretable.* The PC axes are simple linear combinations of the values for each species, as in equation (4.1), so in theory have some potential for interpretation. In practice though, when there are more than a handful of species (as is usual), this rarely leads to any useful information. Environmental data arrays often contain a smaller number of variables however, and interpretation of the PCA axes may be informative in that case (see, for example, Chapter 11).

PCA WEAKNESSES

- 1) *There is little flexibility in defining dissimilarity.* An ordination is essentially a technique for converting *dissimilarities* of community composition between samples into (Euclidean) *distances* between these samples in a 2- or higher-dimensional ordination plot. Implicitly, PCA defines dissimilarity between two samples as their Euclidean distance apart in the full p -dimensional species space; however, as was seen in Chapter 2, this is rather a poor way of defining sample dissimilarity: something like a Bray-Curtis coefficient would be preferred but standard PCA cannot accommodate this. The only flexibility it has is in transforming (and/or normalising) the species axes so that dissimilarity is defined as Euclidean distance on these new scales.
- 2) *Its distance-preserving properties are poor.* Having defined dissimilarity as distance in the p -dimensional species space, PCA converts these distances by *projection* of the samples onto the 2-dimensional ordination plane. This may distort some distances rather badly. Taking the usual visual analogy of a 2-dimensional ordination from three species, it can be seen that samples which are relatively far apart on the PC3 axis can end up being co-incident when projected (perhaps from "opposite sides") onto the (PC1, PC2) plane.



**EXAMPLE: Dosing experiment,
Solbergstrand mesocosm**

An example of this final point for a real data set can be seen in Fig. 4.2. This is of nematode data for the dosing experiment (D) in the Solbergstrand mesocosms, at the GEEP Oslo Workshop. Intact box core samples were collected from Oslofjord and held for three months under four dosing regimes: control, low, medium and high levels of a hydrocarbon and Cu contaminant mixture, continuously dosed to the basin waters. Four replicate box cores were subjected to each treatment and at the end of the period cores for all 16 boxes were examined for nematode communities (amongst other faunistic components). Fig. 4.2 shows the resulting PCA, based on log-transformed counts for 26 nematode genera (those making up more than 3% of the total individuals in at least one core). The interest here, of course, is in whether all replicates from one of the four treatments separate out from other treatments, indicating a change in community composition which could be attributed to a directly *causal* effect of the contaminant dosing. A cursory glance suggests that the high dose replicates (H) may indeed do this. However, closer study shows that the % of variance explained by the first two PC axes is very low: 22% for PC1 and 15% for PC2. The picture is likely to be *very unreliable* therefore, and an examination of the third and higher PCs confirms the distortion: some of the H replicates are much further apart in the full species space than this projection into two dimensions would imply. For example, the right-hand H sample is actually closer to the nearest M sample than it is to the other H samples. The distances in the full species space are therefore *poorly-preserved* in the 2-dimensional ordination.

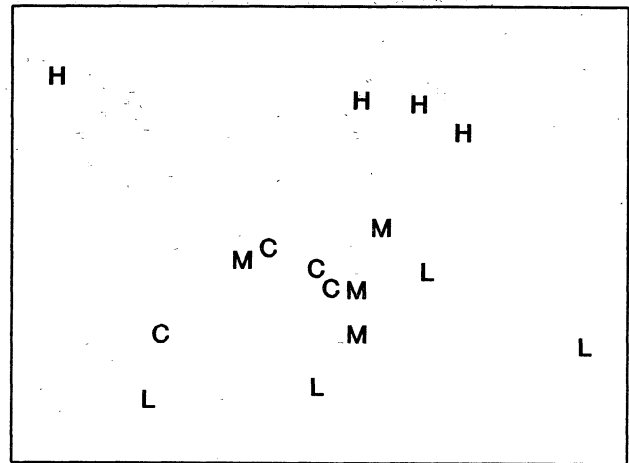


Fig. 4.2. Dosing experiment, Solbergstrand (D). 2-dimensional PCA ordination of log-transformed nematode abundances from 16 box cores (4 replicates from each of control, low, medium and high doses of a hydrocarbon and Cu contaminant mixture). PC1 and PC2 account for 37% of the total variance.

This example is returned to again in Chapter 5, Fig. 5.5, where it is seen that an MDS of the same data makes a much better stab at "dissimilarity preservation", though the data is such that no method will find it easy to represent in two dimensions. The moral here is clear:

- a) be very wary of interpreting any PCA plot which explains so little of the total variability in the original data;
- b) statements about apparent differences in a multivariate community analysis of one site/time/treatment from another should be backed-up by appropriate statistical tests; this is the subject of Chapter 6.

CHAPTER 5: ORDINATION OF SAMPLES BY MULTI-DIMENSIONAL SCALING (MDS)

OTHER ORDINATION METHODS

Principal Co-ordinates Analysis

The two main weaknesses of PCA, identified at the end of Chapter 4, are its inflexibility of dissimilarity measure and its poor distance-preservation. The first problem is addressed in an important paper by Gower (1966), describing an extension to PCA termed *Principal Co-ordinates Analysis* (PCoA), also sometimes referred to as *classical scaling*. This allows a much wider definition of dissimilarity than simple Euclidean distance in the species space (the basis of PCA). Other dissimilarity measures are converted to distances, in high-dimensional space, but the final step is again a projection onto a low-dimensional ordination space (eg a 2-dimensional plane), as in ordinary PCA. Thus, PCA is a special case of PCoA, when the original dissimilarity is just Euclidean distance. It follows that PCoA is still subject to the second criticism of PCA: its lack of emphasis on distance-preservation when the information is difficult to represent in a low number of dimensions.

Detrended Correspondence Analysis

Correspondence analyses are a class of ordination methods featuring strongly in French data-analysis literature (for a review in English see Greenacre, 1984). Key papers in ecology are Hill (1973a) and Hill and Gauch (1980), who introduced *detrended correspondence analysis* (DECORANA). The methods start from the data matrix, rather than a set of dissimilarity coefficients, so are rather inflexible in their definition of sample dissimilarity; in effect, multinomial assumptions generate an implicit dissimilarity measure of "chi-squared" distance. Basic correspondence analysis (CA) has its genesis in a particular model of unimodal species response to underlying (but unmeasured) environmental gradients; an account is outside the scope of this manual but a comprehensive exposition (by C. F. J. ter Braak) of CA and related techniques can be found in Jongman *et al.* (1987).¹

1. A convenient way of carrying out correspondence analyses, and related canonical methods, is to use ter Braak's excellent CANOCO package.

The popular DECORANA version of CA has a primary motivation of straightening out an "arch effect" in a CA ordination, which is expected on theoretical grounds if species abundances have unimodal (Gaussian) responses along a single strong environmental gradient. Where such models are not appropriate, it is unclear what artefacts the algorithms may introduce into the final picture. In the Hill and Gauch (1980) procedure, the detrending is essentially carried out by first splitting the ordination space into segments, stretching or shrinking the scale in each segment and then realigning the segments to remove wide-scale curvature. For some people, this is uncomfortably close to attacking the data with scissors and glue and, though the method is not as subjective as this would imply, some arbitrary decisions about where and how the segmentation and rescaling are defined are rather hidden from the user in the program code. Thus Pielou (1984) and others have criticized DECORANA for its "overzealous" manipulation of the data. It is also a pity that the multivariate techniques which historically have been applied most frequently in the ecological literature are often either inadequately suited to the data or are based on conceptually complex algorithms (e.g. DECORANA and TWINSpan, Hill 1979a, b), erecting a communication barrier between data analyst and ecologist.

The ordination technique which is adopted in this manual's strategy, *non-metric MDS*, is itself a complex numerical algorithm but it can (and will) be argued that it is *conceptually* simple. It makes few (if any) model assumptions about the form of the data or the inter-relationship of the samples, and the link between the final picture and the user's original data is relatively transparent and easy to explain. It addresses both the major criticisms of PCA made earlier: it has *great flexibility* both in the definition and conversion of dissimilarity to distance and its rationale is the preservation of these relationships in the low-dimensional ordination space.

NON-METRIC MULTIDIMENSIONAL SCALING

The method of *non-metric MDS* was introduced by Shepard (1962) and Kruskal (1964), for application to problems in psychology; a useful introductory text is

Kruskal and Wish (1978), though again the applications described are not ecological. Throughout this manual the term MDS refers to Kruskal's non-metric procedure (metric MDS is possible, and is akin to PCoA, but will not be discussed in any detail).

The starting point is the similarity or dissimilarity matrix among samples (Chapter 2). This can be whatever similarity matrix is biologically relevant to the questions being asked of the data. Through choice of coefficient and possible transformation or standardization, one can choose whether to ignore joint absences, to emphasise similarity in common or rare species, to compare only % composition or allow sample totals to play a part etc. In fact, the flexibility of MDS goes beyond this. It recognises the essential arbitrariness of *absolute* similarity values; Chapter 2 showed that the range of values taken could alter dramatically with transformation (often, the more severe the transformation, the higher and more compressed the similarity values become) and there is no absolute interpretation of a statement like "the similarity of sample 1 to sample 2 is 1.5 times that of sample 1 to sample 3". The natural interpretation is in terms of the *relative* values of similarity to each other, e.g. simply that "sample 1 is more similar to sample 2 than it is to sample 3". This is an intuitively appealing and very generally applicable base from which to build a graphical representation of the sample patterns and, in effect, the *ranks* of the similarities are the only information used by a successful non-metric MDS ordination.

The purpose of MDS can therefore be simply stated: it constructs a "map" or *configuration* of the samples, in a specified number of dimensions, which attempts to satisfy all the conditions imposed by the rank (dis)similarity matrix, e.g. if sample 1 has higher similarity to sample 2 than it does to sample 3 then sample 1 will be placed closer on the map to sample 2 than it is to 3.

EXAMPLE: Loch Linnhe macrofauna

This is illustrated in Table 5.1 for the subset of the Loch Linnhe macrofauna data used to demonstrate hierarchical clustering (Table 3.2). Similarities between \sqrt{v} -transformed counts of the four year samples are given by Bray-Curtis similarity coefficients and the corresponding rank similarities are also shown. (The highest similarity has the *lowest* rank, 1, and the lowest similarity the highest rank, $n(n-1)/2$.) The MDS configuration is constructed to *preserve* the similarity

ranking as Euclidean distances in the 2-dimensional plot: samples 2 and 4 are closest, 2 and 3 next closest, then 1 and 4, 3 and 4, 1 and 2 and, finally, 1 and 3 are furthest apart. The resulting figure is a more informative summary than the corresponding cluster analysis in Chapter 3, showing, as it does, a gradation of change from clean (1) to progressively more impacted years (2 and 3) then a reversal of the trend, though not complete recovery to the initial position (4).

Though the mechanism for constructing such MDS plots has not yet been described, two general features of MDS can already be noted.

- 1) MDS plots can be arbitrarily *scaled, located, rotated or inverted*. Clearly, rank order information about which samples are most or least similar can say nothing about which direction in the MDS plot is "up" or "down", or the absolute "distance apart" of two samples: what can be interpreted is *relative* distances apart, of course.
- 2) It is not difficult in the above example to see that four points could be placed in two dimensions in such a way as to satisfy the similarity ranking exactly.² For more realistic data sets, though, this will not usually be possible and there will be some distortion or *stress* between the similarity rankings and the corresponding distance rankings in the ordination plot (even in a high-dimensional configuration). This motivates the principle of the MDS algorithm: to choose a configuration of points which minimises this degree of stress, appropriately measured.

EXAMPLE: Exe estuary nematodes

The construction of an MDS plot is illustrated with data collected by Warwick (1971) and subsequently analysed in this way by Field *et al.* (1982). A total of 19 stations from different sites and tide-levels in the Exe estuary, UK, were sampled bi-monthly at low spring tides between October 1966 and September 1967.

2. In fact, there are rather too many ways of satisfying it and the algorithm described in this chapter will find slightly different solutions each time it is run, all of them equally correct. However, this is not a problem in genuine applications with (say) six or more points. The number of similarities increases roughly with the square of the number of samples and a position is reached very quickly in which not all rank orders can be preserved and this particular indeterminacy disappears.

Table 5.1. Loch Linnhe macrofauna [L] subset. Abundance array after $\sqrt{\cdot}$ -transform, the Bray-Curtis similarities (as in Table 3.2), the rank similarity matrix and the resulting 2-dimensional MDS ordination.

| Year: | 64 | 68 | 71 | 73 | | Sample | 1 | 2 | 3 | 4 | | Sample | 1 | 2 | 3 | 4 | | Sample | 1 | 2 | 3 | 4 | |
|-----------|-----|-----|-----|-----|---|--------|------|------|------|---|---|--------|---|---|---|---|---|--------|---|---|---|---|--|
| Species | | | | | → | 1 | - | | | | → | 1 | - | | | | → | 1 | - | | | | |
| Echinoca. | 1.7 | 0 | 0 | 0 | | 2 | 25.6 | - | | | | 2 | 5 | - | | | | 2 | 5 | - | | | |
| Myrioche. | 2.1 | 0 | 0 | 1.3 | | 3 | 0.0 | 67.9 | - | | | 3 | 6 | 2 | - | | | 3 | 6 | 2 | - | | |
| Labidopl. | 1.7 | 2.5 | 0 | 1.8 | | 4 | 52.2 | 68.1 | 42.0 | - | | 4 | 3 | 1 | 4 | - | | 4 | 3 | 1 | 4 | - | |
| Amaeana | 0 | 1.9 | 3.5 | 1.7 | | | | | | | | | | | | | | | | | | | |
| Capitella | 0 | 3.4 | 4.3 | 1.2 | | | | | | | | | | | | | | | | | | | |
| Mytilus | 0 | 0 | 0 | 0 | | | | | | | | | | | | | | | | | | | |

3
1
2
4

Three replicate sediment cores were taken for meiofaunal analysis on each occasion, and nematodes identified and counted. This analysis considers only the mean nematode abundances across replicates and season (no seasonal differences were evident in a more detailed analysis), so the data matrix consists of 182 species and 19 samples.

This is not an example of a pollution study: the Exe estuary is a relatively unimpacted environment. The aim here is to display the biological relationships among the 19 stations and then to link these to a set of environmental variables (granulometry, interstitial salinity etc.) measured at these sites, to reveal potential determinants of nematode community structure. Fig. 5.1 shows the 2-dimensional MDS ordination of the 19 samples, based on $\sqrt{\cdot}$ -transformed abundances and a Bray-Curtis similarity matrix. Distinct clusters of sites emerge (in agreement with those from a matching cluster analysis), bearing no clear-cut relation to geographical position or tidal level of the samples.

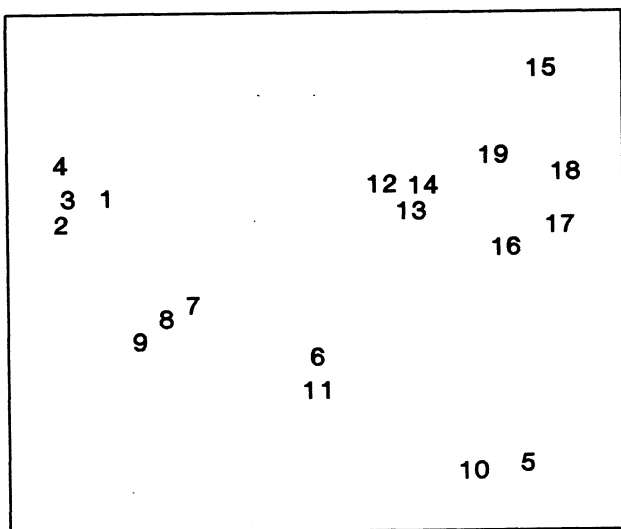


Fig. 5.1. Exe estuary nematodes [X]. MDS ordination of the 19 sites based on $\sqrt{\cdot}$ -transformed abundances and Bray-Curtis similarities (stress = 0.05).

Instead they appear to relate to variables such as sediment type and organic content, and these links are discussed further in Chapter 11. For now the question is: what are stages in the construction of Fig. 5.1?

MDS ALGORITHM

The non-metric MDS algorithm, as employed in Kruskal's original MDSCAL program for example, is an *iterative procedure*, constructing the MDS plot by successively refining the positions of the points until they satisfy, as closely as possible, the dissimilarity relations between samples.³ It has the following steps.

- 1) **Specify the number of dimensions (m)** required for the final ordination plot. If, as will sometimes be desirable, one wishes to compare configurations in two and three dimensions then they have to be constructed separately. For the moment think of m as 2.
- 2) **Construct a starting configuration of the n samples.** This could be the result of an ordination by another method, for example PCA or PCoA, or it could literally be just a random set of n points in m ($= 2$) dimensions.
- 3) **Regress the interpoint distances from this plot on the corresponding dissimilarities.** Let $\{d_{jk}\}$ denote the distance between the j th and k th sample points on the current ordination plot, and $\{\delta_{jk}\}$ the corresponding dissimilarity in the original dissimilarity matrix (of, say, Bray-Curtis coefficients). A scatter plot is then drawn of distance against dissimilarity for all $n(n-1)/2$ such pairs of values. This is termed a *Shepard diagram* and Fig 5.2 shows the type of graph that results. (In fact, this is at a late

3. This is also the algorithm used in the PRIMER program MDS. The required input is a similarity matrix (e.g. as produced by CLUSTER), and the output includes a plot file which can be input to CONPLOT to display the 2-d MDS configuration.

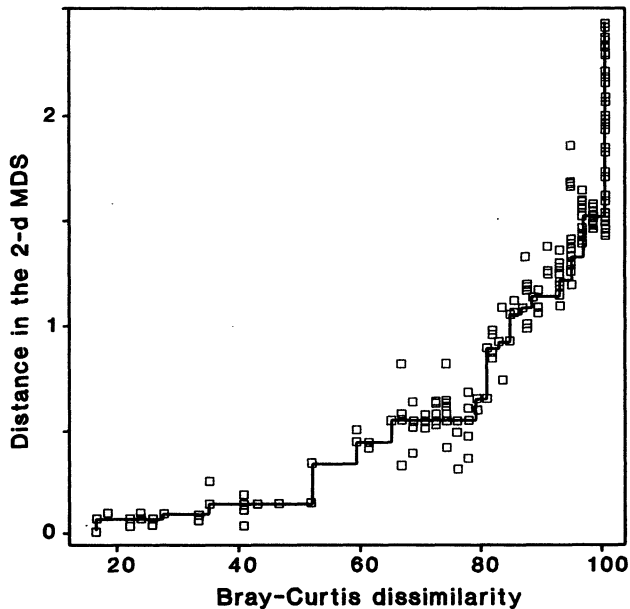


Fig. 5.2. *Exe estuary nematodes (X)*. Shepard diagram of the distances (d) in the MDS plot (Fig. 5.1) against the dissimilarities (δ) in the Bray-Curtis matrix. The line is the fitted non-parametric regression; stress (=0.05) is a measure of scatter about the line.

stage of the iteration, corresponding to the final 2-dimensional configuration of Fig. 5.1; at earlier stages the graph will appear similar though with a greater degree of scatter). The decision that characterises different ordination procedures must now be made: how does one define the underlying relationship between distance in the plot and original dissimilarity? There are two main approaches.

- a) Fit a standard *linear regression* of d on δ , so that final distance is constrained to be *proportional* to original dissimilarity. More flexible would be to fit some form of *curvilinear regression* (perhaps a quadratic, cubic or other polynomial). These are *parametric* models giving rise to the term *metric MDS* for this approach.
- b) Perform a *non-parametric regression* of d on δ giving rise to a *non-metric MDS*. Fig. 5.2 illustrates the non-parametric (monotonic) regression line. This is a "best fitting" line which moulds itself to the shape of the scatter plot, but is always constrained to increase (and therefore consists of a series of steps). The relative success of non-metric MDS, in preserving the sample relationships in the distances of the ordination plot, comes from the flexibility in shape of this non-parametric regression line. A perfect MDS was defined previously as one in which the rank order of dissimilarities was totally

preserved in the rank order of distances; individual points on the Shepard plot must then all be (monotonic) increasing: the larger a dissimilarity, the larger (or equal) the corresponding distance, and the non-parametric regression line is a perfect fit. It follows that the extent to which the scatter points deviate from the line measures the failure to match the rank order dissimilarities, motivating the following definition of *stress*.

- 4) Measure goodness-of-fit of the regression by calculating the *stress* value

$$\text{Stress} = \frac{\sum_j \sum_k (d_{jk} - \hat{d}_{jk})^2}{\sum_j \sum_k d_{jk}^2} \quad (5.1)$$

where \hat{d}_{jk} is the distance predicted from the fitted regression line corresponding to dissimilarity δ_{jk} . If $d_{jk} = \hat{d}_{jk}$ for all the $n(n-1)/2$ distances in this summation, the stress is zero. Large scatter clearly leads to large stress and this can be thought of as measuring the difficulty involved in compressing the sample relationships into two (or a small number) of dimensions. Note that the denominator is simply a scaling term: distances in the final plot have only relative not absolute meaning and the squared distance term in the denominator makes sure that stress is a dimensionless quantity.

- 5) *Perturb the current configuration in a direction of decreasing stress*. This is perhaps the most difficult part of the algorithm to visualise and will not be detailed; it is based on established techniques of numerical optimisation, in particular the method of *steepest descent*. The essential idea is that the regression relation is used to evaluate stress for (small) changes in the position of points on the ordination plot, and points are then moved to new positions in directions which look like they will decrease the stress most rapidly.
- 6) *Repeat steps 3 to 5 until convergence is achieved*. The iteration now cycles around the two stages of a new regression of distance on dissimilarity for the new ordination positions, then further perturbation of the positions in directions of decreasing stress. In most cases, the cycle will stop when further adjustment of the points leads to no improvement in stress.

Features of the algorithm

Like all iterative procedures, especially ones this complex, things can go wrong! By a series of minor adjustments to the parameters at its disposal (the co-ordinate positions in the configuration), the method gradually finds its way down to a minimum of the stress function. This is most easily envisaged in

three dimensions, with just a 2-dimensional parameter space (the x,y plane) and the vertical axis (z) denoting the stress at each (x,y) point. In reality the stress surface is a function of more parameters than this of course, but we have seen before how useful it can be to visualise high-dimensional algebraic operations in terms of 3-dimensional geometry. An appropriate analogy is to imagine a rambler walking across a range of hills in a thick fog(!), attempting to find the *lowest* point within an encircling range of high peaks. A good strategy is always to walk in the direction in which the ground slopes away most steeply (the method of steepest descent, in fact) but there is no guarantee that this strategy will necessarily find the lowest point overall, i.e. the *global minimum* of the stress function. The rambler may reach a low point from which the ground rises in all directions (and the thus the steepest descent algorithm converges) but there may be an even lower point on the other side of an adjacent hill. He is then trapped in a *local minimum* of the stress function. Whether he finds the global or a local minimum depends very much on where he starts the walk, i.e. the starting configuration of points in the ordination plot.

Such local minima do occur in many MDS analyses, usually corresponding to configurations of sample points which are only slightly different from one another. Often this may be because there are one or two points which bear little relation to any of the other samples and there are several choices as to where they may be placed, or perhaps they have a more complex relationship with other samples and may be difficult to fit into (say) a 2-dimensional picture. There is no guaranteed method of ensuring that a global minimum of the stress function has been reached; the practical solution is therefore to repeat the MDS analysis several times starting with different random positions of samples in the initial configuration (step 2 above). If the same (lowest stress) solution re-appears from a number of different starts then there is a strong assurance, though never a total guarantee, that this is indeed the best solution. Note that the easiest way to determine whether the same solution has been reached as in a previous attempt is simply to check for equality of the stress values; remember that the configurations themselves could be arbitrarily rotated or reflected with respect to each other.⁴ In genuine applications, converged stress values are rarely precisely the same if configurations differ materially.

Degenerate solutions can also occur, in which groups of samples collapse to the same point (even though they are not 100% similar), or to the vertices of a

triangle, or are strung out round a circle. In these cases the stress may go to zero. (This is akin to our rambler starting his walk outside the encircling hills, so that he sets off in totally the wrong direction and ends up at the sea!). Artefactual solutions of this sort are relatively rare and easily detected: repetition from different random starts will find many solutions which are more sensible. (In fact, a more likely cause of an ordination in which points tend to be placed around the circumference of a circle is that the input matrix is of similarities when the program is expecting dissimilarities, or vice-versa; in such cases the stress will also be very high.) A much more common form of degenerate solution is repeatable and is a genuine result of a disjunction in the data. For example, if the data divide into two groups, which have no species in common, then there is clearly no yardstick for determining how far apart the groups should be placed in the MDS plot. They are infinitely far apart, in effect, and it is not surprising to find that the samples in each group then collapse to a point. The solution is to split the data and carry out an ordination separately on each group.

Another feature of MDS mentioned earlier is that, unlike PCA, there is not any direct relationship between ordinations in different numbers of dimensions. In PCA, the 2-dimensional picture is just a projection of the 3-dimensional one, and all PC axes can be generated in a single analysis. With MDS, the minimisation of stress is clearly a quite different optimisation problem for each ordination of different dimensionality; indeed, this explains the greater success of MDS in distance-preservation. Samples that are in the same position with respect to (PC1, PC2) axes, though are far apart on the PC3 axis, will be projected on top of each other in a 2-dimensional PCA but they will remain separate, to some degree, in a 2-dimensional as well as a 3-dimensional MDS.

If the ultimate aim is a 2-dimensional ordination, it may still be useful to carry out a 3-dimensional MDS initially. Its first two dimensions will often provide a reasonable *starting point* to the iterative computations

4. The arbitrariness of orientation can be a practical nuisance when comparing different ordinations, and it can be helpful to rotate an MDS so that its direction of maximal variation always lies along the x axis. This can be simply achieved by applying PCA to the 2-d MDS co-ordinates (this is not the same thing as applying PCA to the original data matrix of course!); the PRIMER MDS routine does this automatically but the CONPLOT program also permits user-specified orientation/reflections.

for the 2-dimensional configuration.⁵ In fact, this strategy will tend to reduce the risk of finding local minima or degenerate solutions. The samples are likely to fit more easily into three dimensions, itself reducing the risk of finding a local minimum; the 2-dimensional iteration will then be constrained to start much nearer a global minimum than it would for a purely random initial configuration. Another important reason for obtaining higher-dimensional solutions is to compare their stress with that for two dimensions: this is one of several ways in which the accuracy of a 2-dimensional MDS can be assessed.

ADEQUACY OF MDS REPRESENTATION

1) *Is the stress value small?* By definition, stress increases with reducing dimensionality of the ordination (or in rare cases where a low-dimensional ordination is a perfect representation, stress remains constant). It is helpful therefore to compare the stress values in 2, 3, 4 etc. dimensions: if there is a particularly large drop in stress passing from two to three dimensions (say) and only a modest, steady decrease thereafter, this would imply that a 3-dimensional ordination is likely to be a more satisfactory representation than a 2-dimensional one. However, experience with ecological data suggests that such clear-cut "shoulders" in the plot of minimum stress against dimensionality are rarely seen. It is also undeniable that a 2-dimensional picture will usually be a more useful and accessible summary, so the question is often turned around: not "What is the true dimensionality of the data?" but "Is a 2-dimensional plot a usable summary of the sample relationships, or is it likely to be sufficiently misleading to force its abandonment in favour of a 3- or higher-dimensional plot?" One answer to this is through empirical evidence and simulation studies of stress values. Stress increases not only with reducing dimensionality

5. This procedure is always adopted by the PRIMER MDS program; it also allows the user to specify the number of random re-starts (minimal should be 5 or 6, with the ideal being 10 or more). The output plot file contains the co-ordinates of the best 2-dimensional solution (that with the lowest stress), and the stress values for all 2-dimensional and 3-dimensional repeats are given in the MDS results file. The latter also contains the co-ordinates for the best 3-dimensional solution. These can be copied out to a new file, to input to one of the wide range of 3-dimensional plotting packages now available; note that all PRIMER files are simple ASCII text and therefore easily edited.

but also with increasing quantity of data, but a rough rule-of-thumb for 2-dimensional ordinations, using the stress function (5.1), is as follows.⁶

Stress <0.05 gives an excellent representation with no prospect of misinterpretation (a perfect representation would probably be one with stress <0.01 since numerical iteration procedures often terminate when stress reduces below this value⁷).

Stress <0.1 corresponds to a good ordination with no real prospect of a misleading interpretation; higher-dimensional solutions will not add any additional information about the *overall* structure (though the fine structure of any compact groups may bear closer examination).

Stress <0.2 still gives a potentially useful 2-dimensional picture, though for values at the upper end of this range too much reliance should not be placed on the detail of the plot; a cross-check of any conclusions should be made against those from an alternative technique (e.g. the superimposition of cluster groups suggested in point 5 below).

Stress >0.3 indicates that the points are close to being arbitrarily placed in the 2-dimensional ordination space. In fact, the totally random positions used as a starting configuration for the iteration usually give a stress around 0.35-0.45. Values of stress in the range 0.2-0.3 should therefore be treated with a great deal of scepticism and certainly discarded in the upper half of this range, especially for a small to moderate number of points (<50 say). Other techniques will be certain to highlight inconsistencies and higher-dimensional ordinations should be examined.

2) *Does the Shepard diagram appear satisfactory?* The stress value totals the scatter around the regression line in a Shepard diagram, for example the low stress of 0.05 for Fig. 5.1 is reflected in the low scatter in Fig. 5.2. Outlying points in the plot could be identified with the samples involved; often there are a range of *outliers* all involving dissimilarities with a particular sample and this can indicate a point which really needs a higher-dimensional representation for

6. There are alternative definitions of stress, for example the "stress formula 2" option provided in the MDSCAL and KYST programs. This differs only in the denominator scaling term in (5.1) but is believed to increase the risk of finding local minima and to be more appropriate for other forms of multivariate scaling, e.g. multidimensional unfolding, which are outside the scope of this manual.

7. This is true of the MDS routine in PRIMER, for example.

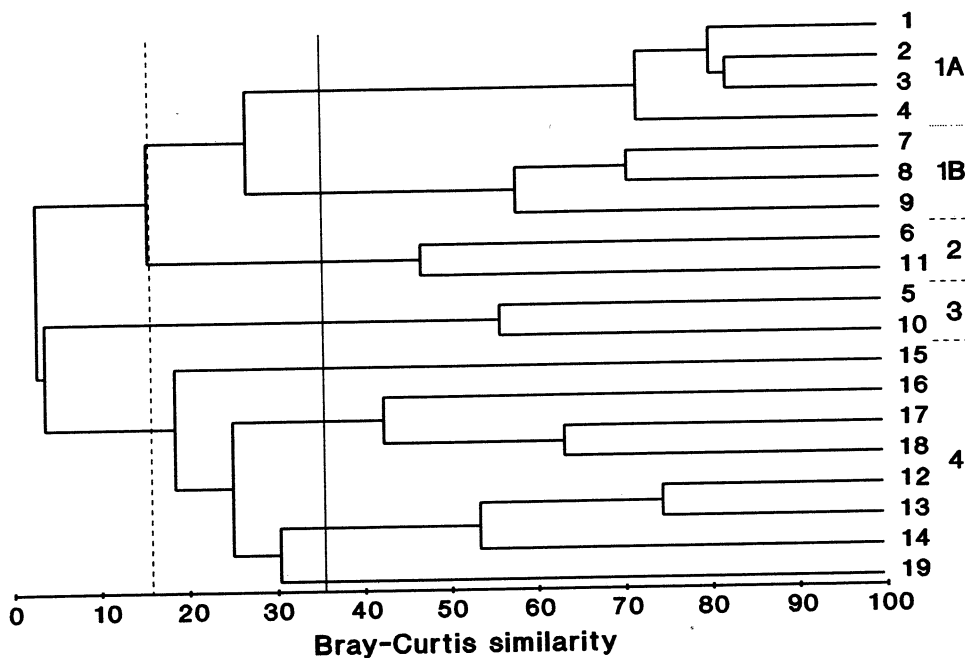


Fig. 5.3. Exe estuary nematodes (X). Dendrogram of the 19 stations, using group-average clustering from Bray-Curtis similarities on $\sqrt{\cdot}$ -transformed abundances. The four groups of stations separated at a 15% similarity threshold (dotted line) are indicated (the two tightly clustered sub-groups within group 1 were designated 1A and 1B by Field et al. 1982).

accurate placement, or simply corresponds to a major error in the data matrix.

3) *Is there distortion when similar samples are connected in the ordination plot?* One simple check on the success of the ordination in dissimilarity-preservation is to identify the top 10% or 20% (say) of values in the similarity matrix and draw a line between the corresponding points on the MDS configuration. An inaccurate representation is indicated if several connections are made between points which are further apart on the plot than other unconnected pairs of points.

4) *Is the "minimum spanning tree" consistent with the ordination picture?* A similar idea to the above is to construct the *minimum spanning tree* (MST, Gower and Ross, 1969). All samples are "connected" by a single line which is allowed to branch but does not form a closed loop, such that one minimises the sum along this line of *dissimilarities* (taken from the original dissimilarity matrix not the distance matrix from the ordination, note). This line is then plotted on the 2-dimensional ordination and inadequacy is again indicated by connections which look unnatural in the context of placement of samples in the MDS configuration.

5) *Do superimposed groups from a cluster analysis distort the ordination plot?* The combination of clustering and ordination analyses can be a very effective way of checking the adequacy and mutual consistency of both representations. Fig. 5.3 shows the dendrogram from a cluster analysis of the Exe estuary nematode data (X) of Fig. 5.1. Two or more (arbitrary)

similarity values are chosen at a spread of hierarchical levels, each determining a particular grouping of samples. In Fig. 5.3, four groups are formed at around a 15% similarity level and eight groups would be determined for any similarity threshold between 30 and 45%. These two sets of groupings are superimposed on the MDS ordination, Fig. 5.4, and it is clear that the agreement between the two techniques is excellent: the clusters are sharply defined and would be determined in much the same way if one were to select clusters by eye from the 2-dimensional ordination alone. The stress for Fig. 5.4 is also low, at 0.05, giving confidence that the 2-dimensional plot is

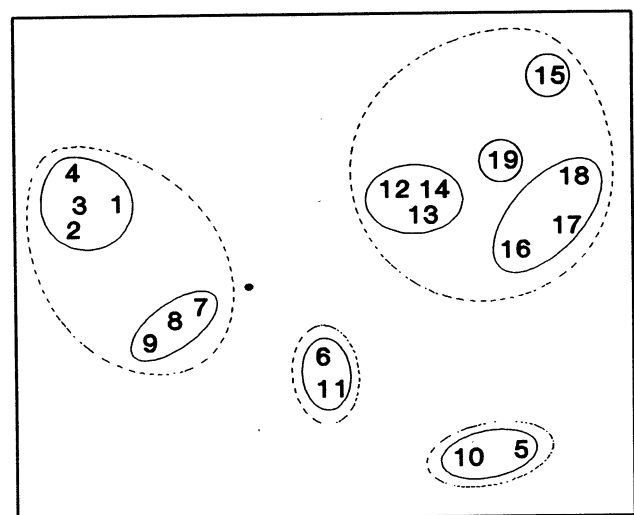


Fig. 5.4. Exe estuary nematodes (X). 2-dimensional MDS configuration, as in Fig. 5.1, with superimposed clusters from Fig. 5.3, at similarity levels of 15% (dashed line) and 30%-45% (continuous line).

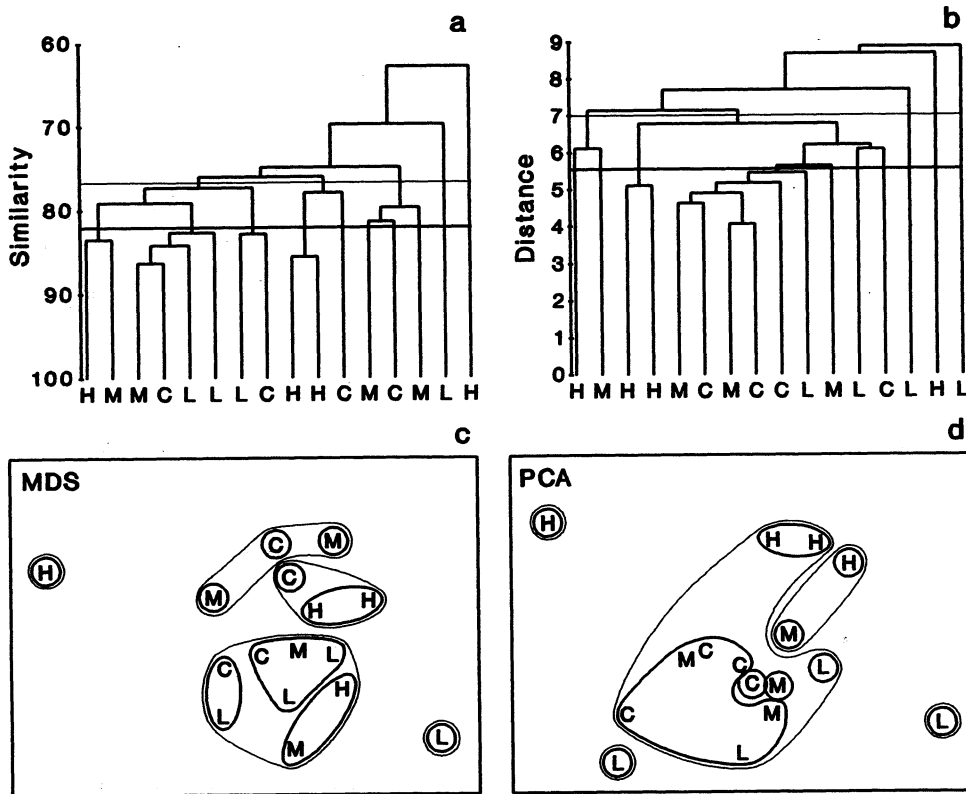


Fig. 5.5. Dosing experiment, Solbergstrand mesocosm (D). Nematode abundances for four replicates from each of four treatments (control, low, medium and high dose of hydrocarbons and Cu) after species reduction and log transformation as in Fig. 4.2. a), c) Group-average clustering from Bray-Curtis similarities; clusters formed at two arbitrary levels are superimposed on the 2-dimensional MDS obtained from the same similarities (stress = 0.16). b), d) Group-average clustering from distances; clusters from two levels are superimposed on the 2-dimensional PCA of Fig. 4.2. Note the greater degree of distortion in the latter.

an accurate representation of the sample relationships. One is not always as fortunate as this, and a more revealing example of the benefits of viewing clustering and ordination in combination is provided by the data of Fig. 4.2.⁸

EXAMPLE: Dosing experiment, Solbergstrand

The nematode abundance data from the dosing experiment (D) at the GEEP Oslo Workshop was previously analysed by PCA, see Fig. 4.2 and accompanying text. The analysis was likely to be unsatisfactory, since the % of variance explained by the first two principal components was very low, at 37%. Fig. 5.5c shows the MDS ordination from the same data, and in order to make a fair comparison with the PCA the data matrix was treated in exactly the same

way prior to analysis.⁹ The stress for the 2-dimensional MDS configuration is moderately high (at 0.16), indicating some difficulty in displaying the relationships between these 16 samples in two dimensions. However, the PCA was positively misleading in its apparent separation of the four high dose (H) replicates in the 2-dimensional space; by contrast the MDS *does* provide a usable summary which is not likely to lead to serious misinterpretation. This can be seen by superimposing the corresponding cluster analysis results, Fig. 5.5a, onto the MDS. Two similarity thresholds have been chosen in Fig. 5.5a such that they (arbitrarily) divide the samples into 5 and 10 groups, the corresponding hierarchy of clusters being indicated in Fig. 5.5c by thin and thick lines respectively. Whilst it is clear that there are no natural groupings of the samples in the MDS plot, and the groupings provided by the cluster analysis must therefore be regarded with a certain degree of caution, the two analyses are not markedly inconsistent.

In contrast, the parallel operation for the PCA ordination clearly illustrates the poorer distance-preserving properties of this method. Fig. 5.5d repeats the

8. One option within PRIMER is to run CLUSTER on the *ranks* of the similarities rather than the similarities themselves. Whilst not of any real merit in itself (and not the default option), Clarke (1993) argues that this could have marginal benefit when performing a group-average cluster analysis solely to see how well the clusters agree with the MDS plot: the argument is that the information utilised by both techniques is then made even more comparable.

9. The same 26 species were retained and a log transformation applied before computation of Bray-Curtis similarities, though, of course, a species reduction would not normally be necessary with MDS or clustering of samples.

2-dimensional PCA of Fig. 4.2 but with superimposed groups from a cluster analysis of the Euclidean distance matrix¹⁰ between the 16 samples (Fig. 5.5b). With the same division into five clusters (thin lines) and ten clusters (thick lines), a much more distorted picture results, with samples that are virtually coincident in the PCA plot being placed in separate groups and samples appearing distant from each other forming a common group.

The outcome one would expect on theoretical grounds is therefore apparent in practice here: MDS can provide a more realistic picture in situations where PCA gives a distorted representation of the true "distances" between samples. In fact, the biological conclusions from this particular study are entirely negative: the test described in Chapter 6 shows that there are no statistically significant differences in community structure between any of the four dosing levels in this experiment.

EXAMPLE: Celtic Sea zooplankton

In situations where the samples are strongly grouped, as in Fig. 5.3 and 5.4, both clustering and ordination analyses will demonstrate this, usually in equally adequate fashion. The strength of ordination is in displaying a *gradation* of community composition across a set of samples. An example is provided by Fig. 5.6, of zooplankton data from the Celtic Sea (C). Samples were collected from 14 depths, separately for day and night time studies at a single site. The changing community composition with depth can be traced on the resulting MDS (from Bray-Curtis similarities). There is a greater degree of variability in community structure of the near-surface samples, with a marked change in composition at about 20–25m; deeper than this the changes are steady but less pronounced and they step in parallel for day and night time samples. Another obvious feature is the strong difference in composition between day and night near-surface samples, contrasted with their relatively higher similarity at greater depth. Cluster analysis of the same data would clearly not permit the accuracy and subtlety of interpretation that is possible from ordination of a gradually changing community pattern.

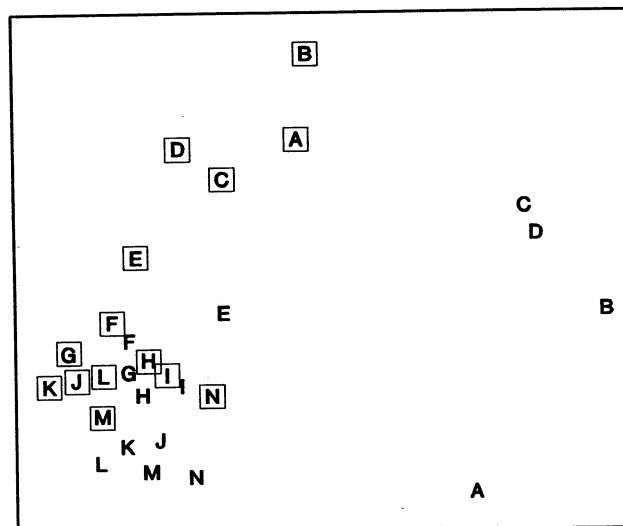


Fig. 5.6. Celtic Sea zooplankton (C). MDS plot for night (boxed) and day time samples from 14 depths (5 to 70m, denoted A, B, ..., N), taken at a single site during September 1978.

MDS STRENGTHS

- 1) *MDS is simple in concept.* Though the numerical algorithm is undeniably complex, it is always clear what MDS is trying to achieve: the construction of a sample map whose inter-point distances have the *same rank order* as the corresponding dissimilarities between samples.
- 2) *It is based on the relevant sample information.* MDS works on the sample dissimilarity matrix not on the original data array, so there is complete freedom of choice to define similarity of community composition in whatever terms are biologically most meaningful.
- 3) *Species deletions are unnecessary.* Another advantage of starting from the sample dissimilarity matrix is that the number of species on which it was based is largely irrelevant to the amount of calculation required. Of course, if the original matrix contained a large number of species whose patterns of abundance across the samples varied widely, and prior transformation (or choice of similarity coefficient) dictated that all species were given equal weight, then the structure in the sample dissimilarities might be more difficult to represent in a low number of dimensions. More usually, the similarity measure will automatically downweight the contribution of species that are rarer (and thus more prone to random and uninterpretable fluctuations). There is then no necessity to delete species, either to obtain realistic low-dimensional

10. As previously noted, Euclidean distance is the dissimilarity measure implicit in a PCA ordination.

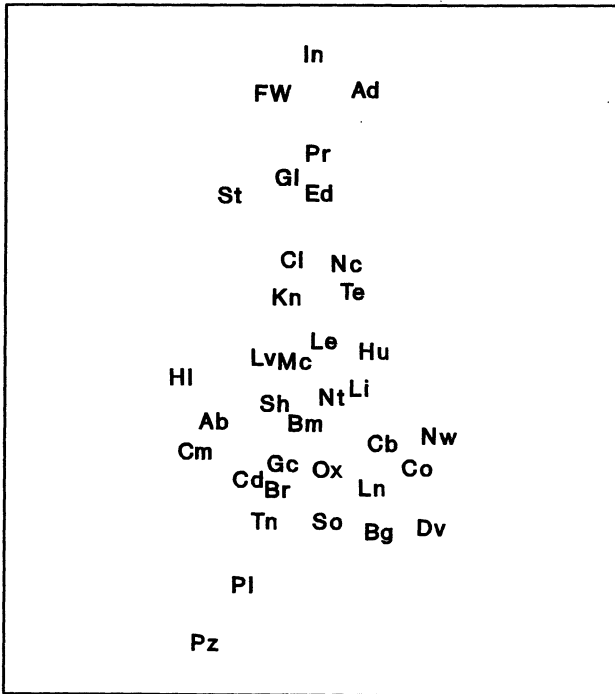


Fig. 5.7. Non-metric MDS configuration of the road distances (partly given in Table 5.2) between selected UK towns and cities (stress = 0.04).

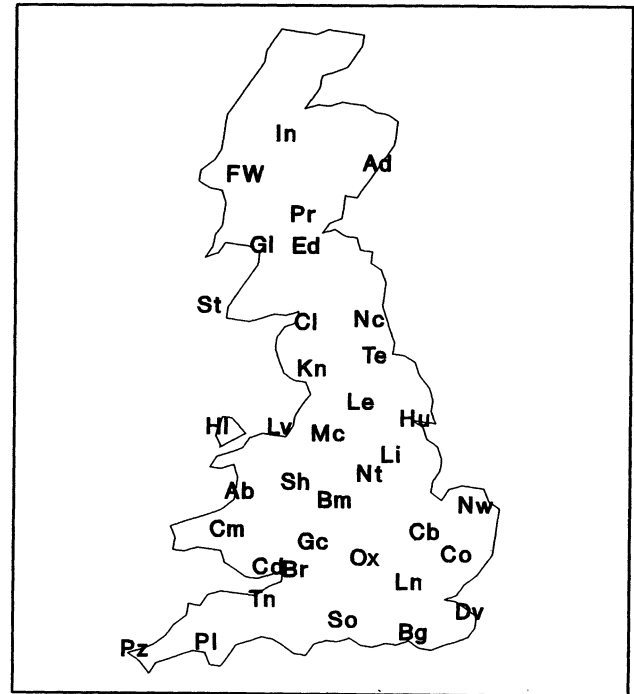


Fig. 5.8. Non-metric MDS configuration of the same towns and cities as in Table 5.2, but starting from the matrix of direct ("as the crow flies") distances between every pair (stress = 0).

5) **Similarities can be given unequal weight.** If some samples are inherently less reliable than others because they are based on smaller amounts of material sampled (perhaps combining the results of fewer replicates), then similarities involving these samples can be given less influence in the construction of the MDS configuration: a weighting term could be added to the definition of stress in equation (5.1). It is also true, though not of practical significance here, that the algorithm can operate perfectly successfully when the similarity matrix is subject to a certain amount of missing data.¹²

MDS WEAKNESS

1) **MDS is computationally demanding.** To generate a single configuration with moderate to large numbers of samples takes some time on a modern personal computer, though speed has become much less of a problem than it once was. However, MDS on much more than $n = 100$ samples is not only rather computationally intensive (processor time increases roughly proportional to n^2) but also increasing sample size generally brings increasing complexity of the sample relationships, and a 2-dimensional representation is unlikely to be adequate in any case. (Of course this last point is

just as true, if not more true, for other ordination methods). This scenario was touched on in Chapter 4, where it was suggested that large data sets can often be sub-divided *a priori*, or on the basis of well-defined subsets from a cluster analysis, and the groups analysed separately by MDS. Representatives (or averages) from each group can then be input to an MDS to display the large-scale structure.

2) **Convergence to the global minimum of stress is not guaranteed.** As we have seen, the iterative nature of the MDS algorithm makes it necessary to repeat each analysis a number of times, from different starting configurations, to be fairly confident that a solution that re-appears several times (with the lowest observed stress) is indeed the global minimum of the stress function. Generally, with higher stress, the greater is the likelihood of

12. This could only be of importance if data were to arise directly as similarities constructed from pairwise comparisons of biological material, and some of those comparisons are not made or are lost. It is not of relevance if similarities are generated from a species-by-samples data matrix since, usually, either all or none of the similarities involving a particular sample can be calculated; if the latter, then there is clearly no way the sample could feature in the ordination!

non-optimal solutions, so a larger number of repeats is required; this adds to the computational burden of course.

3) *The algorithm places most weight on the large distances.* A common feature of most ordination methods (including MDS and PCA) is that more attention is given to correct representation of the *overall* structure of the samples than their *local* structure. For MDS, it is clear from the form of equation (5.1) that the largest contributions to stress will come from incorrect placement of samples which are very distant from each other. Where distances are small, the sum of squared difference terms will also be relatively small and the minimisation process will not be as sensitive to incorrect positioning. This is another reason therefore for repeating the ordination *within* each large cluster: it will lead to a more accurate display of the fine structure. An example is given later in Figs. 6.2a and 6.3, and is fairly typically of the rather minor differences that result: the subset points are given more freedom to expand in a particular direction but their relative positions are only very marginally changed.

RECOMMENDATIONS

- 1) MDS can be recommended as one of the best (arguably *the best*) ordination techniques available (e.g. Everitt, 1978). The few comprehensive studies that have compared ordination methods for
- community data give non-metric MDS a high rating (e.g. Kenkel and Orloci, 1986). In comparison with earlier techniques, such as PCA, MDS has a number of practical advantages stemming from its flexibility and (lack of) assumptions.
- 2) When the inter-sample relationships are relatively simple, e.g. there are a few strong clusters or one strong gradient of change across all samples, most ordination methods will perform adequately and give comparable pictures. The main advantage of MDS is its greater ability to represent more complex relations accurately in low-dimensional space.
- 3) If the stress is low (say <0.1), an MDS ordination is probably a more useful representation than a cluster analysis: when the samples are strongly grouped the MDS will reveal this anyway and when there is a more gradual continuum of change, or some interest in the placement of major groups with respect to each other, MDS will display this in a way that a cluster analysis is quite incapable of doing. For higher values of stress the techniques should be thought of as *complementary* to each other; neither may present the full picture so the recommendation is to *perform both* and *view them in combination*. This may make it clear which points on the MDS are problematic to position (examining some of the local minimum solutions can help here) and an ordination in a higher dimension may prove more consistent with the cluster groupings. Conversely, the MDS plots may make it clear that some groups in the cluster analysis are fairly arbitrary subdivisions of a natural continuum.

CHAPTER 6: TESTING FOR DIFFERENCES BETWEEN GROUPS OF SAMPLES

Many community data sets possess some *a priori* defined structure within the set of samples, for example there may be replicates from a number of different sites (and/or times). A pre-requisite to interpreting community differences between sites should be a demonstration that there *are* statistically significant differences to interpret.

UNIVARIATE TESTS

When the species abundance (or biomass) information in a sample is reduced to a single index, such as Shannon diversity (see Chapter 8), the existence of replicate samples from each of the groups (sites/times etc.) allows formal statistical treatment by analysis of variance (ANOVA). This requires the assumption that the univariate index is normally distributed and has constant variance across the groups, conditions which are normally not difficult to justify (perhaps after transformation, see Chapter 9). A so-called *global test* of the *null hypothesis* (H_0), that there are no differences between groups, involves computing a particular ratio of variability in the group means to variability among replicates within each group. The resulting *F statistic* takes values near 1 if the null hypothesis is true, larger values indicating that H_0 is false; standard tables of the *F* distribution yield a significance level (p) for the observed *F* statistic. Roughly speaking, p is interpreted as the probability that the group means we have observed (or a set of means which appear to differ from each other to an even greater extent) could have occurred if the null hypothesis H_0 is actually true.

Fig. 6.1 and Table 6.1 provide an illustration, for the 6 sites and 4 replicates per site of the Frierfjord macrofauna samples. The mean Shannon diversity for the 6 sites is seen in Fig. 6.1, and Table 6.1 shows that the *F* ratio is sufficiently high that the probability of observing means as disparate as this by chance is $p < 0.001$ (or $p < 0.1\%$), if the true mean diversity at all sites is the same. This is deemed to be a sufficiently unlikely chance event that the null hypothesis can safely be rejected. Convention dictates that values of $p < 5\%$ (say) are sufficiently small, *in a single test*, to discount the possibility that H_0 is true, but there is nothing sacrosanct about this figure: clearly, values of $p = 4\%$ and 6% should elicit the same inference. It is equally clear that repeated significance tests, each of which has

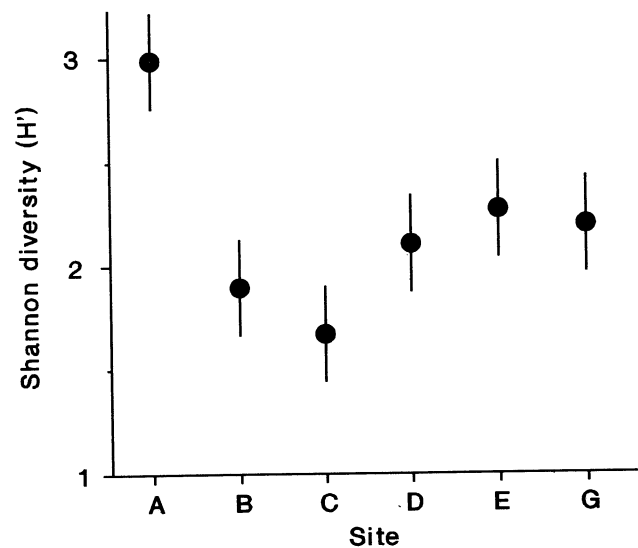


Fig. 6.1. Frierfjord macrofauna (F). Means and 95% confidence intervals of Shannon diversity (H') at the 6 field sites (A–E, G) shown in Fig. 1.1.

(say) a 5% possibility of describing a chance event as a real difference, will cumulatively run a much greater risk of drawing at least one false inference. This is one of the (many) reasons why it is not usually appropriate to handle a multi-species matrix by performing an ANOVA on each species in turn. (More decisive reasons are the complexities of dependence between species and the inappropriateness of normality assumptions).

Fig. 6.1 shows the main difference to be a higher diversity at the outer site, A. The intervals displayed are 95% *confidence intervals* for the true mean diversity at each site; note that these are of equal width because they are based on the assumption of constant variance, that is, they use a pooled estimate of replication variability from the residual mean square in the ANOVA table.

Table 6.1. Frierfjord macrofauna (F). ANOVA table showing rejection (at a significance level of 0.1%) of the global hypothesis of "no site-to-site differences" in Shannon diversity (H').

| | Sum of squares | Deg. of freedom | Mean Square | F ratio | Sig. level |
|----------|----------------|-----------------|-------------|---------|------------|
| Sites | 3.938 | 5 | 0.788 | 15.1 | <0.1% |
| Residual | 0.937 | 18 | 0.052 | | |
| Total | 4.874 | 23 | | | |

Further details of how confidence intervals are determined, why the ANOVA F ratio and F tables are defined in the way they are, how one can allow to some extent for the repeated significance tests in pairwise comparisons of site means etc., are not pursued here. This is the ground of basic statistics, covered by many standard texts, for example Sokal and Rohlf (1981), and such computations are available in all general-purpose statistics packages. This is not to imply that these concepts are elementary; in fact it is ironic that a proper understanding of *why* the univariate F test works requires a level of mathematical sophistication that is not needed for the simple permutation approach to the analogous global test for differences in *multivariate* structure between groups, outlined below.

MULTIVARIATE TESTS

One important feature of the multivariate analyses described in earlier chapters is that they in no way utilise any known structure among the samples, e.g. their division into replicates within groups. (This is in contrast with Canonical Variate Analysis, for example, which deliberately seeks out ordination axes that, in a certain well-defined sense, best separate out the known groups; e.g. Mardia *et al.*, 1979). Thus, the ordination and dendrogram of Fig. 6.2, for the Frierfjord macrofauna data, are constructed only from the pairwise similarities among the 24 samples, treated simply as numbers 1 to 24. By superimposing the group (site) labels A to G on the respective replicates it becomes immediately apparent that, for example, the 4 replicates from the outer site (A) are quite different in community composition from both the mid-fjord sites B, C and D and the inner sites E and G. A statistical test of the hypothesis that there are no site-to-site differences overall is clearly unnecessary, though it is less clear whether sufficient evidence exists to assert that B, C and D differ, for example.

This simple structure of groups, and replicates within groups, is referred to as a *1-way layout*, and it was seen above that 1-way ANOVA would provide the appropriate testing framework *if* the data were univariate (e.g. diversity or total abundance across all species). There is an analogous multivariate analysis of variance (MANOVA, e.g. Mardia *et al.*, 1979), in which the F test is replaced by a test known as Wilks' Λ , but its assumptions will never be satisfied for typical multi-species abundance (or biomass) data. This is the problem referred to in the earlier chapters on choosing similarities and ordination methods; there are typically many more species (variables) than samples

and the probability distribution of counts could never be reduced to approximate (multivariate) normality, by any transformation, because of the dominance of zero values. For example, for the Frierfjord data, as many as 50% of the entries in the species/samples matrix are zero even after reducing the matrix to only the 30 most abundant species!

A valid test can instead be built on a simple non-parametric permutation procedure, applied to the (rank) similarity matrix underlying the ordination or classification of samples, and therefore termed an

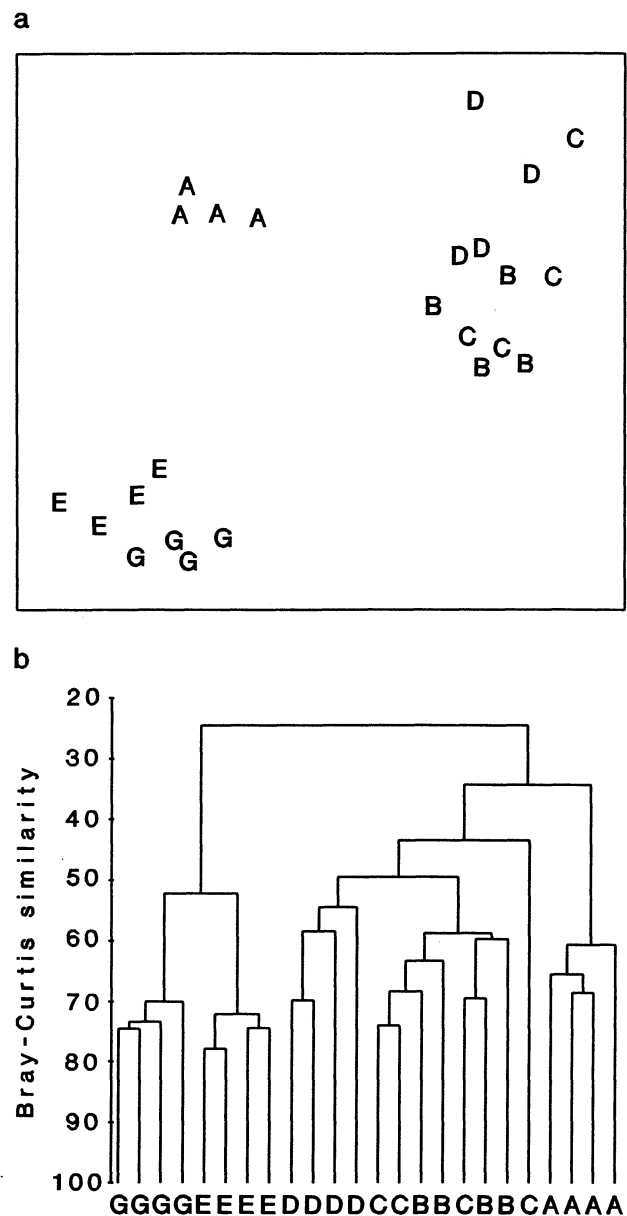


Fig. 6.2. Frierfjord macrofauna (F). a) MDS plot, b) dendrogram, for 4 replicates from each of 6 sites (A-E and G), from Bray-Curtis similarities computed for $\sqrt{\sqrt{\cdot}}$ -transformed species abundances (MDS stress = 0.05).

ANOSIM test (*analysis of similarities*)¹, by analogy with the acronym ANOVA (analysis of variance). The history of such permutation tests dates back to the epidemiological work of Mantel (1967), and this is combined with a general randomization approach to the generation of significance levels (*Monte Carlo tests*, Hope 1968). In the context below, it was described by Clarke and Green (1988).

'ANOSIM' FOR THE 1-WAY LAYOUT

Fig. 6.3 displays the MDS based only on the 12 samples (4 replicates per site) from the B, C and D sites of the Frierfjord macrofauna data. The null hypothesis (H_0) is that there are no differences in community composition at these 3 sites. In order to examine H_0 , there are 3 main steps:

1) *Compute a test statistic* reflecting the observed differences *between* sites, contrasted with differences among replicates *within* sites. Using the MDS plot of Fig. 6.3, a natural choice might be to calculate the average distance between every pair of replicates within a site and contrast this with the average distance apart of all pairs of samples corresponding to replicates from different sites. A test could certainly be constructed from these distances but has a number of drawbacks.

- Such a statistic could only apply to a situation in which the method of display was an MDS rather than, say, a cluster analysis.
- The result would depend on whether the MDS was constructed in two, three or higher dimensions. There is often no "correct" dimensionality and one may end up viewing the picture in several different dimensions—it would be unsatisfactory to generate different test statistics in this way.
- The configuration of B, C and D replicates in Fig. 6.3 also differs slightly from that in Fig. 6.2a, which includes the full set of sites A–E, G. It is again undesirable that a test statistic for comparing *only* B, C and D should depend on which other sites are included in the picture.

These three difficulties disappear if the test is based not on distances between samples in an MDS but on the corresponding (rank) similarities between samples in

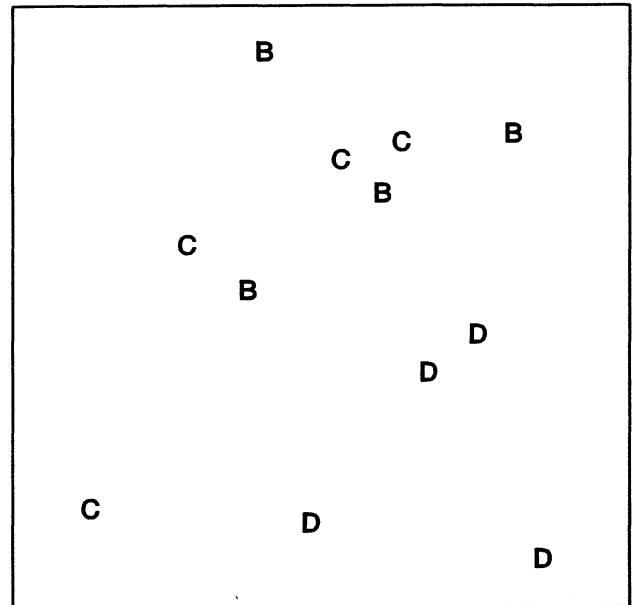


Fig. 6.3. Frierfjord macrofauna {F}. MDS ordination as for Fig. 6.2 but computed only from the similarities involving sites B, C and D (stress = 0.11).

the underlying triangular similarity matrix. If \bar{r}_w is defined as the average of all rank similarities among replicates *within* sites, and \bar{r}_b is the average of rank similarities arising from all pairs of replicates *between* different sites, then a suitable test statistic is

$$R = (\bar{r}_b - \bar{r}_w) / (M/2) \quad (6.1)$$

where $M = n(n-1)/2$ and n is the total number of samples under consideration. Note that the highest similarity corresponds to a rank of 1 (the lowest value), following the usual mathematical convention for assigning ranks.

The denominator constant in equation (6.1) has been chosen so that:

- R can never technically lie outside the range $(-1, 1)$;
- $R = 1$ only if *all* replicates within sites are more similar to each other than *any* replicates from different sites;
- R is approximately zero if the null hypothesis is true, so that similarities between and within sites will be the same on average.

R will usually fall between 0 and 1, indicating some degree of discrimination between the sites. R substantially less than zero is unlikely since it would correspond to similarities across different sites being *higher* than those within sites; such an occurrence is more likely to indicate an incorrect labelling of samples. The R statistic itself is a useful comparative measure of the degree of separation of sites, though the main interest usually centres on whether it is

1. The PRIMER program ANOSIM covers tests for replicates from 1-way and 2-way (nested or crossed) layouts; the program ANOSIM2 tackles the special case of a 2-way layout with no replication, which needs a modified style of test described at the end of this chapter.

significantly different from zero. (Note though that, as with standard univariate tests, it is perfectly possible for R to be significantly different from zero yet inconsequentially small, if there are many replicates at each site).

2) *Recompute the statistic under permutations* of the sample labels. Under the null hypothesis H_0 : "no difference between sites", there will be little effect on average to the value of R if the labels identifying which replicates belong to which sites are arbitrarily re-arranged; the 12 samples of Fig. 6.3 are just replicates from a single site if H_0 is true. This is the rationale for a *permutation test* of H_0 ; all possible allocations of four B, four C and four D labels to the 12 samples are examined and the R statistic recalculated for each. In general there are

$$(kn)! / [(n!)^k k!] \tag{6.2}$$

distinct ways of permuting the labels for n replicates at each of k sites, giving 5775 permutations here. It is computationally possible to examine this number of re-labellings but the scale of calculation can quickly get out of hand with modest increases in replication, so the full set of permutations is randomly sampled (usually with replacement) to give the null distribution of R . In other words, the labels in Fig. 6.3 are randomly reshuffled, R recalculated and the process repeated a large number of times (T).

3) *Calculate the significance level* by referring the observed value of R to its permutation distribution. If H_0 is true, the likely spread of values of R is given by the random rearrangements, so that if the *true* value of R looks unlikely to have come from this distribution there is evidence to reject the null hypothesis. Formally, if only t of the T simulated values of R are as large (or larger than) the *observed* R then H_0 can be rejected at a *significance level* of $100(t + 1) / (T + 1)\%$.

EXAMPLE: Frierfjord macrofauna

The rank similarities underlying Fig. 6.3 are shown in Table 6.2 (note that these are the similarities involving only sites B, C and D, extracted from the matrix for *all* sites and re-ranked). Averaging across the 3 diagonal sub-matrices (within groups B, C and D) gives $\bar{r}_w = 22.7$, and across the remaining (off-diagonal) entries gives $\bar{r}_b = 37.5$. Also $n = 12$ and $M = 66$, so that $R = 0.45$. In contrast, the spread of R values possible from random re-labelling of the 12 samples can be seen in the histogram of Fig. 6.4: the largest of $T = 999$ simulations is less than 0.45 ($t = 0$). An observed value

of $R = 0.45$ is seen to be a most unlikely event, with a probability of less than 1 in a 1000 if H_0 is true, and we can therefore reject H_0 at a significance level of $p < 0.1\%$.

Table 6.2. Frierfjord macrofauna {F}. Rank similarity matrix for the 4 replicates from each of B, C and D, i.e. C3 and C4 are the most, and B1 and C1 the least, similar samples.

| | B1 | B2 | B3 | B4 | C1 | C2 | C3 | C4 | D1 | D2 | D3 | D4 |
|----|----|----|----|----|----|----|----|----|----|----|----|----|
| B1 | - | | | | | | | | | | | |
| B2 | 33 | - | | | | | | | | | | |
| B3 | 8 | 7 | - | | | | | | | | | |
| B4 | 22 | 11 | 19 | - | | | | | | | | |
| C1 | 66 | 30 | 58 | 65 | - | | | | | | | |
| C2 | 44 | 3 | 15 | 28 | 29 | - | | | | | | |
| C3 | 23 | 16 | 5 | 38 | 57 | 6 | - | | | | | |
| C4 | 9 | 34 | 4 | 32 | 61 | 10 | 1 | - | | | | |
| D1 | 48 | 17 | 42 | 56 | 37 | 55 | 51 | 62 | - | | | |
| D2 | 14 | 20 | 24 | 39 | 52 | 46 | 35 | 36 | 21 | - | | |
| D3 | 59 | 49 | 50 | 64 | 54 | 53 | 63 | 60 | 43 | 41 | - | |
| D4 | 40 | 12 | 18 | 45 | 47 | 27 | 26 | 31 | 25 | 2 | 13 | - |

Pairwise tests

The above is a *global* test, indicating that there are site differences *somewhere* that may be worth examining further. Specific pairs of sites can then be compared: for example, the similarities involving only sites B and C are extracted, re-ranked and the test procedure repeated, giving an R value of 0.23. This time there are only 35 distinct relabellings so, under the null hypothesis H_0 that sites B and C do not differ, the full permutation distribution of possible values of R can be

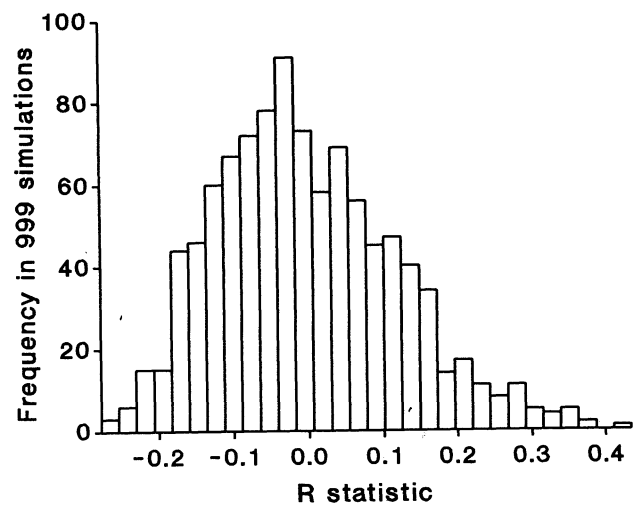


Fig. 6.4. Frierfjord macrofauna {F}. Simulated distribution of the test statistic R (equation 6.1) under the null hypothesis of 'no site differences'; this contrasts with an observed value for R of 0.45.

computed; 12% of these values are equal to or larger than 0.23 so H_0 cannot be rejected. By contrast, $R = 0.54$ for the comparison of B against D, which is the most extreme value possible under the 35 permutations. B and D are therefore inferred to be significantly different at the $p < 3\%$ level. For C against D, $R = 0.57$ similarly leads to rejection of the null hypothesis ($p < 3\%$).

There is a danger in such repeated significance tests which should be noted (although little can be done to ameliorate it here). To reject the null hypothesis at a significance level of 3% implies that a 3% risk is being run of drawing an incorrect conclusion (a *Type I error*, in statistical terminology). If many such tests are performed this risk will cumulate. For example, all pairwise comparisons between 10 sites, each with 4 replicates (allowing 3% level tests at best), would involve 45 tests, and the overall risk of drawing at least one false conclusion is high. For the analogous pairwise comparisons following the global F test in a univariate ANOVA, there exist *multiple comparison* tests which attempt to adjust for this repetition of risk. No such constructs are possible here, and the pragmatic course is to exercise appropriate caution in interpretation and/or enhance the potential significance of the individual tests by a modest increase in the number of replicates. Equation (6.2) shows that 5 replicates from each site would allow a 1% level test for a pairwise comparison (126 permutations), and 6 replicates gives close to a 0.2% level test (462 permutations); compounding these smaller values is clearly preferable to cumulating 3% risks (or the 10% Type I error, at best, from pairwise comparisons of only 3 replicates!).

This also raises the issue of *Type II error* of such a permutation test, related to its *power* to detect a difference between sites if one genuinely exists. Such concepts are not easily examined for non-parametric procedures of this type, which make no distributional assumptions and for which it is difficult to specify a precise non-null hypothesis; all that can be obviously said is that power will improve with increasing replication.

Generality of application

It is evident that few, if any, assumptions have been made about the data in constructing the 1-way ANOSIM test, and it is therefore very generally applicable. It is not restricted to Bray-Curtis similarities or even to similarities computed from species abundance data: it could provide a non-para-

metric alternative to Wilks' Λ test for data which are more nearly multivariate normally distributed, e.g. for testing whether groups (sites or times) can be distinguished on the basis of their environmental data (see Chapter 11). The latter would involve computing a Euclidean distance matrix between samples (after suitable transformation of the environmental variables) and entering this as a dissimilarity matrix to the ANOSIM procedure. Clearly, if multivariate normality assumptions are genuinely justified then the ANOSIM test must lack sensitivity in comparison with standard MANOVA, but this would seem to be more than compensated for by its greater generality.

Note also that there is no restriction to a balanced number of replicates. Some groups could even have only one replicate provided enough replication exists in other groups to generate sufficient permutations for the global test (though there will be a sense in which the power of the test is compromised by a markedly unbalanced design, here as elsewhere). More usefully, note that no assumptions have been made about the variability of within-group replication needing to be similar for all groups. This is seen in the following example, for which the groups in the 1-way layout are not sites but samples from different years at a single site.

EXAMPLE: Indonesian reef-corals

Warwick *et al.* (1990b) examined data from 10 replicate transects across a single coral-reef site in S. Tikus Island, Thousand Islands, Indonesia, for each of the six years 1981, 1983, 1984, 1985, 1987 and 1988. The community data are in the form of % cover of a transect by each of the 58 coral species identified, and the analysis used Bray-Curtis similarities on untransformed data to obtain the MDS of Fig. 6.5. There appears to be a strong change in community pattern between 1981 and 1983 (putatively linked to the 1982/3 El Niño) and this is confirmed by a 1-way ANOSIM test for these two years alone: $R = 0.43$ ($p < 0.1\%$). Note that, though not really designed for this situation, the test is perfectly valid in the face of much greater "variability" in 1983 than 1981; in fact it is mainly a change in variability rather than location in the MDS plot that distinguishes the 1981 and 1983 groups (a point returned to in Chapter 15). This is in contrast with the standard univariate ANOVA (or multivariate MANOVA) test, which will have no power to detect a variability change; indeed it is invalid without an assumption of approximately equal variances (or variance-covariance matrices) across the groups.

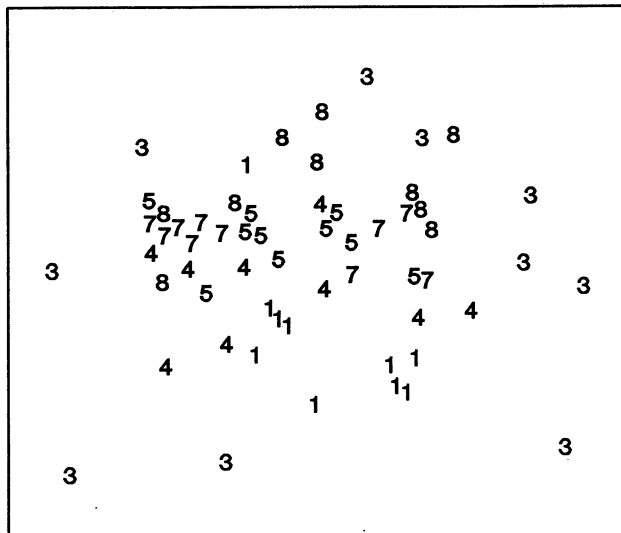


Fig. 6.5. Indonesian reef corals, S. Tikus Island (I). MDS of % species cover from 10 replicate transects in each of 6 years: 1 = 1981, 3 = 1983 etc (stress = 0.19).

The basic 1-way ANOSIM test can also be extended to cater (to some degree) for more complex sample designs, as follows.

ANOSIM FOR TWO-WAY LAYOUTS

Three types of field and laboratory designs are considered here:

- the *2-way nested* case can arise where two levels of spatial replication are involved, e.g. sites are grouped *a priori* to be representative of two "treatment" categories (control and polluted) but there are also replicate samples taken within sites;
- the *2-way crossed* case can arise from studying a fixed set of sites at several times (with replicates at each site/time combination), or from an experimental study in which the same set of "treatments" (e.g. control and impact) are applied at a number of locations ("blocks"), for example in the different mesocosm basins of a laboratory experiment;
- a 2-way crossed case *with no replication* of each treatment/block combination can also be catered for, to a limited extent, by a different style of permutation test.

The following examples of cases a) and b) are drawn from Clarke (1993) and the two examples of case c) are from Clarke and Warwick (1994).

EXAMPLE: Clyde nematodes (2-way nested case)

Lambshhead (1986) analysed meiobenthic communities from three putatively polluted (P) areas of the Firth of Clyde and three control (C) sites, taking three replicate samples at each site (with one exception). The resulting MDS, based on fourth-root transformed abundances of the 113 species in the 16 samples, is given in Fig. 6.6a. The sites are numbered 1 to 3 for both conditions but the numbering is arbitrary – there is nothing in common between P1 and C1 (say). This is what is meant by sites being "nested" within conditions. Two hypotheses are then appropriate:

H1: there are no differences among sites *within* each "treatment" (control or polluted conditions);

H2: there are no differences *between* control and polluted conditions.

The approach to H2 might depend on the outcome of testing H1.

H1 can be examined by extending the 1-way ANOSIM test to a *constrained* randomisation procedure. The presumption under H1 is that there may be a difference between general location of C and P samples in the MDS plots but within each condition there cannot be any pattern in allocation of replicates to the three sites. Treating the two conditions entirely separately, one therefore has two separate 1-way permutation analyses of exactly the same type as for the Frierfjord macrofauna data (Fig. 6.3). These generate test statistics R_C and R_P , computed from equation (6.1), which can be combined to produce an average statistic \bar{R} . This can be tested by comparing it with \bar{R} values from all possible permutations of sample labels permitted under the null hypothesis. This does not mean that all 16 sample labels may be arbitrarily permuted; the randomisation is constrained to take place only within the separate conditions: P and C labels may not be switched. Even so, the number of possible permutations is large (around 20,000). Notice again that the test is not restricted to balanced designs (although lack of balance causes a minor complication in the efficient averaging of R_C and R_P , see Clarke, 1988, 1993). Fig. 6.6b displays the results of 999 simulations (constrained relabellings) from the permutation distribution for \bar{R} under the null hypothesis H1. Possible values range from -0.3 to 0.6, though 95% of the values are <0.27 and 99% are <0.46. The true \bar{R} of 0.75 thus provides a strongly significant rejection of H1.

H2, which will usually be the more interesting of the two hypotheses, can now be examined. The test of H1

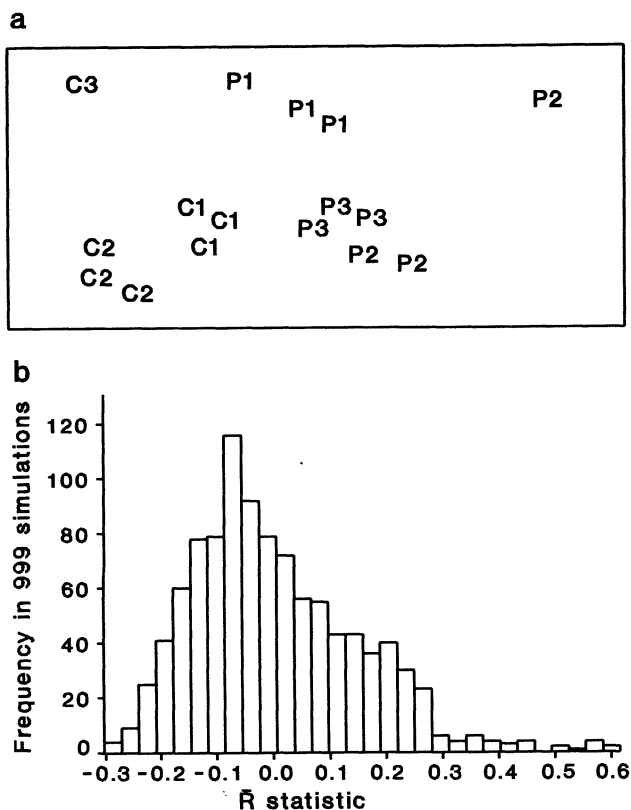


Fig. 6.6. *Clyde nematodes* (Y). a) MDS of species abundances from three 'polluted' (P1-P3) and three 'control' sites (C1-C3), with three replicate samples at most sites (stress = 0.09). b) Simulated distribution of the test statistic \bar{R} , under the hypothesis H1 of 'no site differences' within each condition; the observed \bar{R} is 0.75.

demonstrated that there are, in effect, only three "replicates" (the sites 1-3) at each of the two conditions (C and P). This is a 1-way layout, and H2 can be tested by 1-way ANOSIM but one first needs to combine the information from the three original replicates at each site, to define a similarity matrix for the 6 "new" replicates. Consistent with the overall strategy that tests should only be dependent on the rank similarities in the original triangular matrix, one first averages over the appropriate ranks to obtain a reduced matrix. For example, the similarity between the three P1 and three P2 replicates is defined as the average of the nine inter-group rank similarities; this is placed into the new similarity matrix along with the 14 other averages (C1 with C2, P1 with C1 etc.) and all 15 values are then re-ranked; the 1-way ANOSIM then gives $R = 0.74$. There are only 10 distinct permutations so that, although this is actually the most extreme R value possible, H2 is only able to be rejected at a $p < 10\%$ significance level.

The other scenario to consider is that the first test fails to reject H1; there are then two possibilities for examining H2:

- Proceed with the average ranking and re-ranking exactly as above, on the assumption that even if it cannot be *proved* that there are no differences between sites it would be unwise to *assume* that this is so; the test may have had rather little power to detect such a difference.
- Infer from the test of H1 that there *are* no differences between sites, and treat all replicates as if they were separate sites, e.g. there would be 7 replicates for control and 9 replicates for polluted conditions in Fig. 6.6a.

Which of these two courses to take is a matter for debate, and the argument here is exactly that of whether "to pool" or "not to pool" in forming the residual for the analogous univariate 2-way ANOVA. Option b) will certainly have greater power but runs a real risk of being invalid; option a) is the conservative test and it is certainly unwise to design a study with anything other than option a) in mind.²

EXAMPLE: Eaglehawk Neck meiofauna (2-way crossed case)

An example of a two-way crossed design is given in Warwick *et al.* (1990a) and is introduced more fully here in Chapter 12. This is a so-called *natural experiment*, studying disturbance effects on meiobenthic communities by the continual reworking of sediment by soldier crabs. Two replicate samples were taken from each of four disturbed patches of sediment, and from adjacent undisturbed areas, on a sand flat at Eaglehawk Neck, Tasmania; Fig. 6.7a is a schematic representation of the 16 sample locations. There are two factors: the presence or absence of disturbance by the crabs and the "block effect" of the four different disturbance patches. It might be anticipated that the community will change naturally across the sand flat, from block to block, and it is important to be able to separate this effect from any changes associated with the disturbance itself. There are parallels here with impact studies in which pollutants affect sections of several bays so that matched control and polluted conditions can be compared against a background of changing community pattern across a wide spatial scale. There are presumed to be replicate samples from

2. The ANOSIM program in the PRIMER package always takes this first option.

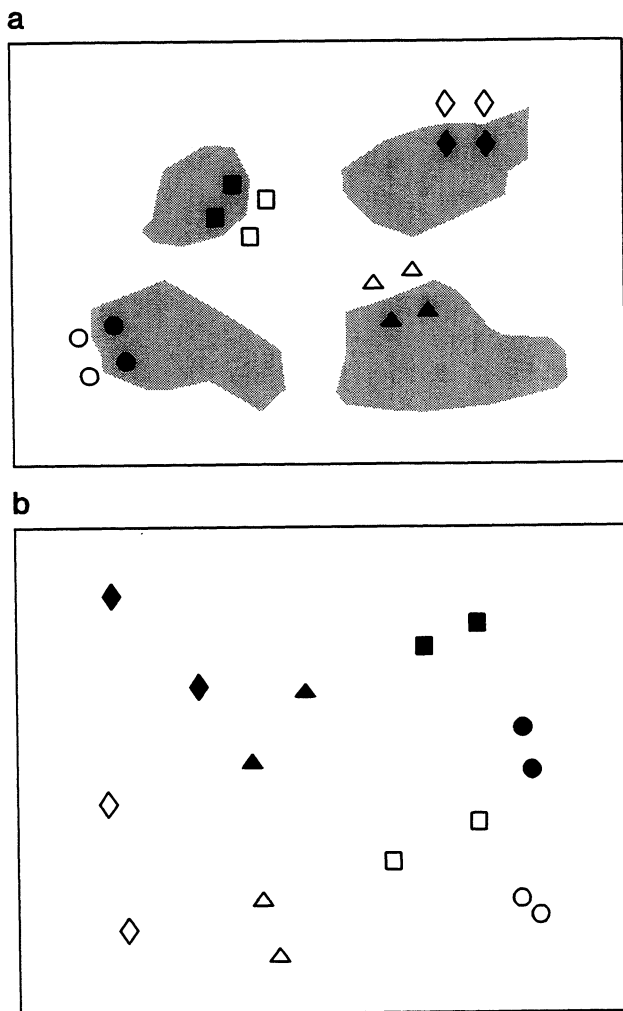


Fig. 6.7. Tasmania, Eaglehawk Neck (T). a) Schematic of the '2-way crossed' sampling design for 16 meiofaunal cores with two disturbed and two undisturbed replicates from each of four patches of burrowing activity by soldier crabs (shaded). b) MDS of species abundances for the 16 samples, showing separation of the blocks on the x-axis and discrimination of disturbed from undisturbed communities on the y-axis (stress = 0.11).

each treatment/block combination (the meaning of the term *crossed*), though balanced numbers are not essential.

For the Eaglehawk Neck data, Fig. 6.7b displays the MDS for the 16 samples (2 treatments \times 4 blocks \times 2 replicates), based on Bray-Curtis similarities from root transformed abundances of 59 meiofaunal species. The pattern is remarkably clear and a classic analogue of what, in univariate two-way ANOVA, would be called an *additive* model. The meiobenthic community is seen to change from area to area across the sand flat but also appears to differ consistently between disturbed and undisturbed conditions. A test for the latter sets up a null hypothesis that there are no disturbance effects, *allowing* for the fact that there may

be block effects, and the procedure is then exactly that of the 2-way ANOSIM test for hypothesis H1 of the nested case. For each *separate* block an R statistic is calculated from equation (6.1), as if for a simple one-way test for a disturbance effect, and the resulting values averaged to give \bar{R} . Its permutation distribution under the null hypothesis is generated by examining all simultaneous re-orderings of the four labels (two disturbed, two undisturbed) *within* each block. There are only three distinct permutations in each block, giving a total of $3^4 = 81$ combinations overall and the observed value of \bar{R} (= 0.94) is the highest value attained in the 81 permutations. The null hypothesis is therefore rejected at a significance level of just over 1%.

The procedure departs from the nested case because of the symmetry in the crossed design. One can now test the null hypothesis that there are no block effects, allowing for the fact that there *are* treatment (disturbance) differences, by simply reversing the roles of treatments and blocks. \bar{R} is now an average of two R statistics, separately calculated for disturbed and undisturbed samples, and there are $8!/(2!)^4! = 105$ permutations of the 8 labels for each treatment. A random selection from the $105^2 = 11,025$ possible combinations must therefore be made. In 1000 simulations the true value of \bar{R} (= 0.85) is again the most extreme and is almost certainly the largest in the full set; the null hypothesis is decisively rejected. In this case the test is inherently uninteresting but in other situations (e.g. a sites \times times study) tests for both factors could be of practical importance.

EXAMPLE: Mesocosm experiment (2-way crossed case with no replication)

Although the above test may still function if a few random cells in the 2-way layout have only a single replicate, its success depends on reasonable levels of replication overall to generate sufficient permutations. A commonly arising situation in practice, however, is where the 2-way design includes no replication at all.³ Typically this could be a sites \times times field study (see next section) but it may also occur in experimental work: an example is given by Austen and Warwick (in press) of a laboratory mesocosm study in which a complex array of treatments was applied to soft-sediment cores taken from a single, intertidal location in the Westerschelde estuary, Netherlands. A total of 64

3. As noted earlier, this case is not covered by the PRIMER ANOSIM program; it uses a separate routine, ANOSIM2.

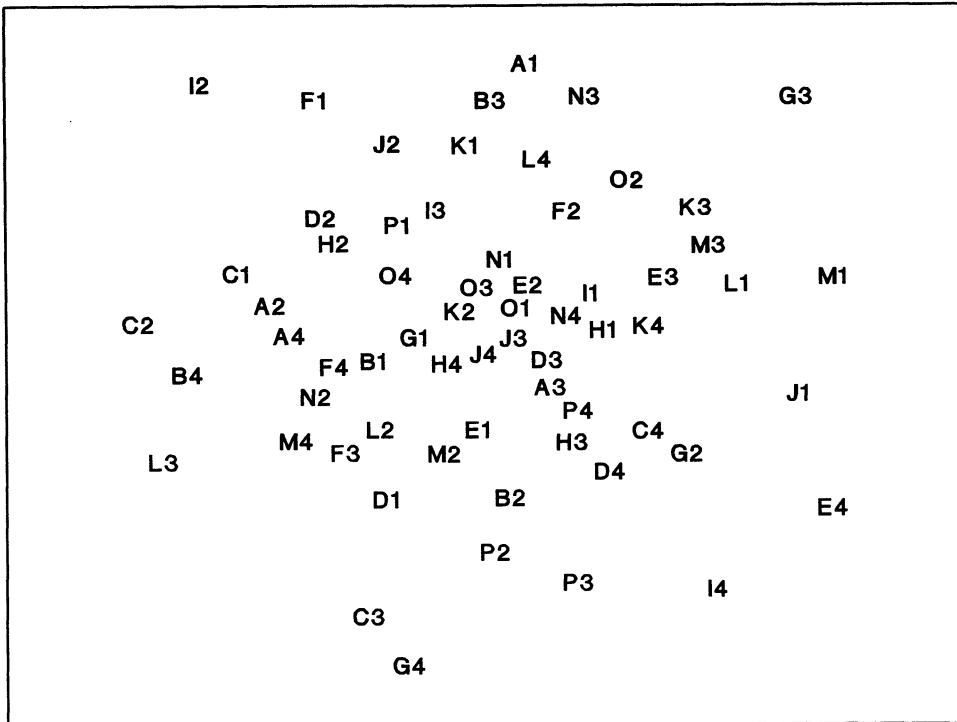


Fig. 6.8. *Westerschelde nematodes experiment (W)*. MDS of species abundances from 16 different nutrient-enrichment treatments, A to P, applied to sediment cores in each of four mesocosm basins, 1 to 4 (stress = 0.28).

cores were randomly divided between 4 mesocosm basins, 16 to a basin. The experiment involved 15 different nutrient enrichment conditions and one control, the treatments being applied to the surface of the undisturbed sediment cores. After 16 weeks controlled exposure in the mesocosm environment, the meiofaunal communities in the 64 cores were identified, and Bray-Curtis similarities on root-transformed abundances gave the MDS of Fig. 6.8. The full set of 16 treatments is repeated in each of the 4 basins (blocks), so the structure is a 2-way treatments \times blocks layout with only one replicate per cell. Little, if any, of this structure is apparent from Fig. 6.8 and a formal test of the null hypothesis

H_0 : there are no treatment differences (but allowing the possibility of basin effects)

is clearly necessary before any interpretation is attempted.

In the absence of replication, a test is still possible in the *univariate* case, under the assumption that interaction effects are small in relation to the main treatment or block differences (Scheffé, 1959). In a similar spirit, a *global* test of H_0 is possible here, relying on the observation that *if* certain treatments are responsible for community changes, in a more-or-less consistent way across blocks, *separate* MDS analyses for each block should show a repeated treatment pattern. This is illustrated schematically in the top half of Fig. 6.9: the fact that treatment A is consistently close to B (and C to D) can only arise if H_0 is false. The analogy with the

univariate test is clear: large interaction effects imply that the treatment pattern differs from block to block and there is little chance of identifying a treatment effect; on the other hand, for a treatment \times block design such as the current mesocosm experiment there is no reason to expect treatments to behave very differently in the different basins.

What is therefore required is a measure of how well the treatment patterns in the ordinations for the different blocks match; this statistic can then be recomputed under all possible (or a random subset of) permutations of the treatment labels within each block. As previously, if the observed statistic does not fall within the body of this (simulated) distribution there is significant evidence to reject H_0 . Note that, as required by the statement of H_0 , the test makes no assumption about the absence of block effects; between-block similarities are irrelevant to a statistic based only on agreement in within-block patterns.

In fact, for the same reasons advanced for the previous ANOSIM tests (e.g. arbitrariness in choice of MDS dimensionality), it is more satisfactory to define agreement between treatment patterns by reference to the underlying similarity matrix and not the MDS locations. Fig. 6.9 indicates two routes, which lead to equivalent formulations. If there are n treatments and thus $N = n(n-1)/2$ similarities within a block, a natural choice for agreement of two blocks j and k is the Spearman correlation coefficient

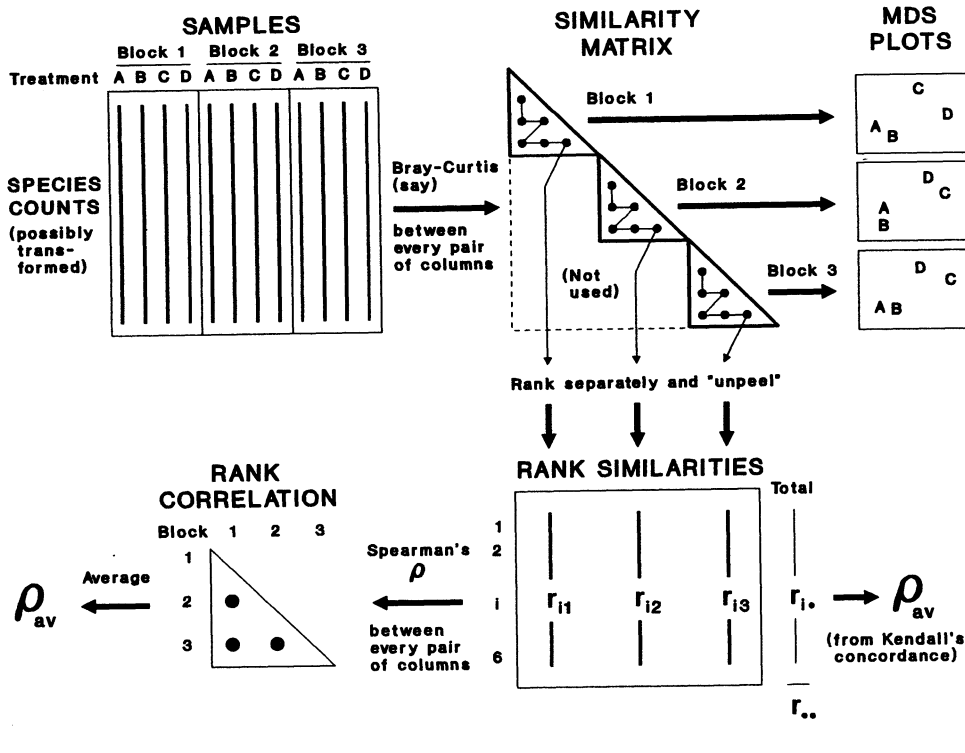


Fig. 6.9. Schematic diagram illustrating the stages in defining concordance of treatment patterns across the blocks, and the two computational routes for ρ_{av} .

$$\rho_{jk} = 1 - \frac{6}{N(N^2-1)} \sum_{i=1}^N (r_{ij} - r_{ik})^2 \quad (6.3)$$

between the matching elements of the two rank similarity matrices $\{r_{ij}, r_{ik}; i=1, \dots, N\}$, since these ranks are the only information used in successful MDS plots. The coefficients can be averaged across all $b(b-1)/2$ pairs from the b blocks, to obtain an overall measure of agreement ρ_{av} on which to base the test. A short cut is to define, from the row totals $\{r_{i.}\}$ and grand total $r_{..}$ shown in Fig. 6.9, Kendall's (1970) coefficient of concordance between the full set of ranks:

$$W = \frac{12}{b^2N(N^2-1)} \sum_{i=1}^N (r_{i.} - \frac{r_{..}}{N})^2 \quad (6.4)$$

and then exploit the known relationship between this and ρ_{av} :

$$\rho_{av} = (bW - 1) / (b - 1) \quad (6.5)$$

As a correlation coefficient, ρ_{av} takes values in the range $(-1, 1)$, with $\rho_{av} = 1$ implying perfect agreement and $\rho_{av} \approx 0$ if the null hypothesis H_0 is true.

Note that standard significance tests and confidence intervals for ρ or W (e.g. as given in basic statistical tables) are totally invalid, since they rely on the ranks $\{r_{ij}; i=1, \dots, N\}$ being from independent variables; this is obviously not true of similarity coefficients calculated from all possible pairs of a set of (independent) samples. This does not make ρ_{av} any the less appropriate as a measure of agreement whose

departure from zero (rejection of H_0) can be tested by permutation.

For the nutrient enrichment experiment, Fig. 6.10 shows the separate MDS plots for the 4 mesocosm basins. Although the stress values are rather high (and the plots therefore slightly unreliable as a summary of the among treatment relationships), there appears to be no commonality of pattern, and this is borne out by a near zero value for ρ_{av} of -0.03 . This is fairly central to the range of simulated values for ρ_{av} under H_0 (obtained by permuting treatment labels separately for each block and recomputing ρ_{av}), so the test provides no evidence of any treatment differences. Note that the symmetry of the 2-way layout also allows a test of the (less interesting) hypothesis that there are no block effects, by looking for any consistency in the among-basin relationships across separate analyses for each of the 16 treatments. The test is again non-significant, with $\rho_{av} = -0.02$. The overall negative conclusion to the tests should bar any further attempts at interpretation of these data.

EXAMPLE: Exe nematodes (no replication and missing data)

A final example demonstrates a positive outcome to such a test, in a commonly occurring case of a 2-way layout of sites and times with the additional feature that observations are *missing* altogether from a small number of the cells. Fig. 6.11 shows again the MDS of

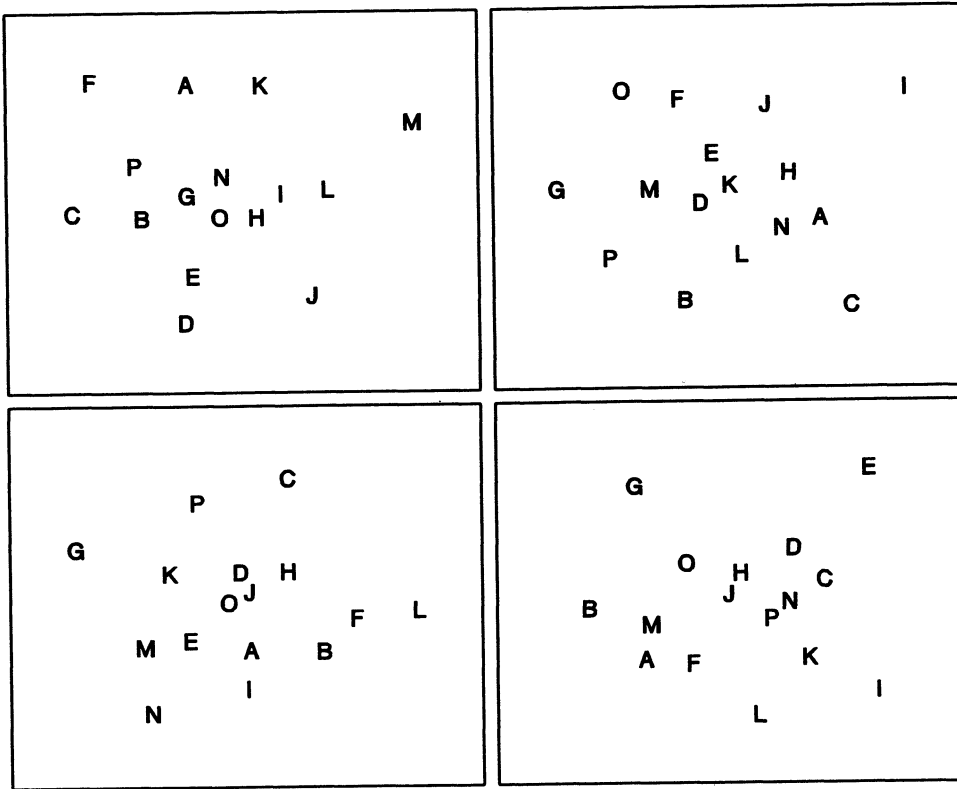


Fig. 6.10. Westerschelde nematodes experiment (W). MDS for the 16 treatments (A to P), performed separately for each of the four basins; no shared treatment pattern is apparent (stress ranges from 0.16 to 0.20).

nematode communities at 19 sites in the Exe estuary, seen in Chapter 5. In fact, this is based on an average of data over 6 successive bi-monthly sampling occasions. For the individual times, the samples remain strongly clustered into the 4 or 5 main groups apparent from Fig. 6.11. Less clear, however, is whether any structure exists within the largest group (sites 12 to 19) or whether the scatter in Fig. 6.11 is simply the consequence of sampling variation.

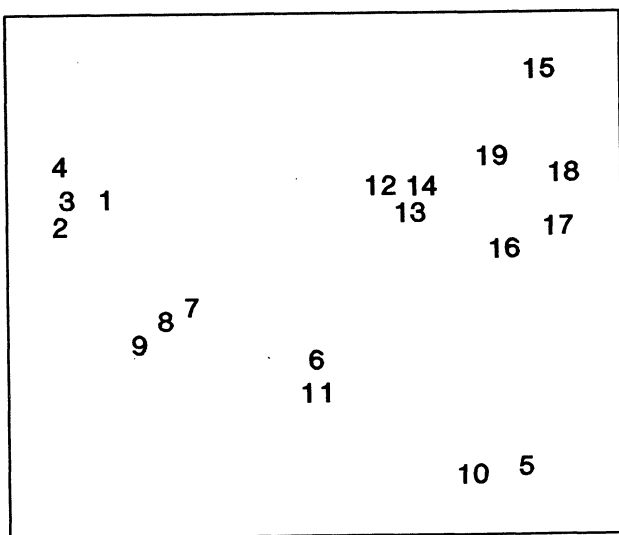


Fig. 6.11. Exe estuary nematodes (X). MDS, for 19 inter-tidal sites, of species abundances averaged over 6 bi-monthly sampling occasions; see also Fig. 5.1 (stress = 0.05).

Rejection of the null hypothesis of "no site to site differences" would be suggested by a common site pattern in the separate MDS plots for the 6 times (Fig. 6.12). At some of the times, however, one of the site samples is missing (site 19 at times 1 and 2, site 15 at time 4 and site 18 at time 6). Instead of removing these sites from *all* plots, in order to achieve matching sets of similarities, one can remove for each *pair* of times only those sites missing for either of that pair, and compute the Spearman correlation ρ between the remaining rank similarities. The ρ values for all pairs of times are then averaged to give ρ_{av} , i.e. the left-hand route is taken in the lower half of Fig. 6.9. This is usually referred to as *pairwise removal* of missing data, in contrast to the *listwise removal* that would be needed for the right-hand route. Though increasing the computation time, pairwise removal clearly utilises more of the available information.

Figure 6.12 shows evidence of a consistent site pattern, for example in the proximity of sites 12 to 14 and the tendency of site 15 to be placed on its own; the fact that site 15 is missing on one occasion does not undermine this perceived structure. Pairwise computation gives $\rho_{av} = 0.36$ and its significance can be determined by a Monte Carlo test, as before. The (non-missing) site labels are permuted amongst the available samples, separately for each time, and these designations fixed whilst all the paired ρ values are computed (using pairwise removal) and averaged. Here, the largest

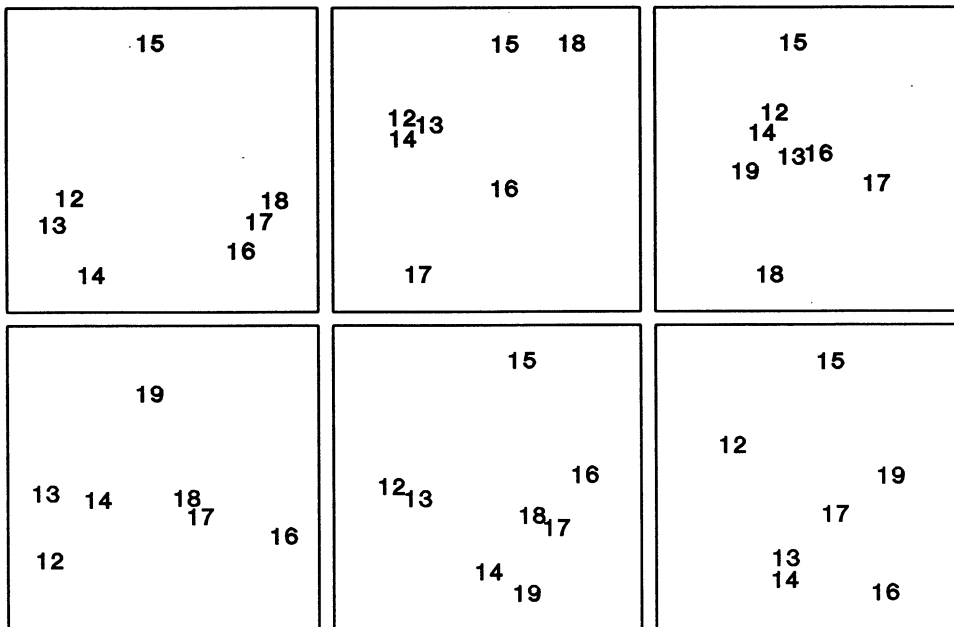


Fig. 6.12. *Exe estuary nematodes (X)*. MDS for sites 12 to 19 only, performed separately for the 6 sampling times (read across rows for time order); in spite of the occasional missing sample some commonality of site pattern is apparent (stress ranges from 0.01 to 0.08).

such ρ_{av} value in 999 simulations was 0.30, so the null hypothesis is rejected at the $p < 0.1\%$ level. In the same way, one can also carry out a test of the hypothesis that there are no differences across *time* for sites 12 to 19. The component plots of the 4 to 6 times for each site display no obvious features and $\rho_{av} = 0.08$ ($p < 18\%$). (The failure to reject this null hypothesis justifies, to some extent, the use of averaged data across the 6 times, in the earlier analyses).

Tests of this form, searching for agreement between two or more similarity matrices, occur also in Chapter 11 (in the context of matching species to environmental data) and Chapter 15 (where they link biotic patterns to some model structure). The discussion there includes use of measures other than a simple Spearman coefficient, for example a weighted Spearman coefficient ρ_w (preferred for reasons explained in Chapter 11), and these adjustments could certainly be implemented here also if desired, using the left-hand route in the lower half of Fig. 6.9. In the present context, this type of "matching" test is clearly an inferior one to that possible where genuine replication exists within the 2-way layout. It cannot cope with follow-up tests for differences between specific pairs of treatments, and it can have little sensitivity if the numbers of treatments and blocks are both small. A test for two treatments is impossible note, since the treatment pattern in all blocks would be identical, by definition.

RECOMMENDATIONS

- 1) For typical species abundance matrices, use an ANOSIM-type permutation test in preference to a classical MANOVA procedure; the latter will almost always be invalid.
- 2) Choice of the level and type of replication should be carefully considered. Though it is difficult to define power for any of the ANOSIM tests, it is clearly important to take sufficient replicates to generate a large enough set of permutations for meaningful significance levels (e.g. a test for two groups, each of three replicates, can only ever attain 10% significance!). Equally important is that replicates should *genuinely* represent the condition being sampled: *pseudo-replication* (Hurlbert, 1984) is commonplace, e.g. analyses of sub-cores of single cores, or sets of spatially contiguous samples which are unrepresentative of the extent of a site. For pseudo-replicates in a 2-way layout, the *only* valid course is to average them and carry out the above global test for the case of "no replication".
- 3) A point that cannot be over-stressed is that the ANOSIM tests only apply to groups of samples specified *prior* to seeing (or collecting) the data. A dangerous misconception is that one can use a cluster analysis of the species abundance data to define sample groupings, whose statistical validity can be established by performing an ANOSIM test for differences between these groups. *This is entirely erroneous*, the argument being completely circular.

CHAPTER 7: SPECIES ANALYSIS

SPECIES CLUSTERING AND MDS

Chapter 2 (page 2-6) describes how the original data matrix can be used to define similarities between every pair of species; two species are thought of as "similar" if their numbers (or biomass) tend to fluctuate in parallel across sites. The resulting *species similarity matrix* can be input to a cluster analysis or ordination in exactly the same way as for *sample similarities*.¹

Fig. 7.1 displays the results of a cluster analysis on Exe estuary nematode data {X}, extensively illustrated in Chapter 5. The dendrogram is based on Bray-Curtis similarities computed on standardised abundances, as given in equations (2.9) and (2.10). Following the recommendations on page 2-6, the number of species was first reduced, retaining only those that accounted for more than 4% of the total abundance at any one site. Cluster analysis with a greater number of species is possible but the "hit-and-miss" occurrence of the

rarer species across the sites tends to confuse the picture. In fact, at a similarity of around 10%, the dendrogram divides fairly neatly into 5 clusters of species, and these groups can be identified with the 5 clusters that emerge from the sample dendrogram, Fig. 5.3. (This identification comes simply from categorising the species by the site groups in which they have the greatest abundance; the correspondence between site and species groupings on this basis is seen to be very close.)

Fig 7.2 shows the 2-dimensional MDS plot of the same species similarities. The groups determined from the cluster analysis are superimposed and indicate a good measure of agreement. However, both clustering and MDS have worked well here because the sites are strongly grouped, with many species characteristic of only one site group. Typically, species cluster analyses are less clearly delineated than this and the corresponding MDS ordinations have high stress. A more informative approach is often to concentrate on the *sample similarities* and highlight the species principally responsible for determining the *sample groupings* in the cluster or ordination analyses.

1. Computation of species similarities is an option available in the PRIMER program CLUSTER, and is referred to as *inverse analysis* by Field et al. (1982).

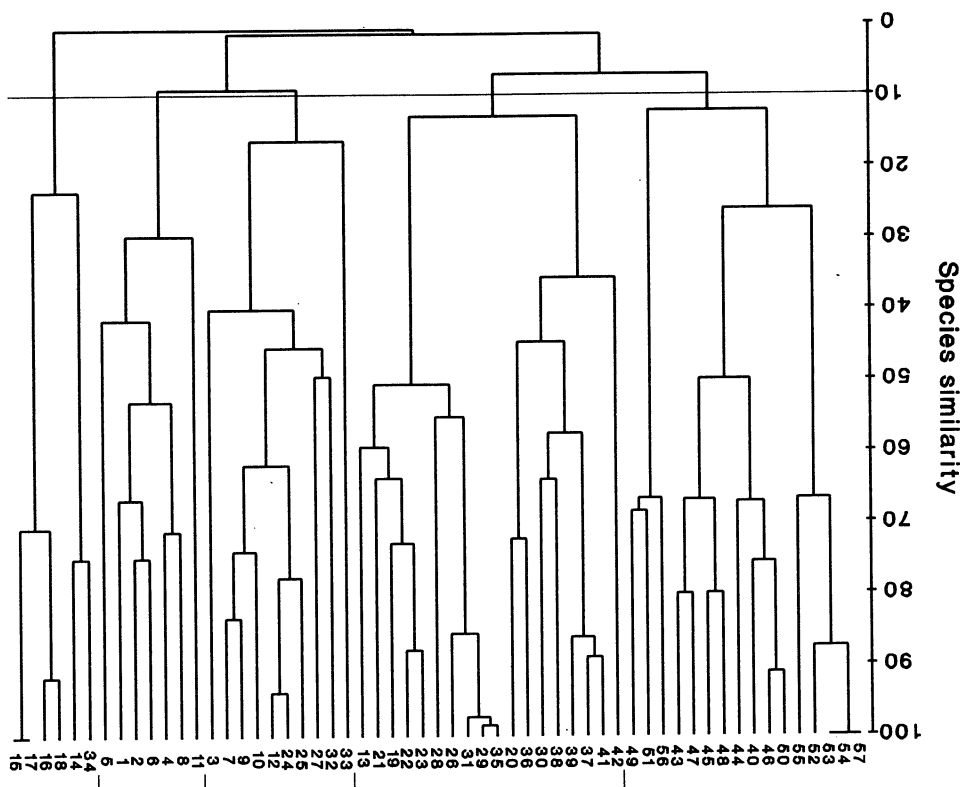


Fig. 7.1. Exe estuary nematodes {X}. Dendrogram using group-average linking on Bray-Curtis species similarities from standardized abundance data; the 57 most important species were retained from an original list of 182. The 5 groups defined at arbitrary similarity level of 10% are indicated.

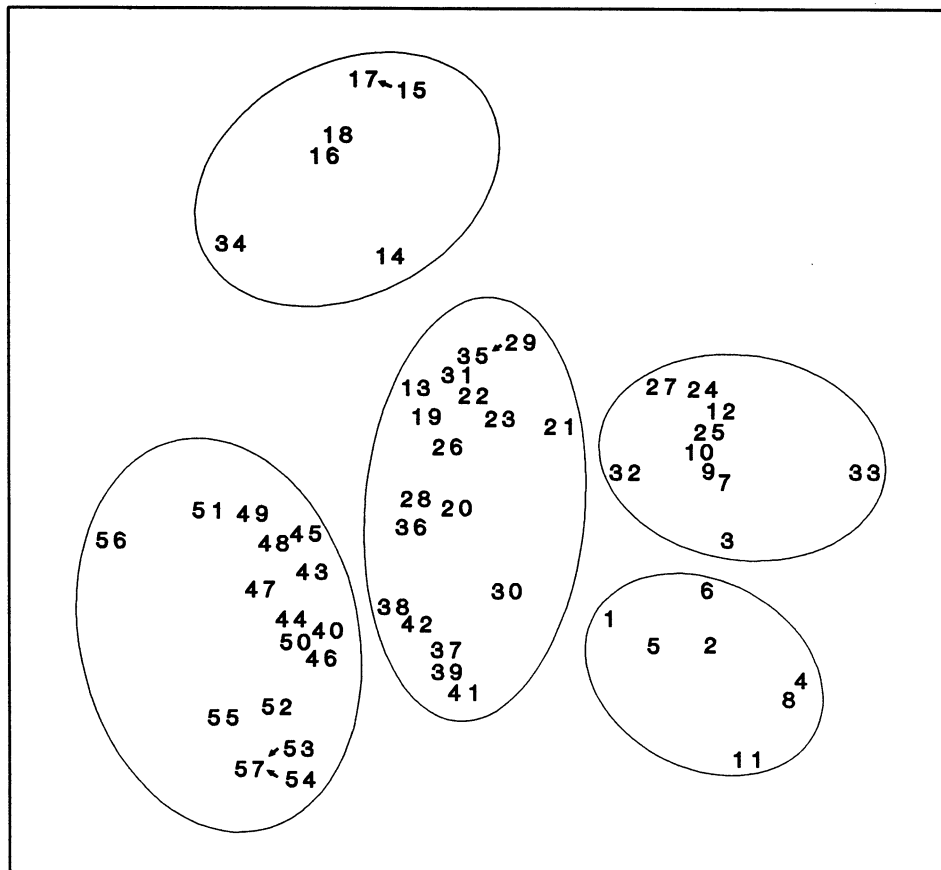


Fig. 7.2. Exe estuary nematodes (X). 2-dimensional MDS of the species similarity matrix, as in Fig. 7.1. The 5 groupings from the cluster analysis are indicated (stress = 0.08).

DETERMINING DISCRIMINATING SPECIES

Shade matrix

With a wide range of sophisticated multivariate techniques at one's disposal, it is all too easy to lose sight of the original data. A full understanding requires the data matrix to be re-examined *in the light* of the multivariate results. In its original form, it can be difficult to trace patterns in the data matrix (indeed, this is the rationale for multivariate analysis in the first place) but a simple *re-ordering of columns* (samples) and *rows* (species) can be an effective way of displaying groupings or gradual changes in species composition.

This is illustrated by the data on Bristol Channel zooplankton {B} met in Chapter 3. The 57 sites are re-ordered according to the groupings in the dendrogram (Fig. 3.3), and the 24 species re-ordered according to their approximate placement across the

species MDS.² The entries in the matrix are *categorized* into seven abundance classes here, approximately to a linear scale for $\sqrt{\sqrt{\cdot}}$ -transformed counts (the transformation used for the multivariate analyses). The increasing abundance classes are denoted by symbols of increasing size and intensity, and the resulting re-ordered data matrix is displayed in Fig. 7.3. The effect is to concentrate the higher abundances in the diagonal region of the matrix, and the representation of abundance by increasing density gives rise to the term *shade matrix* for such plots. It is then relatively easy to identify a number of species which have characteristically different abundance levels between (say) sample groups 1 and 2 (eg species 6, 1, 4, 23, 18, 3).

2. There are more formal ways of re-ordering rows and columns simultaneously in an attempt to achieve some optimal degree of diagonalisation of the matrix but the additional effort does not really seem worthwhile here (though it may be with more extensive data sets). All that is required is a restatement of the matrix in a form in which it is easy to trace the patterns of individual species counts across groups, and a re-arrangement based on perusal of both sample and species cluster and MDS diagrams seems adequate.

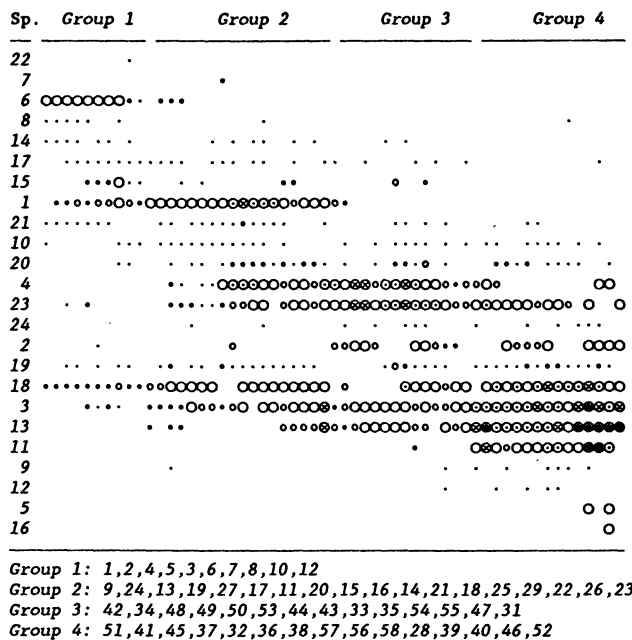


Fig. 7.3. Bristol Channel zooplankton (B). Shade matrix for the 24 species and 57 sites. The original abundances have been categorized and represented by symbols of increasing density, and the rows and columns of the array re-ordered on the basis of cluster and MDS analyses of the sites and species.

Similarity breakdown

An alternative, more analytical way of achieving the same characterisation is to compute the *average dissimilarity* $\bar{\delta}$ between *all* pairs of inter-group samples (i.e. every sample in group 1 paired with every sample in group 2, say) and then break this average down into the separate *contributions from each species* to $\bar{\delta}$.³

For Bray–Curtis dissimilarity δ_{jk} between two samples j and k , the contribution from the i th species, $\delta_{jk}(i)$, could simply be defined as the i th term in the summation of equation (2.11), namely:

$$\delta_{jk}(i) = 100 \cdot |y_{ij} - y_{ik}| / \sum_{i=1}^p (y_{ij} + y_{ik}) \quad (7.1)$$

$\delta_{jk}(i)$ is then averaged over all pairs (j, k), with j in the first and k in the second group, to give the *average contribution* $\bar{\delta}_i$ from the i th species to the overall

3. This is implemented in the PRIMER program SIMPER ("similarity percentages"), both in respect of contributions to average similarity within a group and average dissimilarity between groups.

dissimilarity $\bar{\delta}$ between groups 1 and 2.⁴ Typically, there are many pairs of samples (j, k) making up the average $\bar{\delta}_i$, and a useful measure of how *consistently* a species contributes to $\bar{\delta}_i$ across all such pairs is the *standard deviation* $SD(\delta_i)$ of the $\delta_{jk}(i)$ values.⁵ If $\bar{\delta}_i$ is large and $SD(\delta_i)$ small (and thus the ratio $\bar{\delta}_i/SD(\delta_i)$ is large), then the i th species not only contributes much to the dissimilarity between groups 1 and 2 but it also does so consistently in inter-comparisons of all samples in the two groups; it is thus a good *discriminating species*.

Table 7.1. Bristol Channel zooplankton (B). Breakdown of average dissimilarity between groups 1 and 2 into contributions from each species; species are ordered in decreasing contribution (part only given).

| Sp. Name | $\bar{\delta}_i$ | $SD(\delta_i)$ | $\bar{\delta}_i/SD(\delta_i)$ | $\Sigma \bar{\delta}_i\%$ |
|----------------------------|------------------|----------------|-------------------------------|---------------------------|
| 6 Eurytemora affinis | 7.7 | 2.8 | 2.7* | 13.0 |
| 4 Centropages hamatus | 7.3 | 4.4 | 1.7* | 25.2 |
| 3 Calanus helgolandicus | 6.8 | 4.0 | 1.7* | 36.7 |
| 1 Acartia bifilosa | 5.7 | 4.0 | 1.4* | 46.3 |
| 23 Temora longicornis | 5.6 | 3.3 | 1.7* | 55.6 |
| 18 Pseudocalanus elongatus | 4.7 | 1.5 | 3.1* | 63.5 |
| 13 Paracalanus parvus | 3.3 | 4.2 | 0.8 | 69.1 |
| 15 Pleuorbrachia pileus jv | 3.1 | 2.8 | 1.1 | 74.3 |
| 20 Sagitta elegans jv | 2.9 | 1.9 | 1.6* | 79.1 |
| 19 Sagitta elegans | 2.1 | 1.6 | 1.3 | 82.5 |
| 8 Gastrosaccus spinifer | 2.0 | 1.8 | 1.1 | 85.9 |
| 14 Pleuorbrachia pileus | 1.9 | 1.6 | 1.2 | 89.0 |
| 10 Mesopodopsis slabberi | 1.7 | 1.4 | 1.3 | 91.9 |
| 21 Schistomysis spiritus | 1.6 | 1.4 | 1.1 | 94.5 |
| 17 Polychaete larvae | 1.5 | 1.3 | 1.2 | 97.1 |
| 2 Acartia clausi | 0.7 | 1.8 | 0.4 | 98.3 |
| | ... | ... | ... | |

For the Bristol Channel zooplankton data (B) of Fig. 7.3, Table 7.1 shows the results of breaking down the dissimilarities between sample groups 1 and 2 into species contributions. Species are ordered by their average contribution $\bar{\delta}_i$ to the total average dissimilarity $\bar{\delta} = \Sigma \bar{\delta}_i = 59.5$. Species which are likely to

4. Though this is a natural definition, it should be noted that there is no unambiguous partition of δ_{jk} into contributions from each species, since the standardising term in the denominator of equation (7.1) is a function of all species values.

5. The usual definition of standard deviation from elementary statistics is a convenient measure of variability here, but there is no sense in which the $\delta_{jk}(i)$ values are "independent observations", and one cannot use standard statistical inference to define, say, "95% confidence intervals" for the mean contribution from the i th species.

be good discriminators of groups 1 and 2 are indicated by an asterisk in the $\bar{\delta}_i/SD(\delta_i)$ column. The final column rescales the first column to percentages, i.e. it computes the % of the total dissimilarity $\bar{\delta}$ that is contributed by the i th species, and then cumulates these percentages down the rows of the table. It can be seen that many of the species play some part in determining the dissimilarity between groups 1 and 2, and this is typical of such analyses. Here, nearly 90% of the contribution to $\bar{\delta}$ is accounted for by the first twelve species listed, with over 50% accounted for by the first five. The results are in good agreement, of course, with the pattern observed in the shade matrix of Fig. 7.3.

In much the same way, though perhaps with less practical significance, one can examine the contribution each species makes to the average *similarity within* a group, \bar{S} . The average contribution of the i th species, \bar{S}_i , could be defined by taking the average, over all pairs of samples *within* a group, of the i th term in the similarity definition of equation (2.1) (the second form). The more abundant a species is within a group the more it will contribute to the *intra*-group similarities. It *typifies* that group if it is found at a consistent abundance throughout, so the standard deviation of its contribution $SD(S_i)$ is low, and the ratio $\bar{S}_i/SD(S_i)$ high. Note that this says nothing about whether that species is a good *discriminator* of one group from another; it may be very *typical* of a number of communities.

Such a breakdown is shown for group 1 of the Bristol Channel zooplankton data in Table 7.2. The average similarity within the group is $\bar{S} = 66.3$, with more than two-thirds of this contributed by only three species (6,

18 & 1), the first two of which are found at very consistent levels within the group.

Table 7.2. Bristol Channel zooplankton (B). Breakdown of average similarity within group 1 into contributions from each species (part only given).

| Sp. Name | \bar{S}_i | $SD(S_i)$ | $\bar{S}_i/SD(S_i)$ | $\Sigma \bar{S}_i\%$ |
|-----------------------------------|-------------|-----------|---------------------|----------------------|
| 6 <i>Eurytemora affinis</i> | 19.3 | 6.3 | 3.1* | 29.1 |
| 18 <i>Pseudocalanus elongatus</i> | 14.7 | 2.7 | 5.4* | 51.3 |
| 1 <i>Acartia bifilosa</i> | 12.2 | 6.4 | 1.9* | 69.6 |
| 17 <i>Polychaete larvae</i> | 3.9 | 3.1 | 1.2 | 75.5 |
| 14 <i>Pleuorbrachia pileus</i> | 3.4 | 3.8 | 0.9 | 80.7 |
| 21 <i>Schistomysis spiritus</i> | 3.3 | 3.6 | 0.9 | 85.7 |
| 15 <i>Pleuorbrachia pileus jv</i> | 3.3 | 4.7 | 0.7 | 90.7 |
| | ... | ... | ... | |

RECOMMENDATION

A multivariate display of the samples, either by ordination or clustering, is not the end-point of a community analysis. The species determining the observed sample patterns should be identified either by returning to the data matrix itself in the form of a shade matrix, or in the case of a convincing clustering of samples, by some partitioning of the contributions from each species to the separation of pairs of clusters. Note that this is not a statistical testing framework, just an *exploratory* analysis. It indicates which species are principally responsible *either* for an observed clustering pattern *or* for differences between sets of samples that have been defined *a priori* and are confirmed to differ in community structure by the tests of Chapter 6.

CHAPTER 8: DIVERSITY MEASURES, DOMINANCE CURVES AND OTHER GRAPHICAL ANALYSES

UNIVARIATE MEASURES

A variety of different indices (single numbers) can be used as measures of some attribute of community structure in a sample. These include the total number of individuals (N), total number of species (S), the total biomass (B), and also ratios such as B/N (the average size of an organism in the sample) and N/S (the average number of individuals per species). These indices tend to be less informative than some measure of the way in which the total number of individuals is divided up among the different species, i.e. *diversity indices*.

Indices of diversity and evenness

A single index of species (or higher taxon) diversity is commonly employed in community studies, and is amenable to simple statistical analysis. A bewildering variety of diversity indices has been used, and it is not appropriate here to discuss their relative merits and disadvantages. Good accounts can be found in Heip *et al.* (1988)¹ and Magurran (1991).

Two different aspects of community structure contribute to the concept of community diversity:

- Species richness.** This is a measure related to the total number of species present. Obviously we would consider a sample containing more species than another to be the more diverse.
- Equitability.** This expresses how evenly the individuals are distributed among the different species, and is often termed *evenness*. For example, if two samples each comprising 100 individuals and four species had species abundances of 25, 25, 25, 25 and 97, 1, 1, 1, we would intuitively consider the former to be more diverse although the species richness is the same. The former has high evenness, but low *dominance* (essentially the reverse of evenness), while the latter has low evenness and high dominance (the sample being highly dominated by one species).

Different diversity indices may emphasize the species richness or equitability components of diversity to varying degrees. Several of these indices are included as special cases in a unified series of diversity numbers of different orders proposed by Hill (1973b).² However, these numbers do not as yet seem to have been widely adopted. The most commonly used diversity measure is the *Shannon–Wiener diversity index*:

$$H' = - \sum_i p_i (\log p_i) \quad (8.1)$$

where p_i is the proportion of the total count (or biomass etc) arising from the i th species.

This incorporates both the species richness and equitability components. Note that logarithms to the base 2 are often used in the calculation, giving the diversity units as 'bits per individual'. \log_e is also frequently used, so when comparing published indices it is important to check that the same logarithm base has been used in each case.

Species richness

Species richness is often given simply as the total number of species (S), which is obviously very dependent on sample size (the bigger the sample, the more species there are likely to be). More commonly *Margalef's index* (d) is used, which also incorporates the total number of individuals (N) and is a measure of the number of species present for a given number of individuals:

$$d = (S-1) / \log N \quad (8.2)$$

Equitability

This is most commonly expressed as *Pielou's evenness index*:

$$J' = H'(\text{observed}) / H'_{\max} \quad (8.3)$$

where H'_{\max} is the maximum possible diversity which would be achieved if all species were equally abundant (= $\log S$).

1. Although this book relates specifically to the meiobenthos, the treatment of statistical methods is applicable to all community studies.

2. The PRIMER program DIVERSE permits selection from a dozen or so indices, including Hill numbers and the other richness and equitability measures given here.

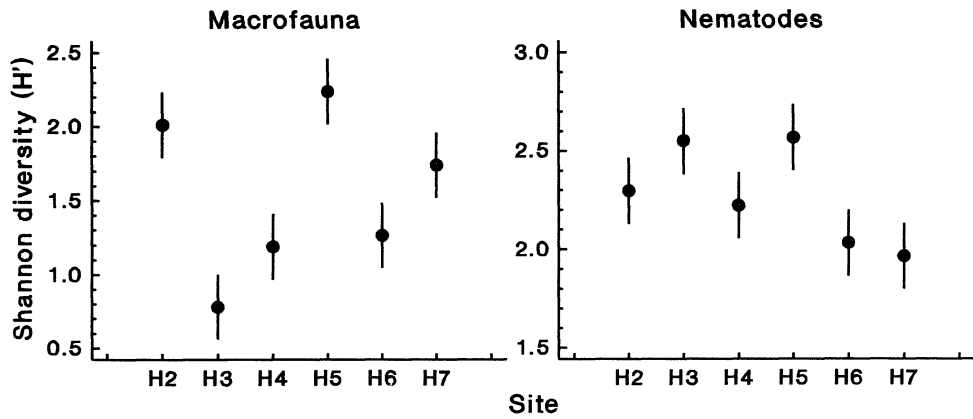


Fig. 8.1. Hamilton Harbour, Bermuda (H). Diversity (H') and 95% confidence intervals for macrobenthos (left) and meiobenthic nematodes (right) at six stations.

Units of measurement

The numbers of individuals belonging to each species are the most common units used in the calculation of the above indices. For internal comparative purposes other units can be used, e.g biomass or total cover of each species along a transect or in quadrats (e.g. for hard-bottom epifauna), but obviously diversity measures using different units are not comparable. Often, on hard bottoms where colonial encrusting organisms are difficult to enumerate, total or percentage cover will be much easier to determine than species abundances.

Representing communities

Changes in univariate indices between sites or over time are usually presented graphically simply as plots of means and confidence intervals for each site or time. For example, Fig. 8.1 graphs the differences in diversity of the macrobenthos and meiobenthic nematodes at six stations in Hamilton Harbour, Bermuda, showing that there are clear differences in diversity between sites for the former but much less obvious differences for the latter. Fig 8.2 graphs the

temporal changes in three univariate indices for reef corals at South Tikus Island, Indonesia, spanning the period of the 1982-3 El Nino (an abnormally long period of high water temperatures which caused extensive coral bleaching in many areas throughout the Pacific). Note the dramatic decline between 1981 and 1983 and subsequent partial recovery in both the number of species (S) and the Shannon diversity (H'), but no obvious changes in evenness (J').

Discriminating sites or times

The significance of differences in univariate indices between sampling sites or times can simply be tested by one-way analysis of variance (ANOVA), followed by t -tests or (preferably) multiple comparison tests for individual pairs of sites³; see the discussion at the start of Chapter 6.

3. These tools are routinely available in all basic statistics packages and are not therefore replicated in PRIMER; the DIVERSE program simply outputs the chosen suite of indices for each sample in a format that can be readily converted (e.g. using the CONFMT routine) for input to many such packages.

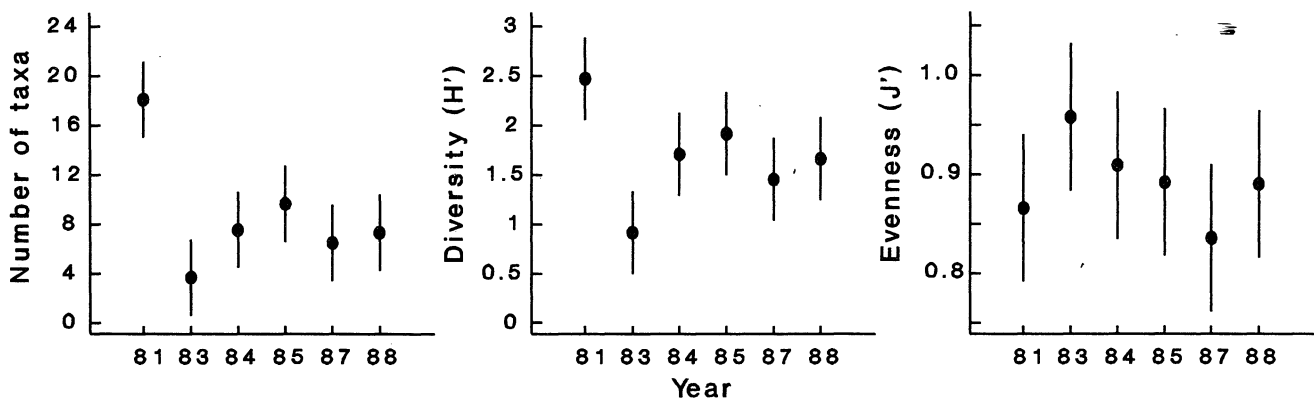


Fig. 8.2. Indonesian reef corals, South Tikus Island (I). Total number of species (S), Diversity (H') and Evenness (J') based on coral species cover data along transects, spanning the 1982-3 El Nino.

Determining stress levels

Increasing levels of environmental stress have generally been considered to *decrease* diversity (e.g. H'), *decrease* species richness (e.g. d) and *decrease* evenness (e.g. J'), i.e. *increase* dominance. This interpretation may, however, be an over-simplification of the situation. More recent theories on the influence of disturbance or stress on diversity suggest that in situations where disturbance is minimal, species diversity is reduced because of competitive exclusion between species; with a slightly increased level or frequency of disturbance competition is relaxed, resulting in an increased diversity, and then at still higher or more frequent levels of disturbance species start to become eliminated by stress, so that diversity falls again. Thus it is at intermediate levels of disturbance that diversity is highest (Connell, 1978; Huston, 1979). Therefore, depending on the starting point of the community in relation to existing stress levels, increasing levels of stress (e.g. induced by pollution) may either result in an increase or decrease in diversity. It is difficult, if not impossible, to say at what point on this continuum the community under investigation exists, or what value of diversity one might expect at that site if the community were not subjected to any anthropogenic stress. Thus, changes in diversity can only be assessed by *comparisons* between stations along a spatial contamination gradient (e.g. Fig. 8.1) or with historical data (Fig. 8.2).

Caswell's neutral model

The equitability component of diversity can, however, be compared with some theoretical expectation of diversity, given the number of individuals and species present. Observed diversity has been compared with predictions from *Caswell's neutral model* (Caswell, 1976). This model constructs an ecologically 'neutral' community with the same number of species and individuals as the observed community, assuming certain community assembly rules (random births/deaths and random immigrations/emigrations) and no interactions between species. The deviation statistic V is then determined which compares the observed diversity (H') with that predicted from the neutral model ($E(H')$):

$$V = \frac{[H' - E(H')]}{S.D. (H')} \quad (8.4)$$

A value of zero for the V statistic indicates neutrality, positive values indicate greater diversity than predicted and negative values lower diversity. Values

>+2 or <-2 indicate significant departures from neutrality. The computer program of Goldman & Lambshead (1989) is useful.⁴

Table 8.1 gives the V statistics for the macrobenthos and nematode component of the meiobenthos from Hamilton Harbour, Bermuda (c.f. Fig. 8.1). Note that the diversity of the macrobenthos at stations H4 and H3 is significantly below neutral model predictions, but the nematodes are close to neutrality at all stations. This indicates that the macrobenthic communities are under some kind of stress at these two stations. However, it must be borne in mind that deviation in H' from the neutral model prediction depends only on differences in equitability, since the species richness is fixed, and that the equitability component of diversity may behave differently from the species richness component in response to stress (see, for example, Fig. 8.2). Also, it is quite possible that the 'intermediate disturbance hypothesis' will have a bearing on the behaviour of V in response to disturbance, and increased disturbance may either cause it to decrease or increase. Using this method, Caswell found that the flora of tropical rain forests had a diversity below neutral model predictions!

Table 8.1. Hamilton Harbour, Bermuda (H). V statistics for summed replicates of macrobenthos and meiobenthic nematode samples at six stations.

| Station | Macrobenthos | Nematodes |
|---------|--------------|-----------|
| H2 | +0.5 | -0.1 |
| H3 | -5.4 | +0.4 |
| H4 | -4.5 | -0.5 |
| H5 | -1.9 | 0.0 |
| H6 | -1.3 | -0.4 |
| H7 | -0.2 | -0.4 |

GRAPHICAL/DISTRIBUTION PLOTS

The purpose of graphical/distributional representations is to extract information on patterns of relative species abundances without reducing that information to a single summary statistic, such as a diversity index. This class of techniques can be thought of as intermediate between *univariate* summaries and full *multivariate* analyses. Unlike multivariate methods, these distributions may extract universal features of community structure which are not a function of the

4. This is implemented in the PRIMER program CASWELL.

specific taxa present, and may therefore be related to levels of biological 'stress'.⁵

- 1) **Rarefaction curves** (Sanders, 1968) were among the earliest to be used in marine studies. They are plots of the number of individuals on the x -axis against the number of species on the y -axis. The more diverse the community is, the steeper and more elevated is the rarefaction curve. The sample sizes (N) may differ widely between stations, but the relevant sections of the curves can still be compared.
- 2) Gray and Pearson (1982) recommend plotting the number of species in $\times 2$ **geometric abundance classes** as a means of detecting the effects of pollution stress. These are plots of the number of species represented by only 1 individual in the sample (class 1), 2–3 individuals (class 2), 4–7 (class 3), 8–15 (class 4) etc. In unpolluted situations there are many rare species and the curve is smooth with its mode well to the left. In polluted situations there are fewer rare species and more abundant species so that the higher geometric abundance classes are more strongly represented, and the curve may also become more irregular or 'jagged' (although this latter feature is more difficult to quantify). Gray and Pearson further suggest that it is the species in the intermediate abundance classes 3 to 5 that are the most sensitive to pollution induced changes and might best illustrate the differences between polluted and unpolluted sites (i.e. this is a way of selecting 'indicator species' objectively).
- 3) **Ranked species abundance (dominance) curves** are based on the ranking of species (or higher taxa) in decreasing order of their importance in terms of abundance or biomass. The ranked abundances, expressed as a percentage of the total abundance of all species, are plotted against the relevant species rank. Log transformations of one or both axes have frequently been used to emphasise or downweight different sections of the curves. Logging the x (rank) axis enables the distribution of the commoner species to be better visualised.

5. Two plotting programs of this type are available in the PRIMER package: GEOPLOT produces a frequency distribution of geometric abundance classes and DOMPLOT generates ranked species abundance (dominance) curves, choosing from ordinary, cumulative or partial forms, abundance-biomass comparisons (ABC), transformation of the y -axis, computation of the W statistic etc., as illustrated in the remainder of this chapter.

- 4) **k -dominance curves** are cumulative ranked abundances plotted against species rank, or log species rank (Lambshhead *et al.*, 1983). This has a smoothing effect on the curves. Ordering of curves on a plot will obviously be the reverse of rarefaction curves, with the most elevated curve having the lowest diversity. To compare dominance separately from the number of species, the x -axis (species rank) may be rescaled from 0–100 (relative species rank), to produce **Lorenz curves**.

EXAMPLES: Garroch Head and Ekofisk macrofauna

Plots of geometric abundance classes along a transect across the Garroch Head (G) sewage-sludge dump site (Fig. 8.3) are given in Fig. 8.4. Note that the curves are very steep at both ends of the transect (the relatively unpolluted stations) with many species represented by only one individual, and they extend across very few abundance classes (6 at station 1 and 3 at station 12). As the dump centre at station 6 is approached the curves become much flatter, extending over many more abundance classes (13 at station 7), and there are fewer rare species.

In Fig. 8.5a, average ranked species abundance curves (with the x -axis logged) are given for the macrobenthos at a group of 6 sampling stations within 250 m of the current centre of oil-drilling activity at the Ekofisk field in the North Sea (E), compared with a group of 10 stations between 250 m and 1 km from the centre (see inset map in Fig. 10.6a for locations of these stations). Note that the curve for the more polluted (inner) stations is J-shaped, showing high dominance of abundant species, whereas the curve for the less

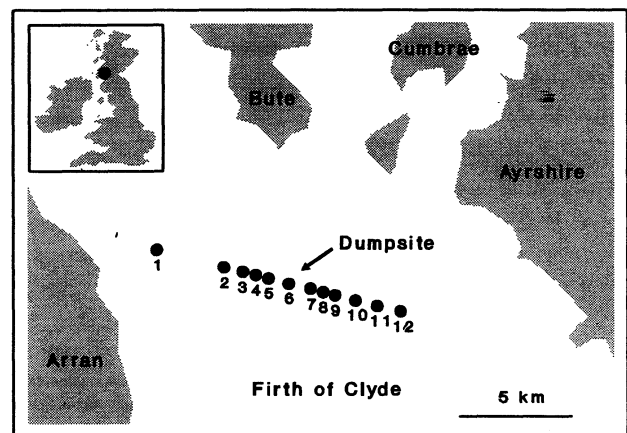


Fig. 8.3. Garroch Head macrofauna (G). Map showing location of dump ground and position of sampling stations (1–12); the dump centre is at station 6.

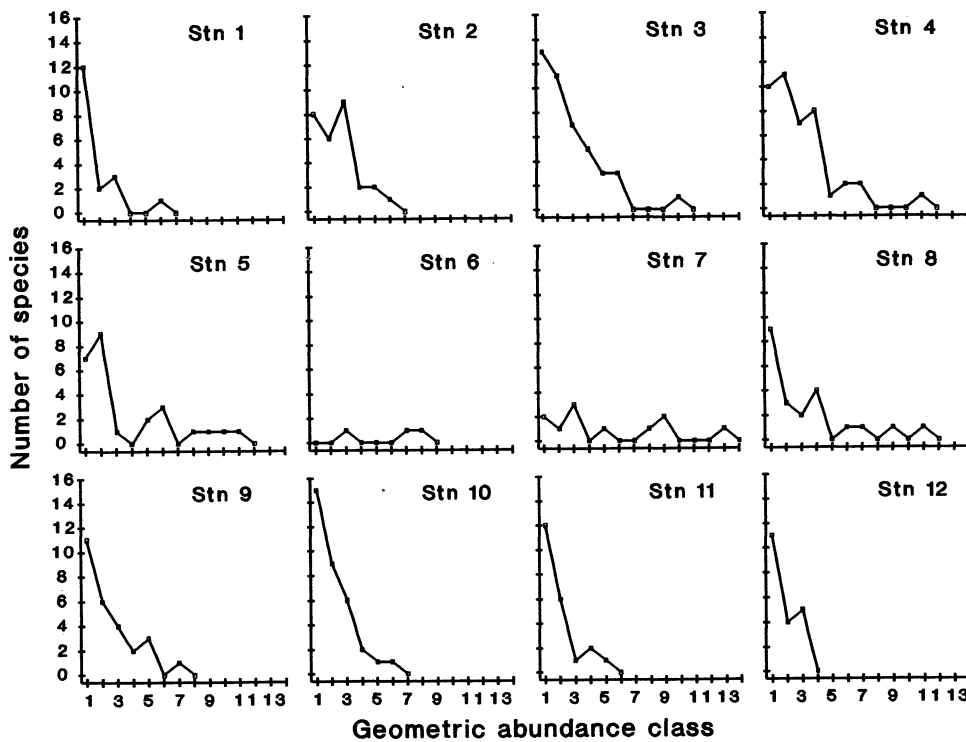


Fig. 8.4. Garroch Head macrofauna (G). Plots of $\times 2$ geometric species abundance classes for the 12 sampling stations shown in Fig. 8.3.

polluted (outer) stations is much flatter, with low dominance. Fig. 8.5b shows k -dominance curves for the same data. Here the curve for the inner stations is elevated, indicating lower diversity than at the 250 m–1 km stations.

Abundance/biomass comparison (ABC) plots

The advantage of distribution plots such as k -dominance curves is that the distribution of species abundances among individuals and the distribution of species biomasses among individuals can be compared on the same terms. Since the two have different units of measurement, this is not possible with diversity indices.

This is the basis of the *Abundance/Biomass Comparison (ABC)* method of determining levels of disturbance (pollution-induced or otherwise) on benthic macrofauna communities. Under stable conditions of infrequent disturbance the competitive dominants in macrobenthic communities are K -selected or conservative species, with the attributes of large body size and long life-span: these are rarely dominant numerically but are dominant in terms of biomass. Also present in these communities are smaller r -selected or opportunistic species with a short life-span, which are usually numerically dominant but do not represent a large proportion of the community biomass. When pollution perturbs a community, conservative species are less favoured and opportunistic species often become the biomass dominants as well as the numerical dominants. Thus,

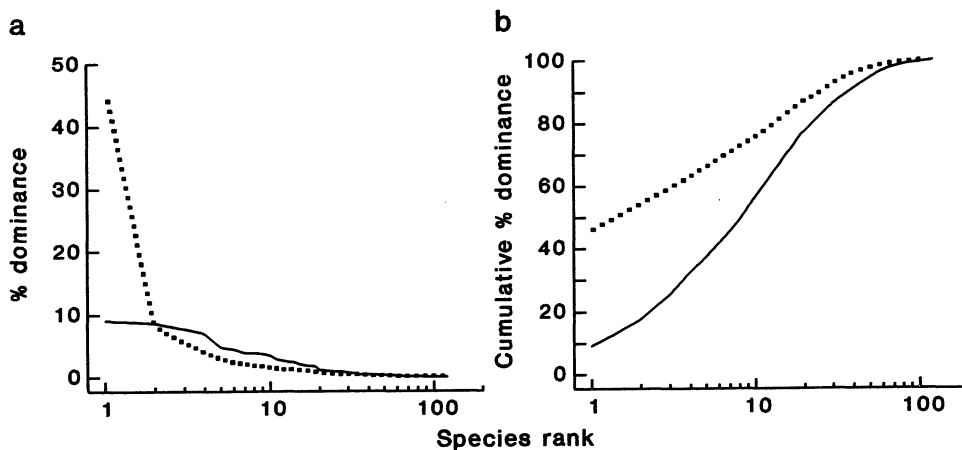


Fig. 8.5. Ekofisk macrobenthos (E). a) Average ranked species abundance curves (x -axis logged) for 6 stations within 250 m of the centre of drilling activity (dotted line) and 10 stations between 250 m and 1 km from the centre (solid line); b) k -dominance curves for the same groups of stations.

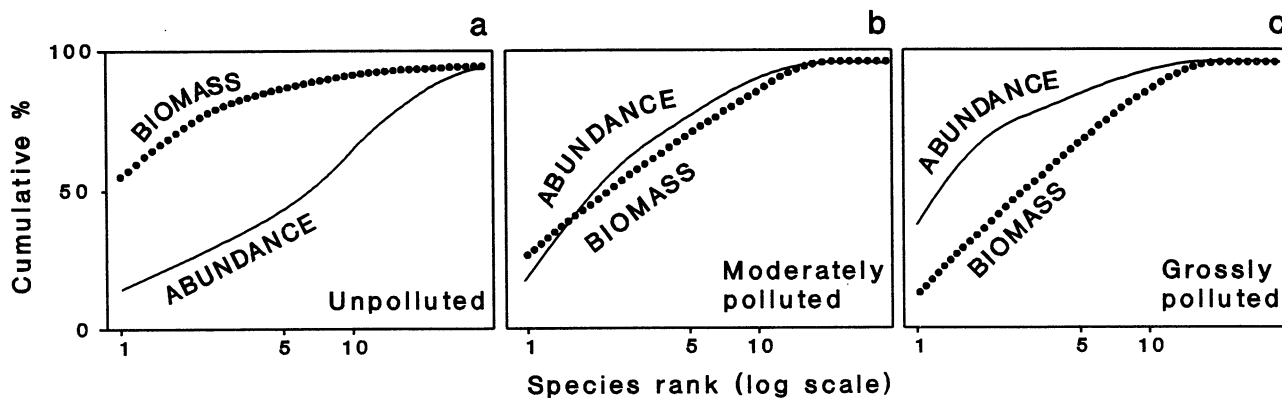


Fig. 8.6. Hypothetical *k*-dominance curves for species biomass and abundance, showing unpolluted, moderately polluted and grossly polluted conditions.

under pollution stress, the distribution of numbers of individuals among species behaves differently from the distribution of biomass among species. The ABC method, as originally described by Warwick (1986), involves the plotting of separate *k*-dominance curves (Lambhead *et al.*, 1983) for species abundances and species biomasses on the same graph and making a comparison of the forms of these curves. The species are ranked in order of importance in terms of abundance or biomass on the *x*-axis (logarithmic scale) with percentage dominance on the *y*-axis (cumulative scale). In undisturbed communities the biomass is dominated by one or a few large species, each represented by rather few individuals, whilst the numerical dominants are small species with a strong stochastic element in the determination of their abundance. The distribution of numbers of individuals among species is more even than the distribution of biomass, the latter showing strong dominance. Thus, the *k*-dominance curve for biomass lies above the curve for abundance for its entire length (Fig. 8.6a). Under moderate pollution, the large competitive dominants are eliminated and the inequality in size between the numerical and biomass dominants is reduced so that the biomass and abundance curves are closely coincident and may cross each other one or more times (Fig. 8.6b). As pollution becomes more severe, benthic communities become increasingly dominated by one or a few very small species and the abundance curve lies above the biomass curve throughout its length (Fig. 8.6c). These three conditions (unpolluted, moderately polluted and grossly polluted) should be recognisable in a community without reference to control samples in time or space, the two curves acting as an "internal control" against each other. Reference to control samples is, however, still desirable. Adequate replication of sampling is a prerequisite of the method,

since the large biomass dominants are often represented by few individuals, which will be liable to a higher sampling error than the numerical dominants.

EXAMPLES: Loch Linnhe and Garroch Head macrofauna

ABC curves for the macrobenthos at site 34 in Loch Linnhe, Scotland (*L*) between 1963 and 1973 are given in Fig. 8.7. The time course of organic pollution from a pulp-mill, and changes in species diversity, are shown top left. Moderate pollution commenced in 1966, and by 1968 species diversity was reduced. Prior to 1968 the ABC curves had the unpolluted configuration. From 1968 to 1970 the ABC plots indicated moderate pollution. In 1970 there was an increase in pollutant loadings and a further reduction in species diversity, reaching a minimum in 1972, and the ABC plots for 1971 and 1972 show the grossly polluted configuration. In 1972 pollution was decreased and by 1973 diversity had increased and the ABC plots again indicated the unpolluted condition. Thus, the ABC plots provide a good 'snapshot' of the pollution status of the benthic community in any one year, without reference to the historical comparative data which would be necessary if species diversity alone was used as the criterion.

ABC plots for the macrobenthos along a transect of stations across the accumulating sewage-sludge dumping ground at Garroch Head, Scotland (*G*) (Fig 8.3) are given in Fig. 8.8. Note how the ABC curves behave along the transect, with the peripheral stations 1 and 12 having unpolluted configurations, those near the dump-centre at station 6 with grossly polluted configurations and intermediate stations showing moderate pollution. Of course, at the dump-centre itself there are only three species present, so that any

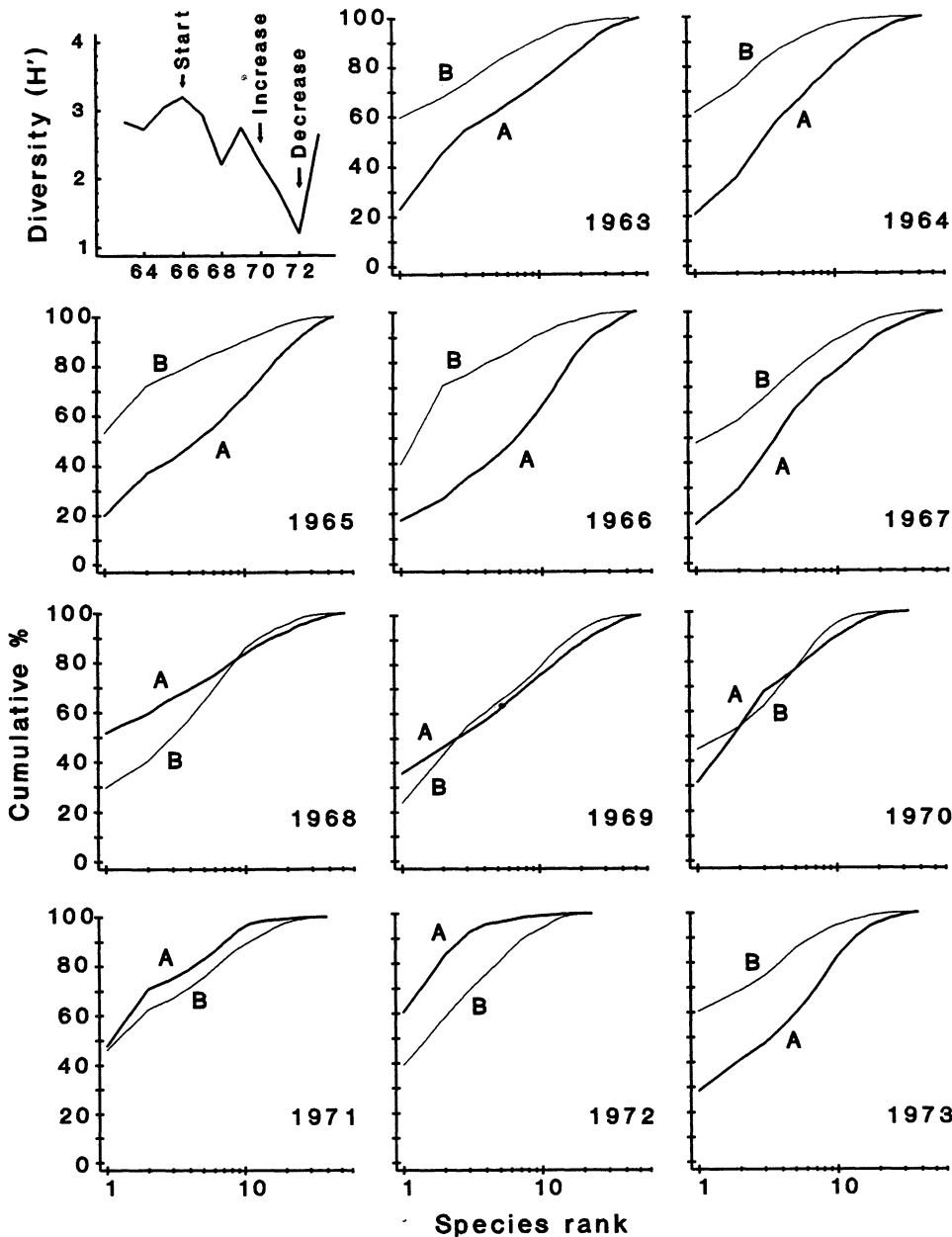


Fig. 8.7. Loch Linnhe macrofauna (L). Shannon diversity (H') and ABC plots over the 11 years, 1963 to 1973. Abundance = thick line, biomass = thin line.

method of data analysis would have indicated gross pollution. However, the biomass and abundance curves start to become transposed at some distance from the dump-centre, when species diversity is still high.

Transformations of k -dominance curves

Very often k -dominance curves approach a cumulative frequency of 100% for a large part of their length, and in highly dominated communities this may be after the first two or three top-ranked species. Thus, it may be difficult to distinguish between the forms of these curves. The solution to this problem is to transform the y -axis so that the cumulative values are

closer to linearity. Clarke (1990) suggests the *modified logistic* transformation:

$$y_i' = \log[(1 + y_i)/(101 - y_i)] \quad (8.5)$$

An example of the effect of this transformation on ABC curves is given in Fig. 8.9 for the macrofauna at two stations in Frierfjord, Norway (F), A being an unimpacted reference site and C a potentially impacted site. At site C there is an indication that the biomass and abundance curves cross at about the tenth species, but since both curves are close to 100% at this point, the crossover is unclear. The logistic transformation enables this crossover to be better visualised, and illustrates more clearly the differences in the ABC configurations between these two sites.

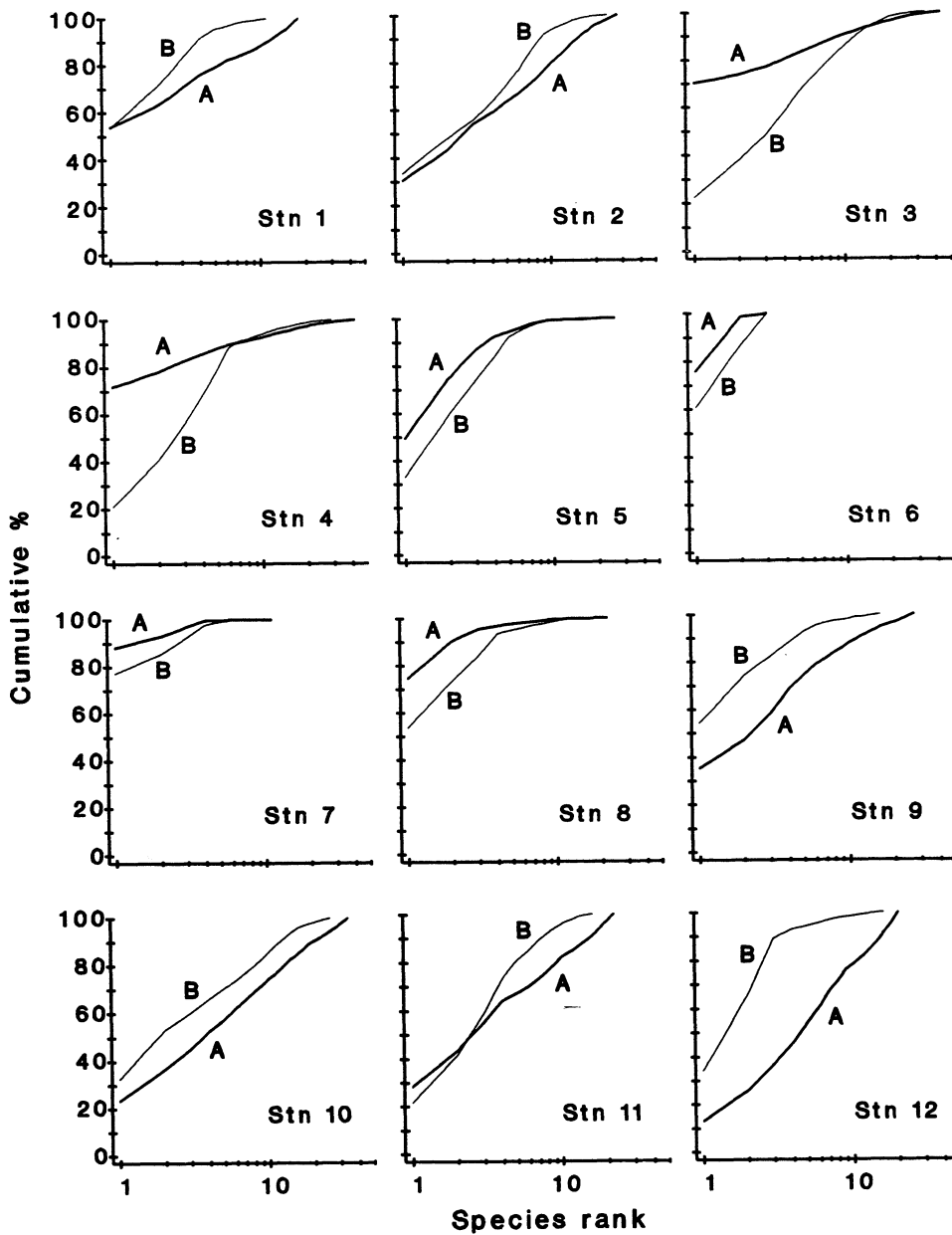


Fig. 8.8. Garroch Head macrofauna (G). ABC plots for macrobenthos in 1983. Abundance = thick line, biomass = thin line.

Partial dominance curves

A second problem with the cumulative nature of k -dominance (and ABC) curves is that the visual information presented is over-dependent on the single most dominant species. The unpredictable presence of large numbers of a species with small biomass, perhaps a heavy spatfall of the young of one species, may give a false impression of disturbance. With genuine disturbance, one might expect patterns of ABC curves to be unaffected by successive removal of the one or two most dominant species in terms of abundance or biomass, and so Clarke (1990) recommended the use of *partial dominance curves*, which compute the dominance of the second ranked species *over the remainder* (ignoring the first ranked species),

the same with the third most dominant etc. Thus if a_i is the absolute (or percentage) abundance of the i th species, when ranked in decreasing abundance order, the partial dominance curve is a plot of p_i against $\log i$ ($i = 1, 2, \dots, S-1$), where

$$p_1 = 100 a_1 / (\sum_{j=1}^S a_j), \quad p_2 = 100 a_2 / (\sum_{j=2}^S a_j), \dots$$

$$\dots, \quad p_{S-1} = 100 a_{S-1} / (a_{S-1} + a_S) \quad (8.6).$$

Earlier values can therefore never affect later points on the curve. The partial dominance curves (ABC) for undisturbed macrobenthic communities typically look like Fig. 8.10, with the biomass curve (dashed) above the abundance curve (solid) throughout its length. The abundance curve is much smoother than

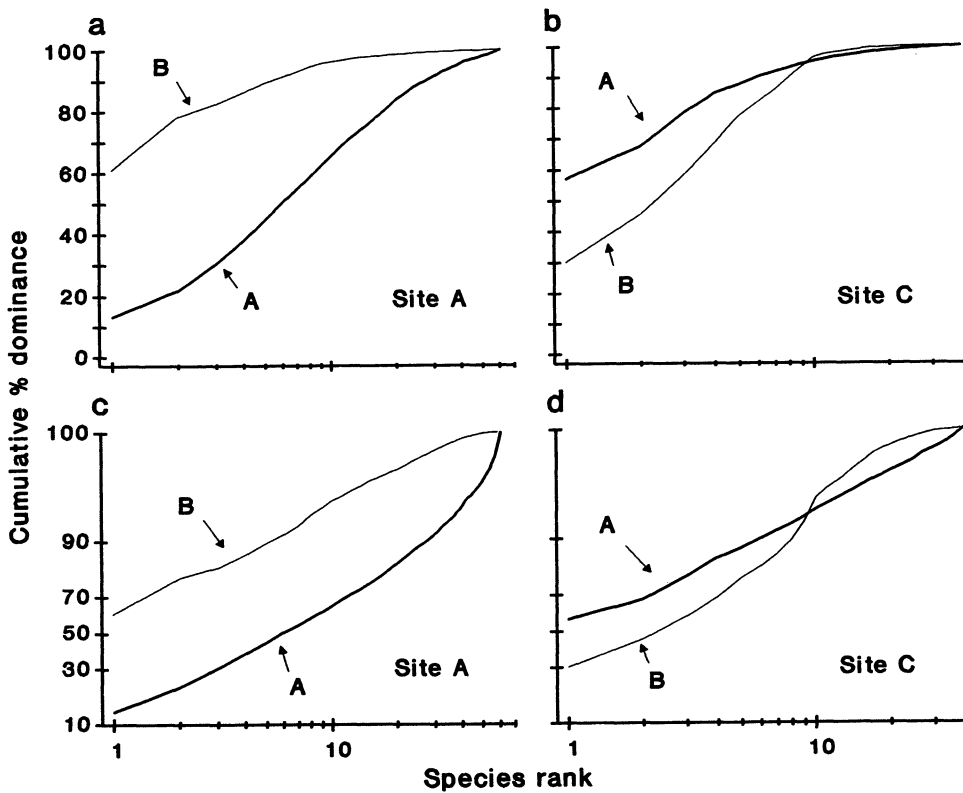


Fig. 8.9. Frierfjord macrofauna (F). a), b) Standard ABC plots for sites A (reference) and C (potentially impacted). c), d) ABC plots for sites A and C with the y-axis subjected to modified logistic transformation. Abundance = thick line, biomass = thin line.

the biomass curve, showing a slight and steady decline before the inevitable final rise.

Under polluted conditions there is still a change in position of partial dominance curves for abundance and biomass, with the abundance curve now above the biomass curve in places, and the abundance curve becoming much more variable. This implies that

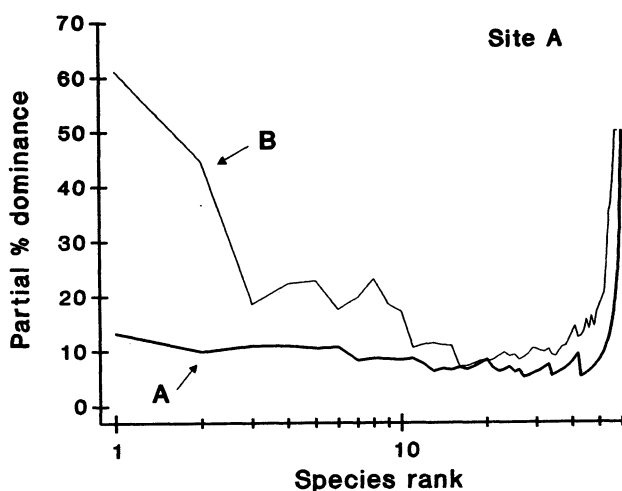


Fig. 8.10. Frierfjord macrofauna (F). Partial dominance curves (abundance/biomass comparison) for reference site A (c.f. Figs 8.9a and c for corresponding standard and transformed ABC plots).

pollution effects are not just seen in changes to a few dominant species but are a phenomenon which pervades the complete suite of species in the community. For example, the time series of macrobenthos data from Loch Linnhe (Fig. 8.11) shows that in the most polluted years 1971 and 1972 the abundance curve is above the biomass curve for most of its length (and the abundance curve is very atypically erratic), the curves cross over in the moderately polluted years 1968 and 1970 and have an unpolluted configuration prior to the pollution impact in 1966. In 1967, there is perhaps the suggestion of incipient change in the initial rise in the abundance curve. Although these curves are not so smooth (and therefore not so visually appealing!) as the original ABC curves, they may provide a useful alternative aid to interpretation and are certainly more robust to random fluctuations in the abundance of a small-sized, numerically dominant species.

Phyletic role in ABC method

Warwick and Clarke (1994) have recently shown that the ABC response results from (i) a shift in the proportions of different phyla present in communities, some phyla having larger-bodied species than others, and (ii) a shift in the relative distributions of abundance and biomass among species within the Polychaeta but not within any of the other major phyla

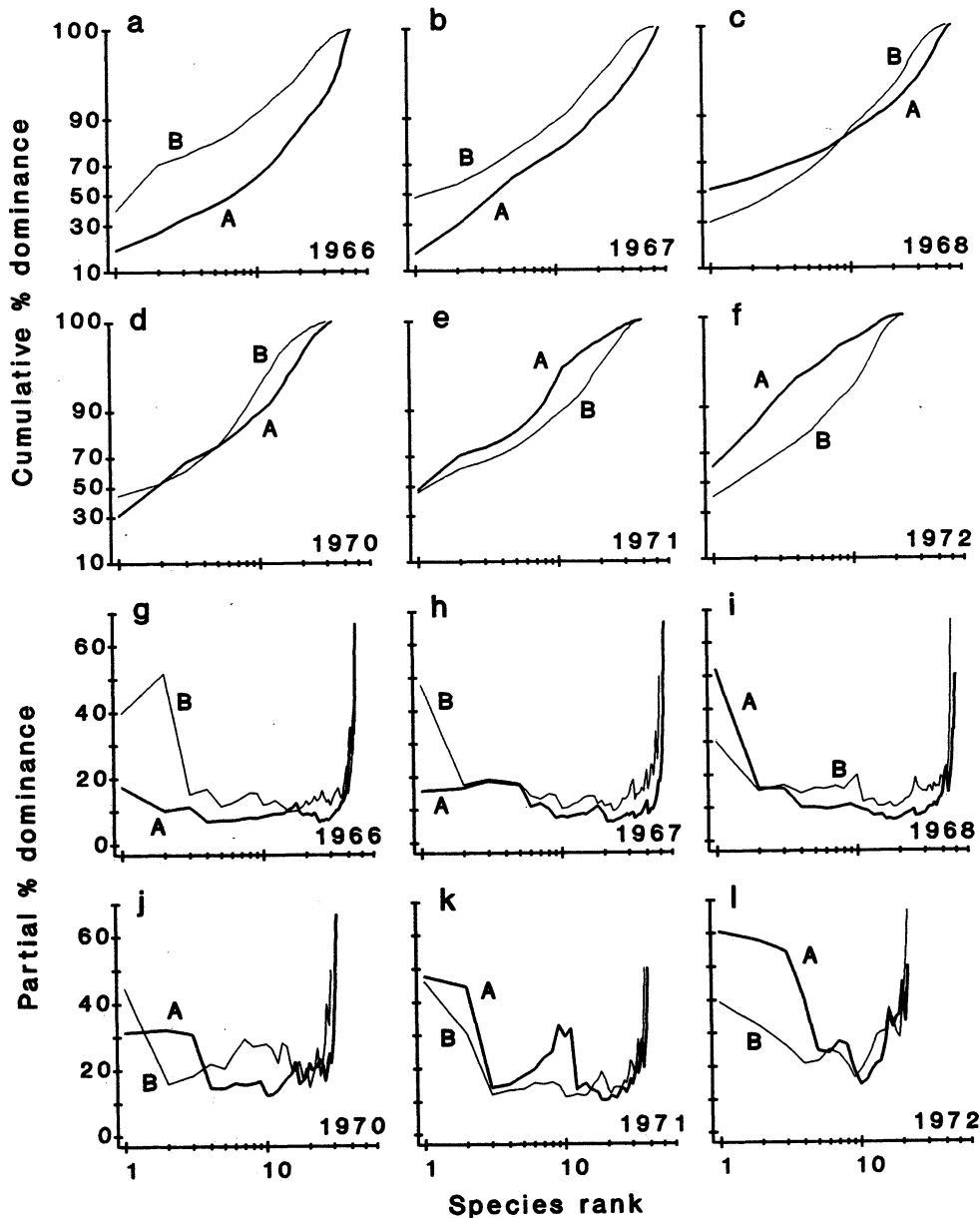


Fig. 8.11. Loch Linnhe macrofauna [L]. Selected years 1966–68 and 1970–72. a)–f) ABC curves (logistic transform). g)–l) Partial dominance curves for abundance (thick line) and biomass (thin line) for the same years.

(Mollusca, Crustacea, Echinodermata).⁶ The shift within polychaetes reflects the substitution of larger-bodied by smaller-bodied species, and not a change in the average size of individuals within a species. In most instances the phyletic changes reinforce the trend in species substitutions within the polychaetes, to produce the overall ABC response, but in some cases they may work against each other. In cases where the ABC method has not succeeded as a measure of the pollution status of marine macrobenthic communities,

it is because small non-polychaete species have been dominant. Prior to the Amoco Cadiz oil-spill, small ampeliscid amphipods (crustacea) were present at the Pierre Noire station in relatively high abundance (Dauvin, 1984), and their disappearance after the spill confounded the ABC plots (Ibanez and Dauvin, 1988). It was the erratic presence of large numbers of small amphipods (*Corophium*) or molluscs (*Hydrobia*) which confounded these plots in the Wadden Sea (Beukema, 1988). These small non-polychaetous species are not indicative of polluted conditions, as Beukema points out. Indications of pollution or disturbance detected by this method should therefore be viewed with caution if the species responsible for the polluted configurations are not polychaetes.

6. This initially used the PRIMER program SELBYIND to select sub-matrices of the data for each phylum, identified by a taxonomic coding in an 'aggregation matrix', see Chapter 10.

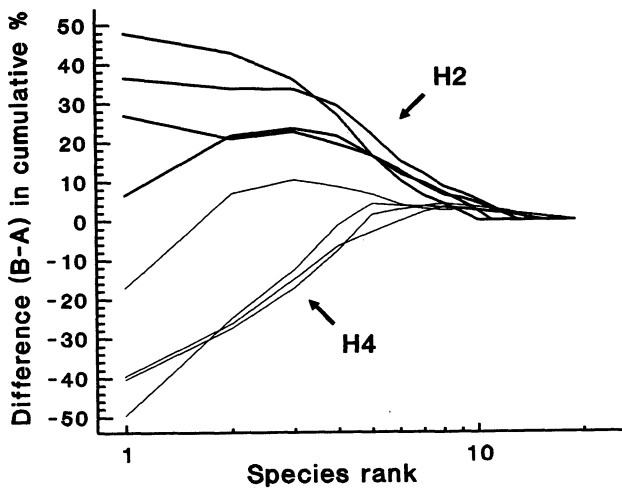


Fig. 8.12. Hamilton Harbour macrobenthos (H). Difference (B-A) between cumulative dominance curves for biomass and abundance for four replicate samples at stations H2 (thick line) and H4 (thin line).

W statistics

When the number of sites, times or replicates is large, presenting ABC plots for every sample can be cumbersome, and it would be convenient to reduce each plot to a single summary statistic. Clearly, some information must be lost in such a condensation: one plots cumulative dominance curves rather than quoting a diversity index precisely because of a reluctance to reduce the diversity information to a single statistic. Nonetheless, Warwick's (1986) contention that the biomass and abundance curves increasingly overlap with moderate disturbance, and transpose altogether for the grossly disturbed condition, is a unidirectional hypothesis and very amenable to quantification by a single summary statistic.

Fig. 8.12 displays the difference curves B-A for each of four replicate macrofauna samples from two stations (H2 and H4) in Hamilton Harbour, Bermuda; these are simply the result of subtracting the abundance (A_i) from the biomass (B_i) value for each species rank (i) in an ABC curve.⁷

For all four replicates from H2, the biomass curve is above the abundance curve throughout its length, so the sum of the $B_i - A_i$ values across the ranks i will be strongly positive. In contrast, this sum will be strongly

negative for the replicates at H4, for which abundance and biomass curves are largely transposed. Intermediate cases in which A and B curves are intertwined will tend to give $\sum(B_i - A_i)$ values near zero. The summation requires some form of standardisation to a common scale, so that comparisons can be made between samples with differing numbers of species, and Clarke (1990) proposes the W (for Warwick) statistic:

$$W = \sum_{i=1}^S (B_i - A_i) / [50(S-1)] \quad (8.7)$$

It can be shown algebraically that W takes values in the range (-1, 1), with $W \rightarrow +1$ for even abundance across species but biomass dominated by a single species, and $W \rightarrow -1$ in the converse case (though neither limit is likely to be attained in practice).

An example is given by the changing macrofauna communities along the transect across the sludge-dumping ground at Garroch Head (G). Fig. 8.13 plots the W values for each of the 12 stations against the station number. These summarise the 12 component ABC plots of Fig. 8.8 and clearly delineate a similar pattern of gradual change from unpolluted to disturbed conditions, as the centre of the dumpsite is approached.

Hypothesis testing for dominance curves

There are no replicates in the Garroch Head data to allow testing for statistical significance of observed changes in ABC patterns but, for studies involving replication, the W statistic provides an obvious route to hypothesis testing. For the Bermuda samples of Fig. 8.12, W takes values 0.431, 0.253, 0.250 and 0.349 for the

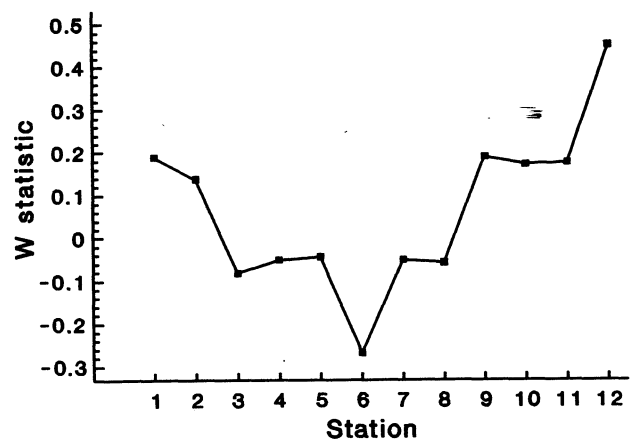


Fig. 8.13. Garroch Head macrofauna (G). W values corresponding to the 12 ABC curves of Fig. 8.8, plotted against station number; station 6 is the centre of the dump ground (Fig. 8.3).

7. Note that, as always with an ABC curve, B_i and A_i do not necessarily refer to values for the same species; the ranking is performed separately for abundance and biomass.

four replicates at H2 and -0.082 , 0.053 , -0.081 and -0.068 for the four H4 samples. These data can be input into a standard univariate ANOVA (equivalent in the case of two groups to a standard 2-sample t -test), showing that there is indeed a clearly established difference in abundance–biomass patterns between these two sites ($F = 45.3$, $p < 0.1\%$).

More general forms of hypothesis testing are possible, likely to be particularly relevant to the comparison of k -dominance curves calculated for replicates at a number of sites, times or conditions (or in some two-way layout, as discussed in Chapter 6). A

measure of "similarity" could be constructed between any pair of k -dominance (or $B-A$) curves, for example based on their *absolute* distance apart, summed across the species ranks. When computed for all pairs of samples in a study this furnishes a (ranked) triangular similarity matrix, essentially similar in structure to that from a multivariate analysis; thus the 1-way and 2-way ANOSIM tests (Chapter 6) can be used in exactly the same way to test hypotheses about differences between *a priori* specified groups of samples. Clarke (1990) discusses some appropriate definitions of "similarity" for use with dominance curves.

CHAPTER 9: TRANSFORMATIONS

There are two distinct roles for transformations in community analyses:

- a) to validate statistical assumptions for parametric techniques – in the approach of this manual such methods are restricted to *univariate* tests;
- b) to weight the contributions of common and rare species in the (non-parametric) *multivariate* representations.

The second reason is the only one of relevance to the preceding chapters, with the exception of Chapter 8 where it was seen that standard parametric analysis of variance (ANOVA) could be applied to diversity indices computed from replicate samples at different sites or times. Being composite indices, derived from all species counts in a sample, some of these will already be approximately continuous variates with symmetric distributions, and others can be readily transformed to the normality and constant variance requirements of standard ANOVA. Also, there may be interest in the abundance patterns of individual species, specified *a priori* (e.g. keystone species), which are sufficiently common across most sites for there to be some possibility of valid parametric analysis after transformation.

UNIVARIATE CASE

For purely illustrative purposes, Table 9.1 extracts the counts of a single *Thyasira* species from the Frierfjord macrofauna data {F}, consisting of four replicates at each of six sites.

Table 9.1. Frierfjord macrofauna {F}. Abundance of a single species (*Thyasira* sp.) in four replicate grabs at each of the six sites (A–E, G).

| Site: | A | B | C | D | E | G |
|-------------|-----|-----|-----|-----|------|------|
| Replicate | | | | | | |
| 1 | 1 | 7 | 0 | 1 | 62 | 66 |
| 2 | 4 | 0 | 0 | 8 | 102 | 68 |
| 3 | 3 | 3 | 0 | 5 | 93 | 52 |
| 4 | 11 | 2 | 3 | 13 | 69 | 36 |
| Mean | 4.8 | 3.0 | 0.8 | 6.8 | 81.8 | 55.5 |
| Stand. dev. | 4.3 | 2.9 | 1.5 | 5.1 | 18.7 | 14.8 |

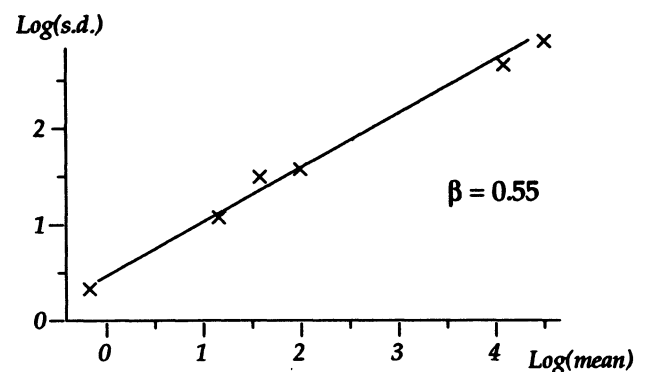
Two features are apparent:

- 1) the replicates are not symmetrically distributed (they tend to be right-skewed);
- 2) the replication variance tends to increase with increasing mean, as is clear from the mean and standard deviation (*s.d.*) values given in Table 9.1.

The lack of symmetry (and thus approximate normality) of the replication distribution is probably of less importance than the large difference in variability; ANOVA relies on an assumption of constant variance across the groups. Fortunately, both defects can be overcome by a simple transformation of the raw data; a power transformation (such as a square root), or a logarithmic transformation, have the effect both of reducing right-skewness and stabilising the variance.

Power transformations

The *power transformations* $y^* = y^\lambda$ form a simple and useful family, in which decreasing values of λ produce increasingly severe transformations. The log transform, $y^* = \log_e(y)$, can also be encompassed in this series (technically, $(y^\lambda - 1)/\lambda \rightarrow \log_e y$ as $\lambda \rightarrow 0$). Box and Cox (1964) give a formal maximum likelihood procedure for optimal selection of λ but, in practice, a precise value is not important, and indeed rather artificial if one were to use slightly different values of λ for each new analysis. The aim should be to select a transformation of the right order for all data of a particular type, choosing only from, say: none, square root, 4th root or logarithmic. It is *not* necessary for a valid ANOVA that the variance be precisely stabilised or the non-normality totally removed, just that gross departures from the parametric assumptions (e.g. the order of magnitude change in *s.d.* in Table 9.1) are avoided. One useful technique is to plot $\log s.d.$ against $\log \text{mean}$ and estimate the approximate slope of this relationship (β). This is shown here for the data of Table 9.1.



It can be shown that, approximately, if λ is set roughly equal to $1 - \beta$, the transformed data will have constant variance. That is, a slope of zero implies no transformation, 0.5 implies the square root, 0.75 the 4th root and 1 the log transform. Here, the square root is indicated and Table 9.2 gives the mean and standard

deviations of the root-transformed abundances: the s.d. is now remarkably constant in spite of the order of magnitude difference in mean values across sites. An ANOVA would now be a valid and effective testing procedure for the hypothesis of "no site-to-site differences", and the means and 95% confidence intervals for each site can be back-transformed to the original measurement scales for a more visually helpful plot.

Table 9.2. Frierfjord macrofauna (F). Mean and standard deviation over the four replicates at each site, for root-transformed abundances of *Thyasira* sp.

| Site: | A | B | C | D | E | G |
|----------|------|------|------|------|------|------|
| Mean(y*) | 2.01 | 1.45 | 0.43 | 2.42 | 9.00 | 7.40 |
| S.d.(y*) | 0.97 | 1.10 | 0.87 | 1.10 | 1.04 | 1.04 |

Like all illustrations, though genuine enough, this one works out too well to be typical! In practice, there is usually a good deal of scatter in the \log s.d. versus \log mean plots; more importantly, most species will have many more zero entries than in this example and it is impossible to "transform these away": species abundance data are simply not normally distributed and can only rarely be made so. Another important point to note here is that it is never valid to "snoop" in a data matrix of, perhaps, several hundred species for one or two species that display apparent differences between sites (or times), and then test the "significance" of these groups for that species. This is the problem of *multiple comparisons* referred to in Chapter 6; a purely random abundance matrix will contain *some* species which fallaciously appear to show differences between groups in a standard 5% significance level ANOVA (even were the ANOVA assumptions to be valid). The best that such "snooping" can do, in hypothesis testing terms, is identify one or two potential key or indicator species that can be tested with an entirely independent set of samples.

These two difficulties between them motivate the only satisfactory approach to most community data sets: a properly multivariate one in which all species are considered in combination in non-parametric methods of display and testing, which make no distributional assumptions at all about the individual counts.

MULTIVARIATE CASE

There being no necessity to transform to attain distributional properties, transformations play an entirely separate (but equally important) role in the clustering and ordination methods of the previous

chapters, that of defining the balance between contributions from common and rarer species in the measure of similarity of two samples.

Returning to the simple example of Chapter 2, a subset of the Loch Linnhe macrofauna data, Table 9.3 shows the effect of a 4th root transformation of these abundances on the Bray-Curtis similarities. The rank order of the similarity values is certainly changed from the untransformed case, and one way of demonstrating how dominated the latter is by the single most numerous species (*Capitella capitata*) is shown in Table 9.4. Leaving out each of the species in turn, the Bray-Curtis similarity between samples 2 and 4 fluctuates wildly when *Capitella* is omitted in the untransformed case, though changes much less dramatically under 4th root transformation, which downweights the effect of single species.

Table 9.3. Loch Linnhe macrofauna (L) subset. Untransformed and 4th root-transformed abundances for some selected species and samples (years), and the resulting Bray-Curtis similarities between samples.

| Untransformed | | | | | | | | |
|---------------------------|-----|-----|-----|-----|----------|----|----|----|
| Sample: | 1 | 2 | 3 | 4 | | | | |
| Species | | | | | Sample 1 | 2 | 3 | 4 |
| <i>Echinoca.</i> | 9 | 0 | 0 | 0 | 1 | - | | |
| <i>Myrioche.</i> | 19 | 0 | 0 | 3 | 2 | 8 | - | |
| <i>Labidopl.</i> | 9 | 37 | 0 | 10 | 3 | 0 | 42 | - |
| <i>Amaeana</i> | 0 | 12 | 144 | 9 | 4 | 39 | 21 | 4 |
| <i>Capitella</i> | 0 | 128 | 344 | 2 | | | | |
| <i>Mytilus</i> | 0 | 0 | 0 | 0 | | | | |
| $\sqrt[4]{}$ -transformed | | | | | | | | |
| Sample: | 1 | 2 | 3 | 4 | | | | |
| Species | | | | | Sample 1 | 2 | 3 | 4 |
| <i>Echinoca.</i> | 1.7 | 0 | 0 | 0 | 1 | - | | |
| <i>Myrioche.</i> | 2.1 | 0 | 0 | 1.3 | 2 | 26 | - | |
| <i>Labidopl.</i> | 1.7 | 2.5 | 0 | 1.8 | 3 | 0 | 68 | - |
| <i>Amaeana</i> | 0 | 1.9 | 3.5 | 1.7 | 4 | 52 | 68 | 42 |
| <i>Capitella</i> | 0 | 3.4 | 4.3 | 1.2 | | | | |
| <i>Mytilus</i> | 0 | 0 | 0 | 0 | | | | |

Transformation sequence

The previous remarks about the family of power transformations apply equally here: they provide a continuum of effect from $\lambda = 1$ (no transform), for which only the common species contribute to the similarity, through $\lambda = 0.5$ (square root), which allows the intermediate abundance species to play a part, to $\lambda = 0.25$ (4th root), which takes some account also of rarer species. As noted earlier, $\lambda \rightarrow 0$ can be thought of as equivalent to the $\log_e(y)$ transformation and the latter would therefore be more severe than the 4th root

transform. However, in this form, the transformation is impractical because the (many) zero values produce $\log(0) \rightarrow -\infty$. Thus, common practice is to use $\log(1+y)$ rather than $\log(y)$, since $\log(1+y)$ is always positive for positive y and $\log(1+y) = 0$ for $y = 0$. The modified transformation no longer falls strictly within the power sequence; on large abundances it does produce a more severe transformation than the 4th root but for small abundances it is less severe than the 4th root. In fact, there are rarely any practical differences between cluster and ordination results performed following $y^{0.25}$ or $\log(1+y)$ transformations; they are effectively equivalent in focusing attention on patterns within the whole community, mixing contributions from both common and rare species.¹

Table 9.4. *Loch Linnhe macrofauna (L) subset.* The changing similarity between samples 2 and 4 (of Table 9.3) as each of the six species is omitted in turn, for both untransformed and 4th root-transformed abundances.

| Untransformed | | | | | | | |
|----------------------------|------|----|----|----|----|----|----|
| Species omitted: | None | 1 | 2 | 3 | 4 | 5 | 6 |
| Bray-Curtis (S): | 21 | 21 | 21 | 14 | 13 | 54 | 21 |
| $\sqrt[4]{y}$ -transformed | | | | | | | |
| Species omitted: | None | 1 | 2 | 3 | 4 | 5 | 6 |
| Bray-Curtis (S): | 68 | 68 | 75 | 61 | 59 | 76 | 68 |

The logical end-point of this transformation sequence is therefore not the log transform but a reduction of the quantitative data to *presence/absence*, the Bray-Curtis coefficient (say) being computed on the resulting matrix of 1's (presence) and 0's (absence). This computation is illustrated in Table 9.5 for the subset of the Loch Linnhe macrofauna data used earlier. Comparing with Table 9.3, note that the rank order of similarities again differs, though it is closer to that for the 4th root transformation than for the untransformed data. In fact, reduction to presence/absence can be thought of as the ultimate transformation in down-

weighting the effects of common species. Species which are sufficiently ubiquitous to appear in *all* samples (producing a 1 in all columns) clearly cannot discriminate between the samples in any way, and thus do not contribute to the final multivariate description. The emphasis is therefore shifted firmly towards patterns in the intermediate and rarer species, the generally larger numbers of these tending to over-ride the contributions from the few numerical or biomass dominants.

Table 9.5. *Loch Linnhe macrofauna (L) subset.* Presence (1) or absence (0) of the six species in the four samples of Table 9.3, and the resulting Bray-Curtis similarities.

| Presence/absence | | | | | Sample 1 | 2 | 3 | 4 |
|------------------|---|---|---|---|----------|----|----|----|
| Sample: | 1 | 2 | 3 | 4 | Sample 1 | 2 | 3 | 4 |
| Species | | | | | 1 | - | | |
| <i>Echinoca.</i> | 1 | 0 | 0 | 0 | 2 | 33 | - | |
| <i>Myrioche.</i> | 1 | 0 | 0 | 1 | 3 | 0 | 80 | - |
| <i>Labidopl.</i> | 1 | 1 | 0 | 1 | 4 | 57 | 86 | 67 |
| <i>Amaeana</i> | 0 | 1 | 1 | 1 | | | | |
| <i>Capitella</i> | 0 | 1 | 1 | 1 | | | | |
| <i>Mytilus</i> | 0 | 0 | 0 | 0 | | | | |

One inevitable consequence of "widening the franchise" in this way, allowing many more species to have a say in determining the overall community pattern, is that it will become increasingly harder to obtain 2-d ordinations with low stress: the "view" we have chosen to take of the community is inherently high-dimensional. This can be seen in Fig. 9.1, for the dosing experiment (*D*) in the Solbergstrand mesocosm (GEEP Oslo workshop), previously met in Figs 4.2 and 5.5. Four levels of contaminant dosing (designated Control, Low, Medium, High) were each represented by four replicate samples of the resulting nematode communities, giving the MDS ordinations of Fig. 9.1. Note that as the severity of transformation increases, through none, root, 4th root and presence/absence (Fig. 9.1a to 9.1d respectively), the stress values rise from 0.08 to 0.19. It is important to realise that this is *not* an argument for deciding against transformation of the data. Fig. 9.1a is not a *better* representation of the between-sample relationships than the other plots: it is a *different* one. The choice of transformation is determined by which aspects of the community we wish to study. If interest is in the response of the whole community then we have to accept that it may be more difficult to capture this in a low-dimensional picture (a 3-d or higher-dimensional MDS may be desirable). On the other hand, if the data are totally dominated by one or two species, and it is these that are of key biological interest, then of course it will be possible to visualise in a 1- or 2-d picture how their numbers (or

1. Though practical differences are likely to be negligible, on purely theoretical grounds it could be argued that the 4th root is the more satisfactory of the two transformations because Bray-Curtis similarity is then invariant to a scale change in y . Similarity values would be altered under a $\log(1+y)$ transformation if abundances were converted from absolute values to numbers per m^2 of the sampled substrate, or if biomass readings were converted from mg to g. This does not happen with a strict power transformation; it is clear from equation (2.1) that any multiplying constant applied to y will cancel on the top and bottom lines of the summations.

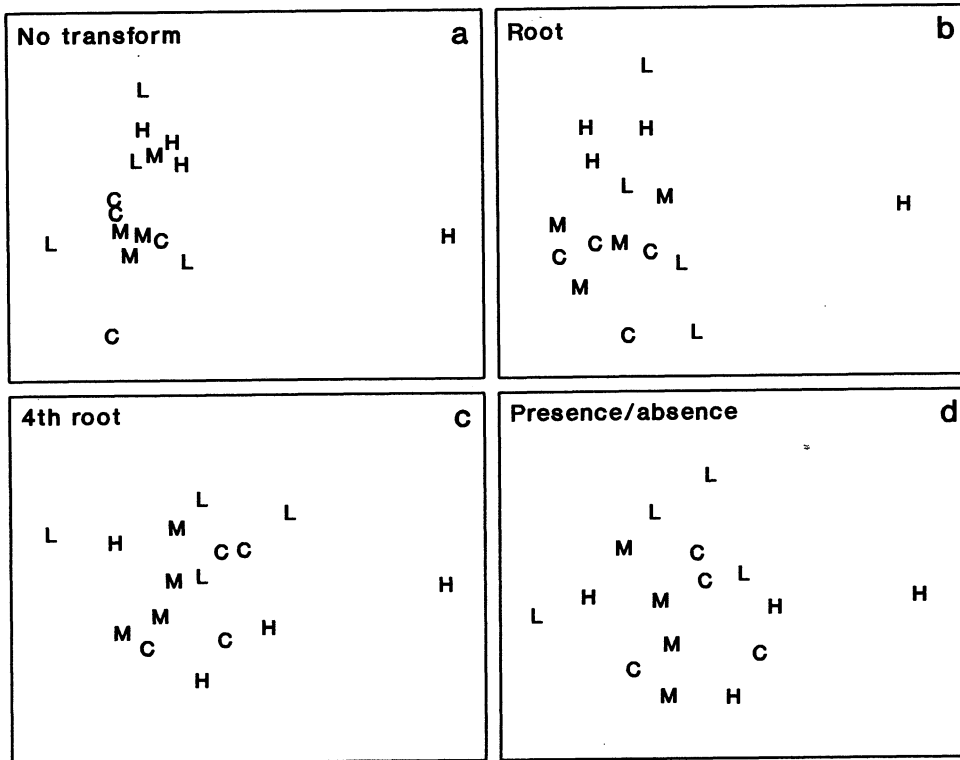


Fig. 9.1. Dosing experiment, Solbergstrand (D). MDS of nematode communities in four treatments (C = control, L = low, M = medium, H = high dose of a hydrocarbon/copper contaminant mixture dosed to mesocosm basins), based on Bray-Curtis similarities from transformed data: a) no transform (stress = 0.08), b) $\sqrt{\quad}$ (stress = 0.14), c) $\sqrt[4]{\quad}$ (stress = 0.18), d) presence/absence (stress = 0.19).

biomass) vary between samples: in that case an ordination on untransformed data will be little different from a simple scatter plot of the counts for the two main species.

CONCLUSIONS

1) The transformation sequence in a multivariate analysis, corresponding to a progressive downweighting of the common species, is effectively:

$$\text{None} \rightarrow \sqrt{y} \left\langle \begin{array}{c} \sqrt[4]{y} \\ \log(1+y) \end{array} \right\rangle \rightarrow \text{pres/abs}$$

The choice of transformation from this sequence *can* affect the conclusions of an analysis (in fact, it may sometimes have more effect than choice of similarity coefficient or ordination method). In many respects the choice of transformation is more a *biological* than a statistical question: which "view" of the community do we wish to take (*shallow* or *deep*), given that there are potentially many different 2-dimensional summaries of this high-dimensional data?

2) Statistical considerations do enter, however, particularly in relation to the reliability of sampling. The numerical (and especially the biomass) dominants may be highly spatially variable. With untransformed data, a single chance capture of a very large-bodied species can totally distort a biomass MDS; similar

effects will result from sampling small-bodied species (e.g. larvae or opportunist colonisers) with a strong degree of spatial clustering, such that replicate samples at the same location give counts varying from 10's to 1000's. At the other extreme, an analysis which places a lot of weight on species which are typically only ever found as single individuals in one sample is highly susceptible to the "noise" introduced by the arbitrary chance capture of rarer species.

3) The practical choice is therefore often between a moderate ($\sqrt{\quad}$) and fairly severe ($\sqrt[4]{\quad}$ or log) transform, retaining the hard-won quantitative information but downplaying the species dominants; the 4th root transform is widely used in this manual. Note that the latter is largely equivalent to reducing the original data to about a 6 or 7 point scale: 0 = absent, $\frac{1}{5}$ = one individual, 2 = handful, 3 = sizeable number, 4 = abundant, ≥ 5 = very abundant. Rounding the transformed counts to this discrete scale will, in many cases, make rather little difference to the multivariate ordination (though this would not be the case for some of the univariate and graphical methods of Chapter 8). The scale may appear crude but is not unrealistic; individual species counts are often highly variable in (genuine) replicate sampling at one location and, if the primary requirement is a multivariate description, effort expended in deriving precise counts from a single sample could often be better spent in analysing more samples, to a less exacting level of detail. This is also a central theme of the following chapter.

CHAPTER 10: SPECIES REMOVAL AND AGGREGATION

SPECIES REMOVAL

For some univariate and graphical/distributional methods of data analysis it is important to include all species present at each site, since the omission of some of them will affect the outcome of the analysis. (This is obviously true for diversity measures such as species richness, for example). In certain circumstances, however, it is not possible or not advisable to include all species in multivariate analyses. There are two main circumstances where *eliminating species* is necessary:

- a) *Sample PCA (not MDS) ordinations.* The species number must be reduced to (say) <50 species, or else there will be problems with computing eigenvalues (see Chapter 4).¹
- b) *Species ordinations.* Although MDS and cluster analyses are possible for all species, the rarer species, whose occurrence at a particular station may largely be due to chance, must be excluded for an interpretable outcome (see Chapter 7).²

The way in which species are eliminated requires careful consideration. A commonly employed method is to remove those species which are rare in respect of their total abundance at all stations in the survey, for example those species comprising less than 1 or 2% of the total number of individuals. This however can be dangerous in situations where total abundance between stations is very variable, as is often the case. Situations frequently arise where certain stations have a very low overall abundance of organisms, but there may be many species which are absolutely characteristic of those stations. Using the above method of species reduction, all these species could be eliminated! To overcome this problem it is recommended that species

1. As discussed in Chapter 4, PCA is not normally recommended for species data. If required, however, PRIMER can perform it by first running REDUCE, to retain only the 50 most important species (in the sense defined below), and then using SWAP to transpose the matrix for entry into the PCA routine, which expects variables as columns.

2. This could also be carried out by an initial run of REDUCE but a better option generally is to specify reduction of species (/samples) directly in CLUSTER. This will retain knowledge of the original row (/column) numbers in the derived species (/samples) similarity matrix

accounting for > p % of the total score (abundance or biomass) in *any one sample* are retained (p is chosen to reduce species to the required number; typically $p = 3$ or 4).

SPECIES REDUNDANCY

We have already seen (Chapters 4 & 5) that sample relationships can often be well summarised in a 2-dimensional ordination, which is reduced from a very much higher-dimensional species space. This implies that many species must be *interchangeable* in the way they characterise the samples, and that an analysis of a small subset of the total number of species may give a similar result to that for the full species analysis. This can be confirmed by performing MDS on a randomly chosen subset of species. Gray *et al.* (1988), for example, compared the configurations produced from an MDS of 110 species of macrobenthos at six stations in Frierfjord, Norway with a similar analysis using just 19 randomly selected species (Fig. 10.1). Note that the ordinations are remarkably similar in the way in which they discriminate between sites (although there is a slight difference in that the replicate samples at stations G and E are transposed in location).

Thus, there appears to be considerable *redundancy* in the species which characterise the community composition. Although the above example, extracting a random subset of species, is of no real practical interest, attempts have been made to exploit this redundancy in the context of taxonomic aggregation.

SPECIES AGGREGATION

The painstaking work involved in sorting and identifying samples to the species level has resulted in community analysis for environmental impact studies being traditionally regarded as labour-intensive, time-consuming and therefore relatively expensive. One practical means of overcoming this problem is to exploit the redundancy in community data by analysing the samples to higher taxonomic levels such as family or phyla, rather than to species. If results from identifications to higher taxonomic levels are comparable to a full species analysis, this means that:

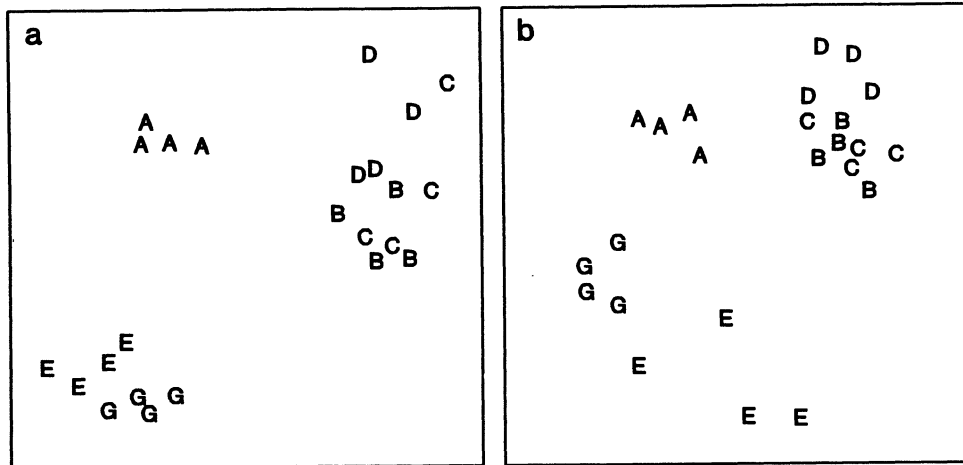


Fig. 10.1. Frierfjord macrofauna (F). Sample MDS using Bray-Curtis similarities on $\sqrt{1}$ -transformed counts for: a) all 110 species, b) 19 randomly selected species (stress = 0.14, 0.13 respectively).

- a) A great deal of *labour can be saved*. Several groups of marine organisms are taxonomically difficult, for example (in the macrobenthos) several families of polychaetes and amphipods; much time can be spent in separating a few of these difficult groups into species as the entire remainder of the sample, even in Northern Europe where taxonomic keys for identification are most readily available.
- b) *Less taxonomic expertise* is needed. Many taxa really require the skills of specialists to separate them into species, and this is especially true in parts of the world where fauna is poorly described. For certain groups of marine organisms, e.g. the meiobenthos, the necessary expertise required to identify even the major taxa (nematodes and copepods) to species is lacking in most laboratories which are concerned with the monitoring of marine pollution, so that these components of the biota are rarely used in such studies, despite their many inherent advantages (see Chapter 13).

For the marine macrobenthos and meiobenthos, aggregations of the species data to higher taxonomic levels have been made and the resultant data matrices have been subjected to several forms of statistical analysis to see how much information has been lost compared with the full species-level analysis.³ Although such experiments have not often been done for other components of the marine biota (e.g. plankton), results from the benthic studies are remarkably encouraging in that very little information appears to be lost in the aggregation process.

3. Simple aggregation can be performed in PRIMER using the AGGREG routine, which inputs the species data matrix and an aggregation file giving, for example, genus, family and phyla designations for each species.

Methods amenable to aggregation

- 1) *Multivariate methods*. Although taxonomic levels higher than that of species can be used to some degree for all types of statistical analysis of community data, it is probably for multivariate methods that this is most appropriate, in view of the redundancy discussed above. All ordination/clustering techniques are amenable to aggregation, and empirical evidence is increasing that identification at least to the family level makes very little difference to the results (Figs. 10.2 – 10.6). There are also certain possible theoretical advantages to conducting multivariate analyses at the level of major groups (e.g. phyla) for pollution impact studies. Natural environmental variables which also affect community structure are rarely constant in surveys designed to detect pollution effects over relatively large geographical areas. In the case of the benthos, these 'nuisance' variables include water depth and sediment granulometry. However, these variables usually influence the fauna more by species replacement than by changes in the proportions of the major taxa present. Each major group, in its adaptive radiation, has evolved species which are suited to rather narrow ranges of natural environmental conditions, whereas the advent of pollution by man has been too recent for the evolution of suitably adapted species. Ordinations of abundance or biomass data of these major taxa are therefore more likely to correlate with a contamination gradient than are species ordinations, the latter being more complicated by the effects of natural environmental variables when large heterogeneous geographical areas are considered. In short, higher taxa may well reflect well-defined pollution gradients *more closely* than species.

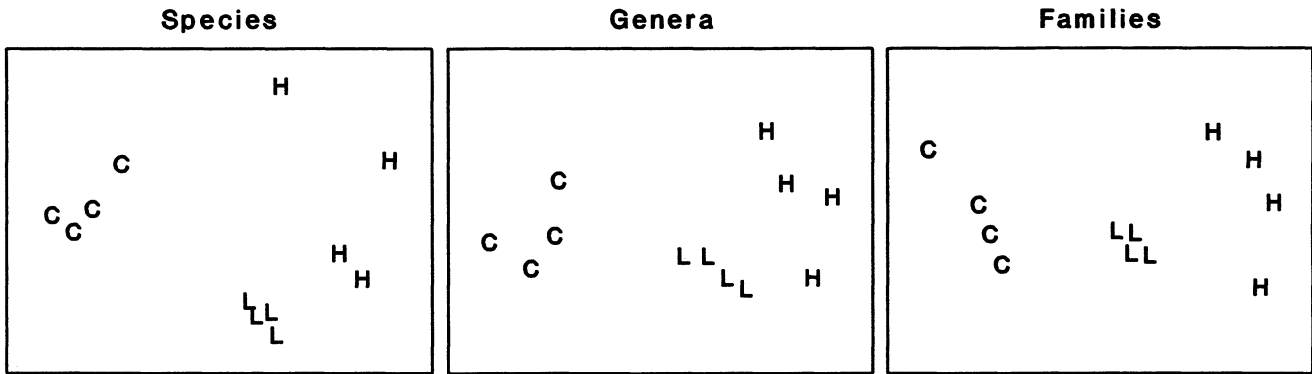


Fig. 10.2. Nutrient-enrichment experiment, Solbergstrand (N). MDS plot of copepod abundances ($\sqrt{\sqrt{\cdot}}$ -transformed, Bray-Curtis similarities) for four replicates from each of three treatments; species data aggregated into genera and families (stress = 0.09, 0.09, 0.08).

- 2) **Distributional methods.** Aggregation for ABC curves is possible, and family level analyses are often identical to species level analyses (see Fig. 10.7).
- 3) **Univariate methods.** The concept of pollution indicator groups rather than indicator species is well-established. For example, at organically enriched sites, polychaetes of the family Capitellidae become abundant (not just *Capitella capitata*), as do meiobenthic nematodes of the family Oncholai-

midae. The *nematode/copepod ratio* (Raffaelli and Mason, 1981) is an example of a pollution index based on higher taxonomic levels. Such indices are likely to be of more general applicability than those based on species level information. Diversity indices themselves can be defined at hierarchical taxonomic levels for internal comparative purposes, although this is not commonly done in practice.

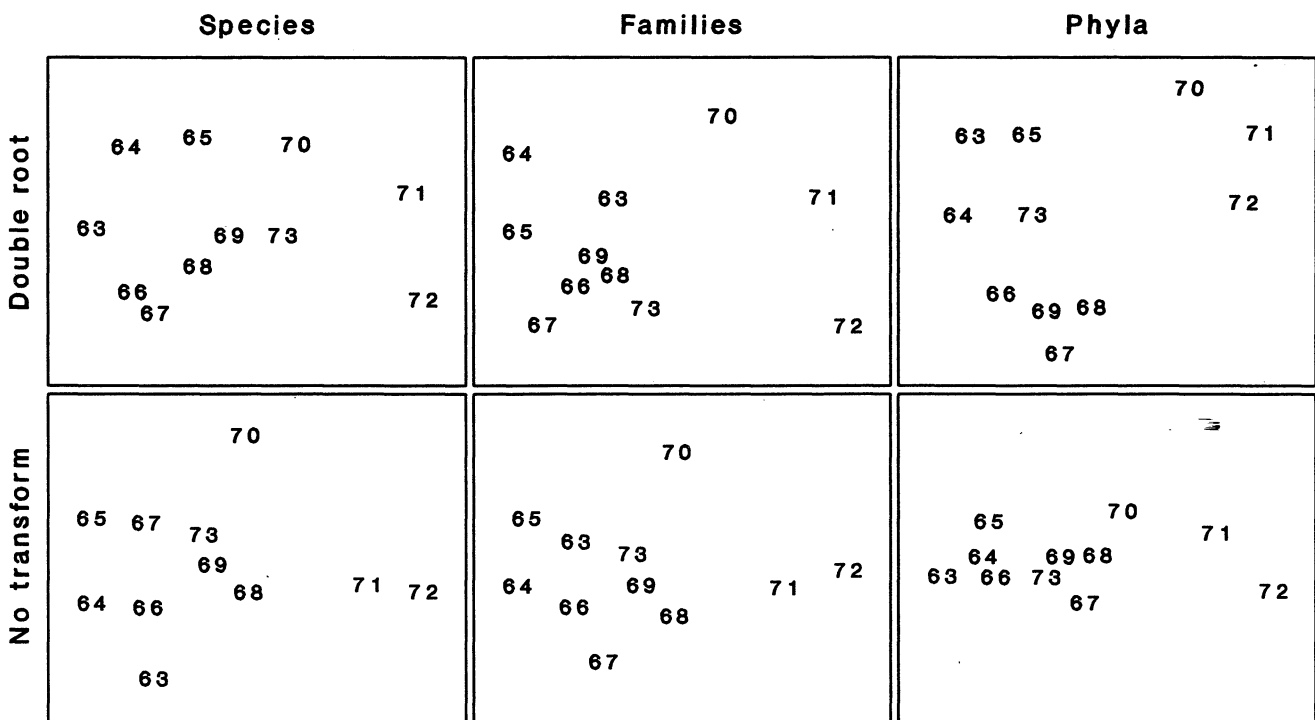


Fig. 10.3. Loch Linnhe macrofauna (L). MDS (using Bray-Curtis similarities) of samples from 11 years. Abundances are $\sqrt{\sqrt{\cdot}}$ -transformed (top) and untransformed (bottom), with 115 species (left), aggregated into 45 families (middle) and 9 phyla (right). (Reading across rows, stress = 0.09, 0.09, 0.10, 0.09, 0.09, 0.02).

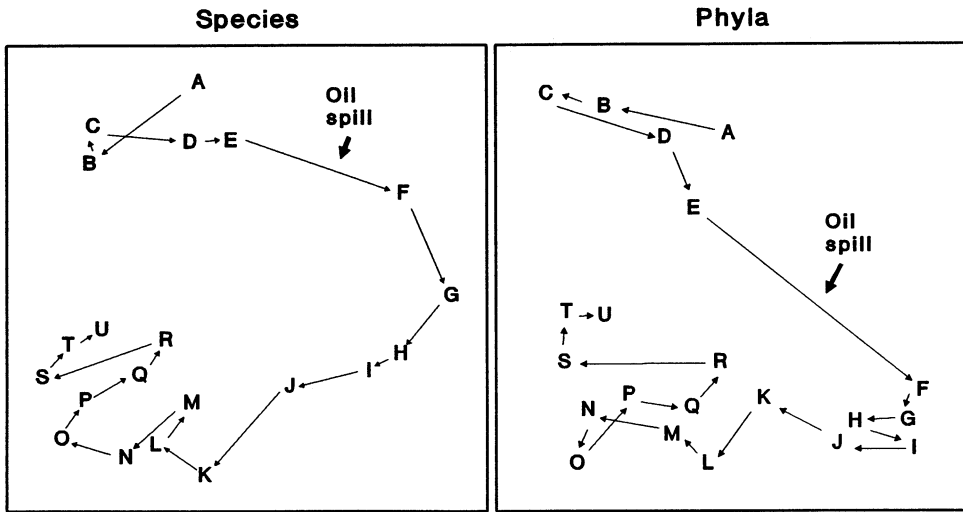


Fig. 10.4. Amoco-Cadiz oil spill (A). MDS for macrobenthos at station "Pierre Noire" in the Bay of Morlaix. Species data (left) aggregated into phyla (right). Sampling months are A:4/77, B:8/77, C:9/77, D:12/77, E:2/78, F:4/78, G:8/78, H:11/78, I:2/79, J:5/79, K:7/79, L:10/79, M:2/80, N:4/80, O:8/80, P:10/80, Q:1/81, R:4/81, S:8/81, T:11/81, U:2/82. The oil-spill was during 3/78, (stress = 0.09, 0.07).

MULTIVARIATE EXAMPLES

Nutrient-enrichment experiment

In the soft-bottom mesocosms at Solbergstrand, Norway (N), box-cores of sublittoral sediment were subjected to three levels of particulate organic enrichment (L = low dose, H = high dose and C = control), there being four replicates from each treatment. After 56 days the meiobenthic communities were analysed. Fig 10.2 shows that, for the copepods, there were clear differences in community structure between treatments at the species level, which were equally evident when the species data were aggregated into genera and families. (Indeed, at the family

level the configuration is arguably more linearly related to the pollution gradient than at the species level).

Loch Linnhe macrofauna

MDS ordinations of the Loch Linnhe macrobenthos are given in Fig. 10.3, using both double square root and untransformed abundance data. Information on the time-course of pollution events and changes in diversity are given in Fig. 10.7 (top left). The ordinations have been performed separately using all 115 species, the 45 families and the 9 phyla. In all ordinations there is a separation to the right of the years 1970, 1971 and 1972 associated with increasing pollution levels and community stress, and a return to

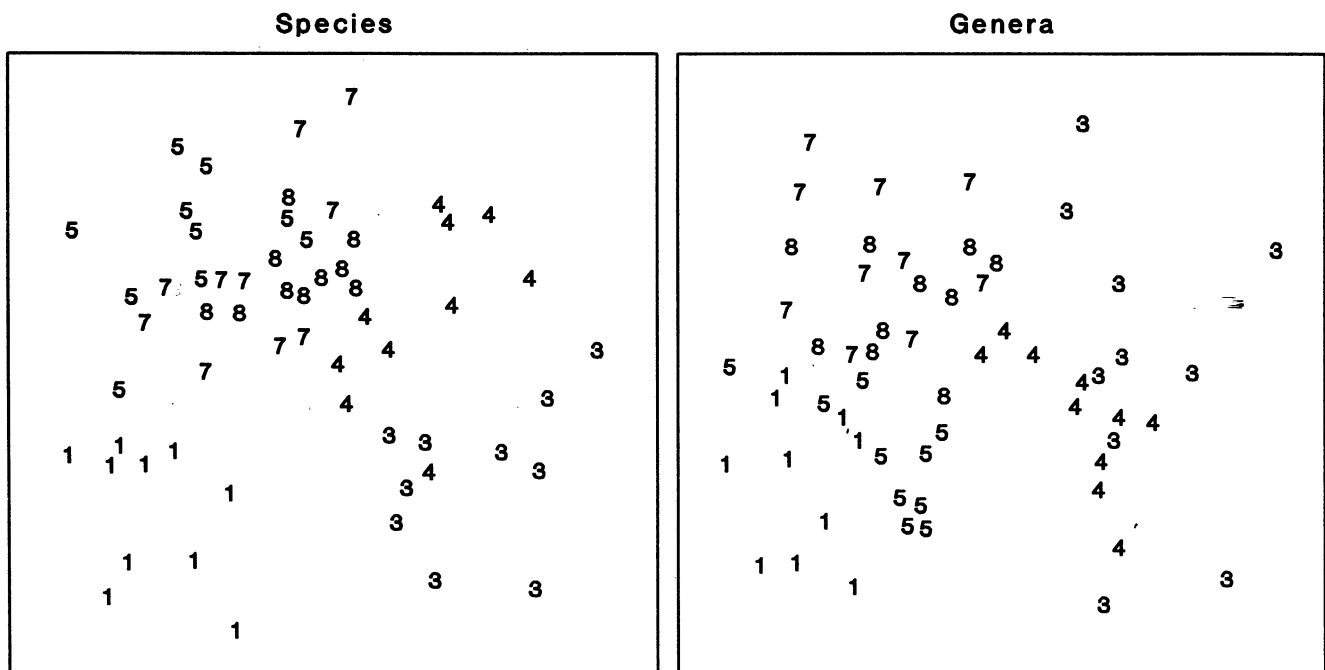


Fig. 10.5. Indonesian reef corals (I). MDS for species ($p=75$) and genus ($p=24$) data at South Pari Island (Bray-Curtis similarities on untransformed % cover). The El Nino occurred in 1982-3. 1=1981, 3=1983 etc. (stress = 0.25).

the left in 1973 associated with reduced pollution levels and community stresses. This pattern is equally clear at all levels of taxonomic aggregation. Again, the separation of the most polluted years is most distinct at the phylum level, at least for the double square root transformed data (and the configuration is more linear with respect to the pollution gradient at the phylum level for the untransformed data).

Amoco-Cadiz oil-spill

Macrofauna species were sampled at station 'Pierre Noire' in the Bay of Morlaix on 21 occasions between April 1977 and February 1982, spanning the period of the wreck of the 'Amoco-Cadiz' in March 1978. The species abundance MDS has been repeated with the data aggregated into five 'phyla': Annelida, Mollusca, Arthropoda, Echinodermata and 'others' (Fig. 10.4). The analysis of phyla closely reflects the timing of pollution events, the configuration being slightly more

linear than in the species analysis. All pre-spill samples (A-E) are in the top left of the configuration, the immediate post-spill sample (F) shifts abruptly to the bottom right after which there is a gradual recovery in the pre-spill direction. Note that in the species analysis, although results are similar, the immediate post-spill response is rather more gradual. The community response at the phylum level is remarkably sensitive, considering that the sampling site was some 40 km away from the oil-spill.

Indonesian reef corals

The El Niño of 1982-3 resulted in extensive bleaching of reef corals throughout the Pacific. Fig. 10.5 shows the coral community response at South Pari Island over six years in the period 1981-1988, based on ten replicate line transects along which coral species cover was determined. Note the immediate post-El Niño location shift on the species MDS and a circuitous

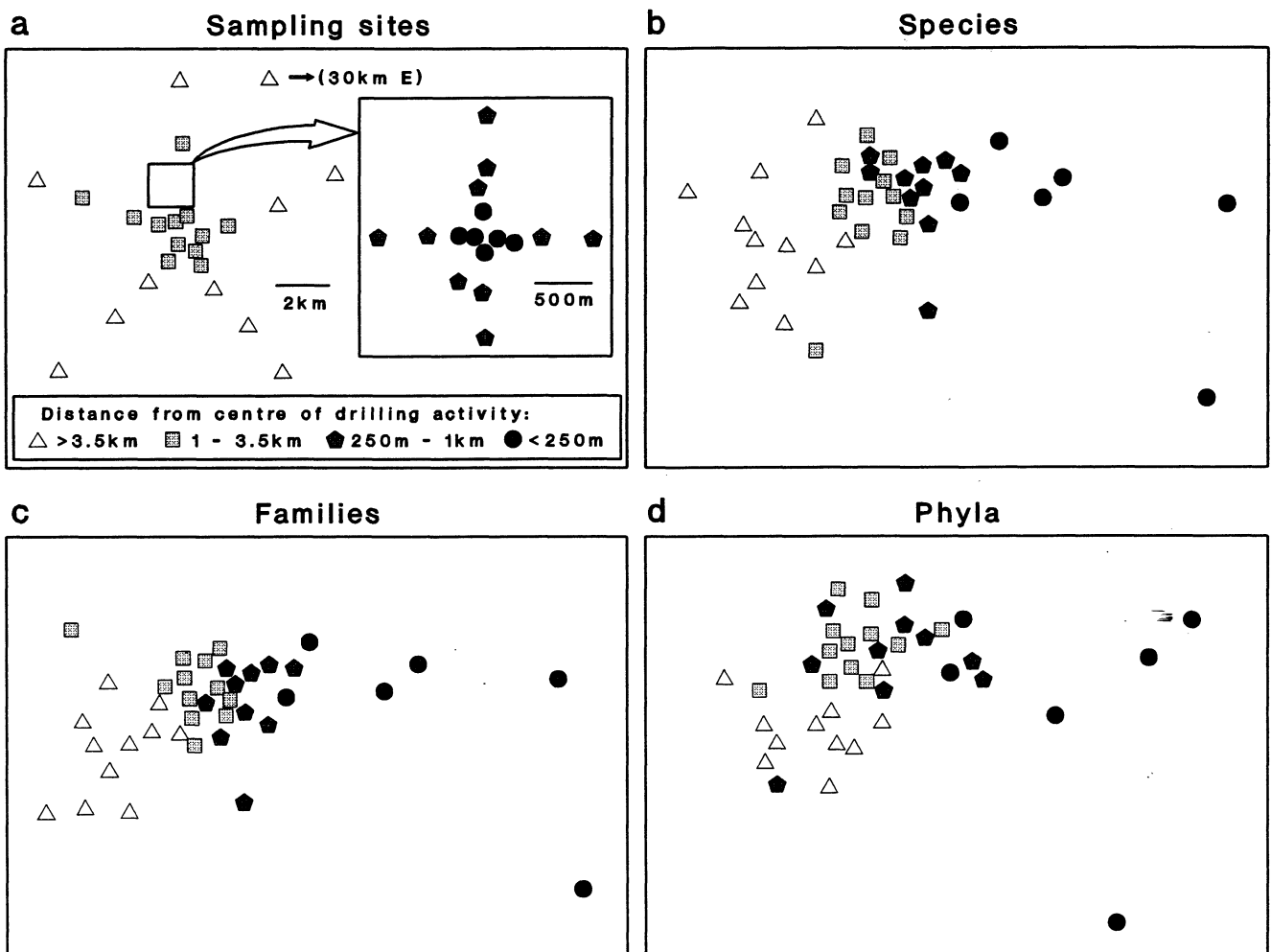


Fig. 10.6. Ekofisk oil-platform macrobenthos {E}. a) Map of station positions, indicating symbol/shading conventions for distance zones from the centre of drilling activity; b)-d) MDS for root-transformed species, family and phyla abundances respectively (stress = 0.12, 0.11, 0.13).

return towards the pre-El Niño condition. This is closely reflected in the genus level analysis.

Ekofisk oil-platform macrobenthos

Changes in community structure of the soft-bottom benthic macrofauna in relation to oil drilling activity at the Ekofisk platform in the North Sea (E) have been studied by Gray *et al.* (1990), Warwick and Clarke (1991). The positions of the 39 sampling stations around the rig are coded by different symbol and shading conventions in Fig. 10.6a, according to their distance from the current centre of drilling activity. In the MDS species abundance analysis (Fig. 10.6b), community composition in all of the zones is distinct,

and there is a clear gradation of change from the (black circle) inner to the (open triangle) outer zones. Formal significance testing (using the methods of Chapter 6) confirms statistically the differences between all zones. The MDS has been repeated with the species data aggregated into families (Fig. 10.6c) and phyla (Fig. 10.6d). The separation of sites is still clear, and pairwise comparisons confirm the statistical significance of differences between all zones, even at the phylum level, which does show some deterioration of the pattern. This is in contrast to (species-level) univariate and graphical/distributional measures, in which only the inner zone (less than 250m from the rig) was significantly different from the other three zones (see Chapter 14). Thus, phylum level analyses are

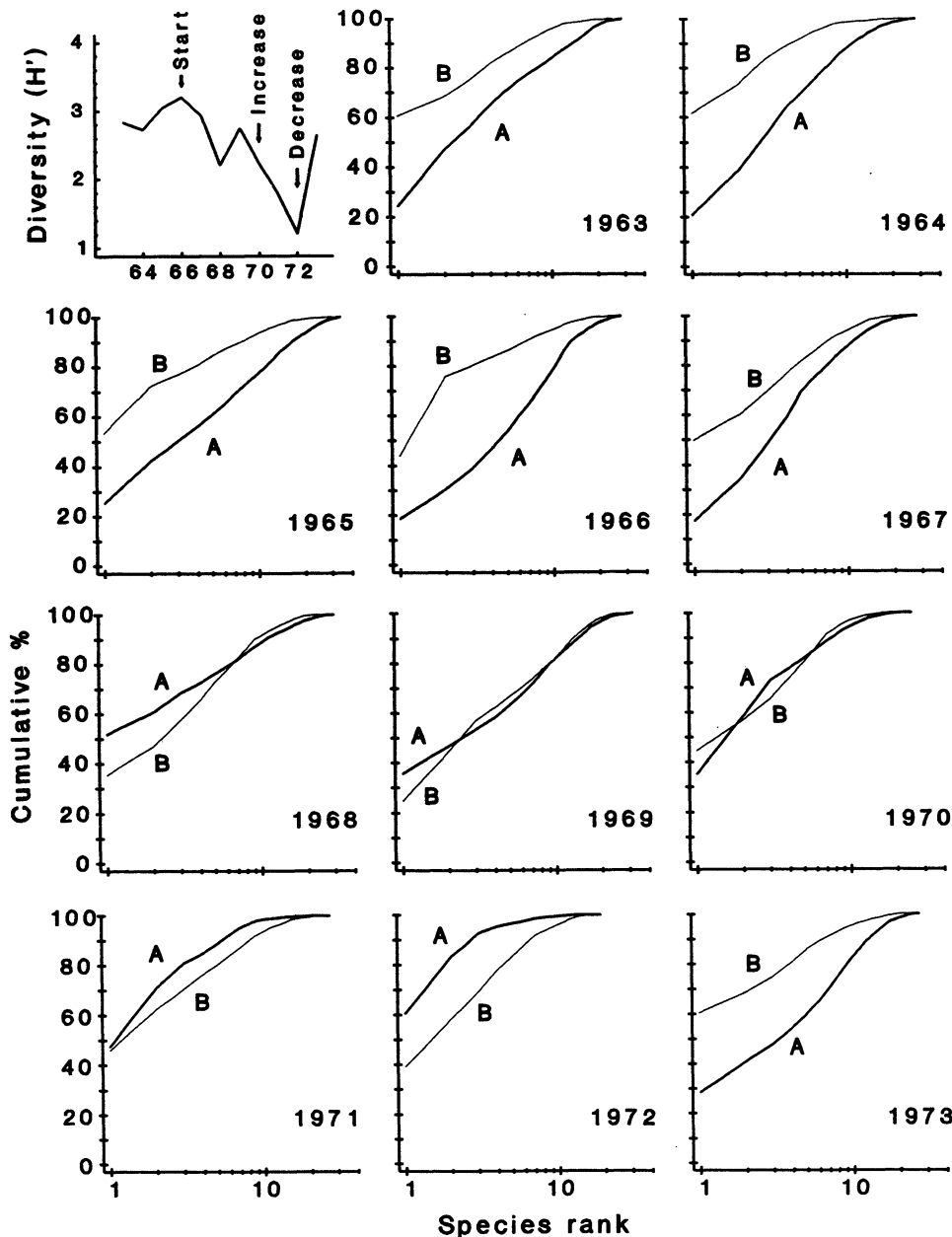


Fig. 10.7. Loch Linnhe macrofauna (L). Shannon diversity (H') and ABC plots over the 11 years, 1963 to 1973, for data aggregated to family level (c.f. Fig. 8.7). Abundance = thick line, biomass = thin line.

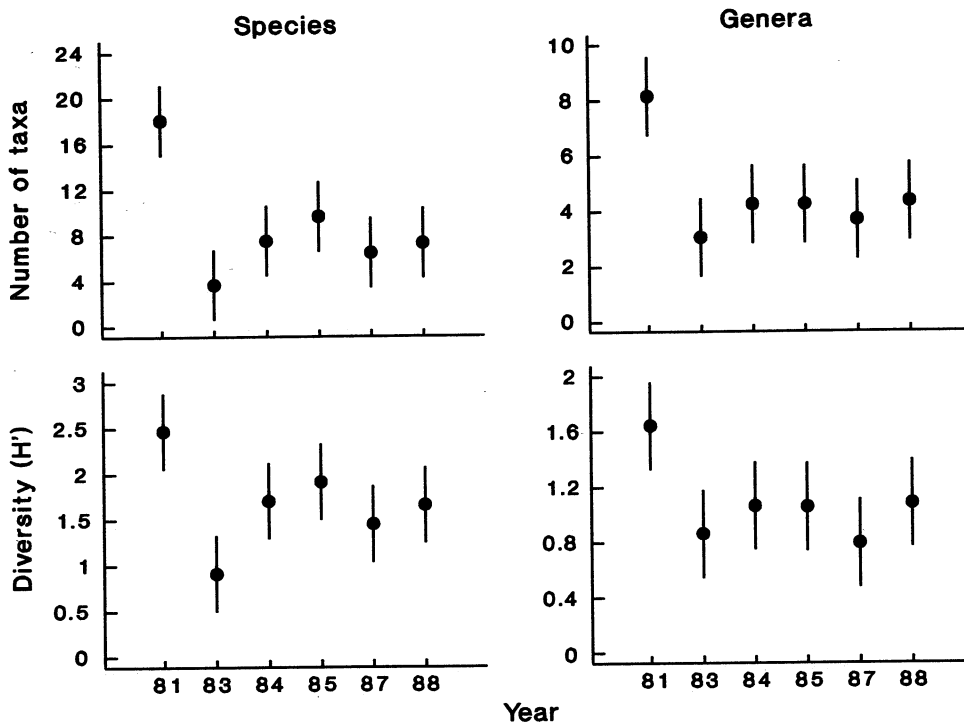


Fig. 10.8. Indonesian reef corals (I). Means and 95% confidence intervals for number of taxa and Shannon diversity at South Tikus Island, showing the impact and partial recovery from the 1982–3 El Niño. Species data (left) have been aggregated into genera (right).

again shown to be surprisingly sensitive in detecting pollution-induced community change.

GRAPHICAL EXAMPLES

Loch Linnhe macrofauna

ABC plots for the Loch Linnhe macrobenthos species data are given in Chapter 8, Fig. 8.7, where the performance of these curves with respect to the time-course of pollution events is discussed. In Fig. 10.7 the species data are aggregated to family level, and it is seen that the curves are virtually identical to the species level analysis, so that there would have been no loss of information had the samples only been sorted originally into families.

Similar results were produced by replotting the ABC curves for the Garroch Head sewage sludge dumping ground macrobenthos (G) (Fig. 8.8) at the family level (Warwick, 1988b).

UNIVARIATE EXAMPLE

Indonesian reef corals

Fig. 10.8 shows results from another survey of 10 replicate line transects for coral cover over the period

1981–1988, in this case at South Tikus Island, Indonesia (I). Note the similarity of the species and genus analyses for the number of taxa and Shannon diversity, with an immediate post-El Niño drop and subsequent suggestion of partial recovery.

RECOMMENDATION

Clearly the operational taxonomic level for environmental impact studies is another factor to be considered when planning such a survey, along with decisions about the number of stations to be sampled, number of replicates, types of statistical analysis to be employed etc. The choice will depend on several factors, particularly the time, manpower and expertise available and the extent to which that component of the biota being studied is known to be robust to taxonomic aggregation, for the type of statistical analysis being employed and the type of perturbation expected. Thus, it is difficult to give any firm recommendations and each case must be treated on its individual merits. For routine monitoring of organic enrichment situations using macrobenthos, one can be relatively certain that family level analysis will be perfectly adequate, but for other components of the fauna, and for other types of perturbation, sufficient evidence has not yet accumulated to be sure of this.

CHAPTER 11: LINKING COMMUNITY ANALYSES TO ENVIRONMENTAL VARIABLES

APPROACH

In many studies, the biotic data is matched by a suite of environmental variables measured at the same set of sites. These could be *natural variables* describing the physical properties of the substrate (or water) from which the samples were taken, e.g. median particle diameter, depth of the water column, salinity etc., or they could be *contaminant variables* such as sediment concentrations of heavy metals. The requirement here is to examine the extent to which the physico-chemical data is related to ("*explains*") the observed biological pattern.

The approach adopted is firstly to analyse the biotic data and then ask how well the information on environmental variables, taken either singly (Field *et al.*, 1982) or in combination (Clarke and Ainsworth, 1993), matches this community structure¹. The motivation here, as in earlier chapters, is to retain simplicity and transparency of analysis, by letting the species and environmental data "tell their own stories" (under minimal model assumptions) before judging the extent to which one provides an "explanation" of the other.

ANALYSIS OF ENVIRONMENTAL DATA

An analogous range of multivariate methods is available for display and testing of environmental samples as has been described for faunistic data: species are simply replaced by physical/chemical variables. However, the matrix entries are now of a rather different type and lead to different analysis choices. No longer do zeros predominate; the readings are usually more nearly continuous and, though their distributions are often right-skewed (with variability increasing with the mean), it is often possible to transform them to approximate normality (and

stabilise the variance) by a simple root or logarithmic transformation, see Chapter 9. Under these conditions, Euclidean distance is an appropriate measure of dissimilarity and PCA (Chapter 4) is an effective ordination technique, though note that this will need to be performed on the correlation rather than the covariance matrix, i.e. the variables will usually have different units of measurement and need normalising to a common scale (see the discussion on p4-6).

In the typical case of samples from a spatial contaminant gradient, it is also usually true that the number of variables is either much smaller than for a biotic matrix or, if a large number of chemical determinations has been made (e.g. GC/MS analysis of a range of specific aromatic hydrocarbons, PCB congeners etc.), they are often highly inter-correlated, tending to preserve a fixed relation to each other in a simple dilution model. A PCA can thus be expected to do an adequate job of representing in (say) two dimensions a pattern which is inherently low-dimensional to start with.

In a case where the samples are replicates from different groups, defined *a priori*, the ANOSIM tests of Chapter 6 are equally available for testing environmental hypotheses, e.g. establishing differences between sites, times, conditions etc., where such tests are meaningful.² The appropriate (rank) dissimilarity matrix would use Euclidean distances.

EXAMPLE: Garroch Head macrofauna

For the 12 sampling stations (Fig. 8.3) across the sewage-sludge dump ground at Garroch Head (G), the biotic information was supplemented by sediment chemical data on metal concentrations (Cu, Mn, Co, ...) and organic loading (% carbon and nitrogen); also recorded was the water depth at each station. The data matrix is shown in Table 11.1; it follows the normal convention in classical multivariate analysis of the

1. Methods such as canonical correlation (e.g. Mardia *et al.*, 1979), and the important technique of canonical correspondence (ter Braak, 1986), take the rather different stance of embedding the environmental data within the biotic analysis, motivated by specific gradient models defining the species-environment relationships.

2. The ANOSIM tests in the PRIMER package are not now the only possibility; the data will have been transformed to approximate normality so, if the number of variables is not large, classical multivariate (MANOVA) tests such as Wilks' Λ (e.g. Mardia *et al.*, 1979) are valid, and will generally have greater power.

Table 11.1. Garroch Head dump ground {G}. Sediment metal concentrations (ppm), water depth at the site (m) and organic loading of the sediment (% carbon and nitrogen), for the transect of 12 stations across the sewage-sludge dump site (centre at station 6), see Fig. 8.3.

| Station | Cu | Mn | Co | Ni | Zn | Cd | Pb | Cr | Dep | %C | %N |
|---------|-----|------|----|----|-----|-----|-----|-----|-----|------|------|
| 1 | 26 | 2470 | 14 | 34 | 160 | 0 | 70 | 53 | 144 | 3 | 0.53 |
| 2 | 30 | 1170 | 15 | 32 | 156 | 0.2 | 59 | 15 | 152 | 3 | 0.46 |
| 3 | 37 | 394 | 12 | 38 | 182 | 0.2 | 81 | 77 | 140 | 2.9 | 0.36 |
| 4 | 74 | 349 | 12 | 41 | 227 | 0.5 | 97 | 113 | 106 | 3.7 | 0.46 |
| 5 | 115 | 317 | 10 | 37 | 329 | 2.2 | 137 | 177 | 112 | 5.6 | 0.69 |
| 6 | 344 | 221 | 10 | 37 | 652 | 5.7 | 319 | 314 | 82 | 11.2 | 1.07 |
| 7 | 194 | 257 | 11 | 34 | 425 | 3.7 | 175 | 227 | 74 | 7.1 | 0.72 |
| 8 | 127 | 246 | 10 | 33 | 292 | 2.2 | 130 | 182 | 70 | 6.8 | 0.58 |
| 9 | 36 | 194 | 6 | 16 | 89 | 0.4 | 42 | 57 | 64 | 1.9 | 0.29 |
| 10 | 30 | 326 | 11 | 26 | 108 | 0.1 | 44 | 52 | 80 | 3.2 | 0.38 |
| 11 | 24 | 439 | 12 | 34 | 119 | 0.1 | 58 | 36 | 83 | 2.1 | 0.35 |
| 12 | 22 | 801 | 12 | 33 | 118 | 0 | 52 | 51 | 83 | 2.3 | 0.45 |

variables appearing as columns and the samples as rows.³

No replication is available for the 12 stations so the variance-to-mean plots suggested in Chapter 9 are not possible, but simple scatter plots of all pairwise combinations of variables (*draftsman plots*, see Fig. 7 of Clarke and Ainsworth, 1993, for these data) suggest that log transformations are appropriate for the chemical concentration variables, though not for water depth. The criteria here are that variables should not show marked skewness across the samples, enabling meaningful normalisation, and that relationships between them should be approximately linear; the standard product-moment correlations between variables and Euclidean distances between samples are then satisfactory summaries. In pursuit of this, note that whilst each variable could in theory be subjected to a different transformation it is more logical to apply the same transformation to all variables of the same type. Thus the decision to log all the metal data stems not just from the draftsman plots but also from past experience that such concentration variables often have standard deviations proportional to their means; i.e. a roughly constant *percentage* variation is log transformed to a stable *absolute* variance.

Fig. 11.1 displays the first two axes (PC1 and PC2) of a PCA ordination on the transformed data of Table 11.1.

3. This is in contrast with abundance matrices which, because of their generally larger number of variables (species) are usually transposed, i.e. the samples are displayed as columns. Note that the PRIMER software package follows both these conventions: it expects biotic matrices to be species-by-samples and abiotic ones to be samples-by-variables. A transposition routine (SWAP) is available if needed.

In fact, the first component accounts for much of the variability (61%) in the full matrix, the first two components accounting for 88%, so the 2-dimensional picture provides an accurate summary of the sample relationships. Broadly speaking, PC1 represents an axis of increasing contaminant load:

$$PC1 = 0.38 Cu' - 0.22 Mn' - 0.08 Co' + 0.15 Ni' + 0.37 Zn' + 0.33 Cd' + 0.37 Pb' + 0.35 Cr' - 0.12 Dep' + 0.37 C' + 0.33 N' \quad (11.1),$$

since most of the sizeable coefficients are positive. The dashes denote that the variables have been log transformed (excepting Dep) and normalised to zero mean and unit standard deviation. Fig. 11.1 shows a

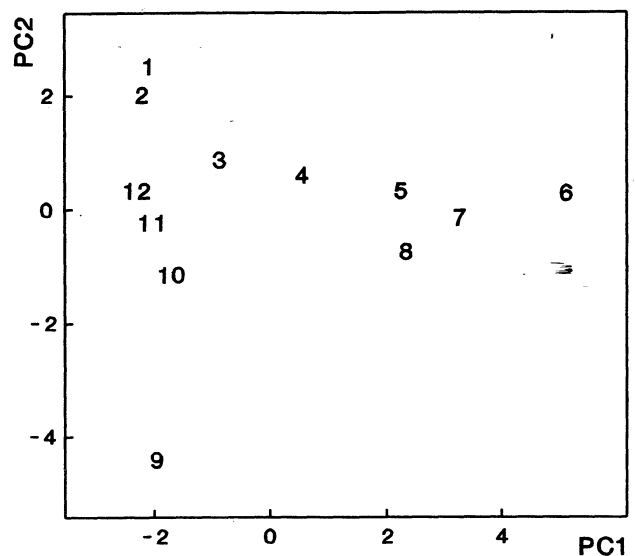


Fig. 11.1. Garroch Head dump ground {G}. Two-dimensional PCA ordination of the 11 environmental variables of Table 11.1 (transformed and normalised), for the stations (1-12) across the sewage-sludge dump site centred at station 6 (% variance explained = 88%).

strong pattern of incremental change on moving from the ends of the transect to the centre of the dump site, which (unsurprisingly) has the greatest levels of organic enrichment and metal concentrations (a significant exception being Mn).

LINKING BIOTA TO UNIVARIATE ENVIRONMENTAL MEASURES

Univariate community measures

If the biotic data are best summarised by one, or a few, simple univariate measures (such as diversity indices), one possibility is to attempt to correlate these with a similarly small number of environmental variables, taken one at a time. The summary provided by a principal component from a PCA of environmental variables can be exploited in this way. In the case of the Garroch Head dump ground, Fig. 11.2 shows the relation between Shannon diversity of the macrofauna samples at the 12 sites and the overall contaminant load, as reflected in the first PC of the environmental data (Fig. 11.1). Here the relationship appears to be a simple linear decrease in diversity with increasing load, and the fitted linear regression line clearly has a significantly non-zero slope ($\beta = -0.29$, $p < 0.1\%$).

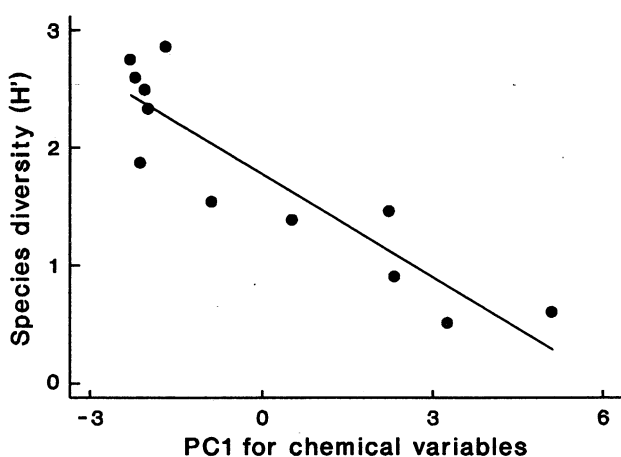


Fig. 11.2. Garroch Head macrofauna [G]. Linear regression of Shannon diversity (H'), at the 12 sampling stations, against the first PC axis score from the environmental PCA of Fig. 11.1, which broadly represents an axis of increasing contaminant load (equation 11.1).

Multivariate community measures

In most cases however, the biotic data is best described by a multivariate summary, such as an MDS ordination. Its relation to a univariate environmental measure can then be visualized by representing the values of this variable as symbols of differing size and superimposing these symbols on the biotic ordination of the corresponding samples. This, or the simpler superimposition of coded values for the variable, can be an effective means of noting any consistent differences in the environmental variable between biotic clusters or observing a smooth relationship with ordination gradients (Field *et al.*, 1982).⁴

EXAMPLE: Bristol Channel zooplankton

The cluster analysis of zooplankton samples from 57 sites in the Bristol Channel [B] was seen in Chapter 3, and the dendrogram suggested a division of the samples into 4 or 5 main clusters (Fig. 3.3). The matching MDS (Fig. 11.3), whilst in good agreement with the cluster analysis, reveals a more informative picture of a strong gradient of change from the Inner Channel to the Celtic Sea sites. This is seen most graphically by superimposing a code representing the salinity levels for each sample (Fig. 11.4). Biological considerations suggest that a simple linear coding is not appropriate: one would expect species turnover to be much greater through a salinity differential of 1 ppt in fully saline water than the turnover from a similar 1 ppt change at (say) 25 ppt. This motivates application of a *reverse logarithmic* transformation, $\log(36-s)$, or more precisely:

$$s^* = a - b \log(36 - s) \quad (11.2)$$

where $a = 8.33$, $b = 3$ are simple constants chosen for this data to constrain the transformed variable s^* to the range 1 (low) to 9 (high salinity). Fig. 11.4 then clearly

4. Superimposing environmental data onto an ordination is an option provided in the PRIMER program CONPLOT, which displays MDS configurations. The technique can also be useful in a wider context: Field *et al.* (1982) superimpose morphological characteristics of each species onto a *species* MDS of the type seen in Chapter 7, and Warwick and Clarke (1993a; see also Fig. 15.3) give an example of superimposition of biotic variables drawn from the same data matrix as used to create the MDS. The latter can provide insight into the role of individual taxa in shaping the biotic picture, especially when the number of taxa is small, as is the case for the phylum-level "meta-analysis" of Chapter 15.

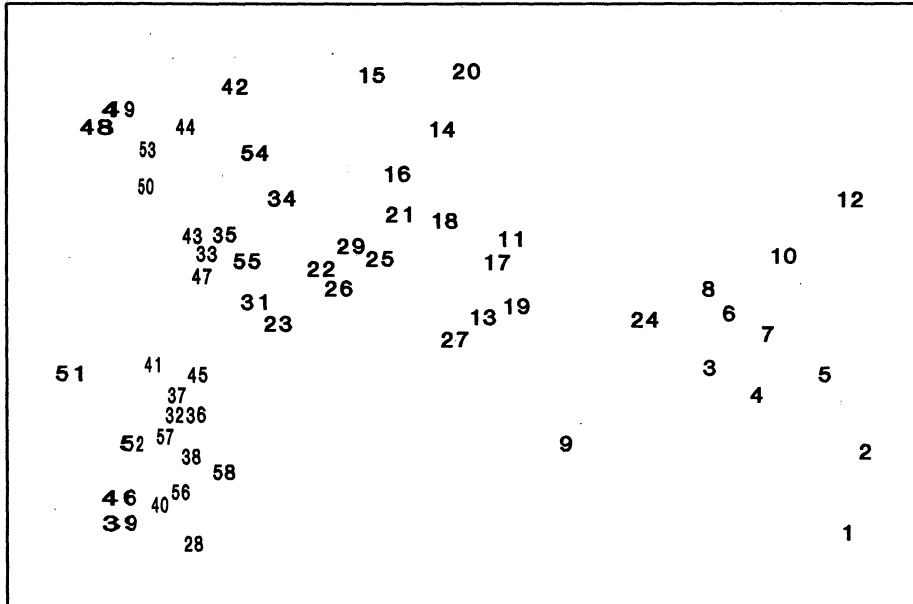


Fig. 11.3. Bristol Channel zooplankton {B}. Biotic MDS for the 57 sampling sites (1-29, 31-58) mapped in Fig. 3.2, from the same Bray-Curtis similarities on $\sqrt{1}$ -transformed abundances used for the cluster analysis of Fig. 3.3 (stress = 0.11).

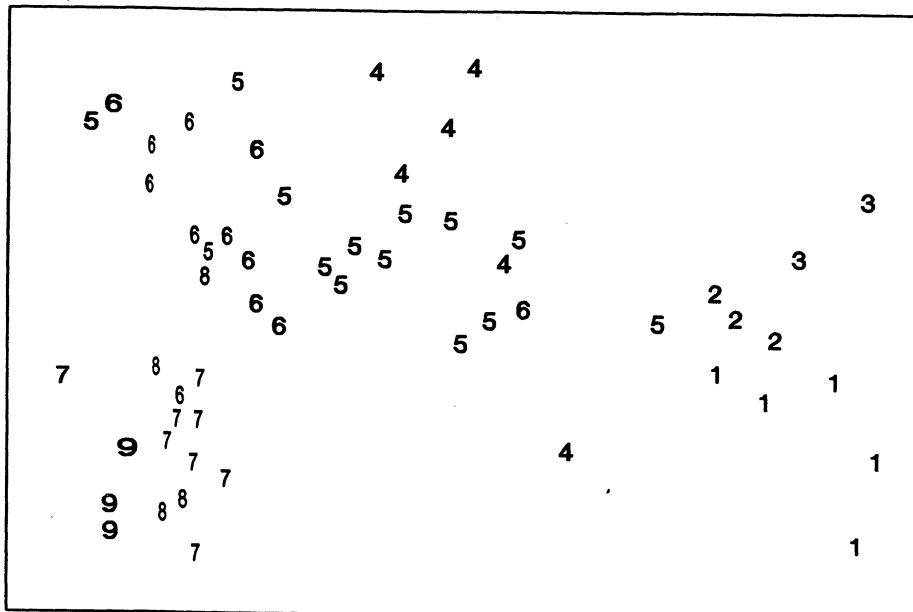


Fig. 11.4. Bristol Channel zooplankton {B}. MDS of Fig. 11.3, with superimposed codes representing increasing salinity levels at the 57 sites; 1: <26.3, 2: (26.3, 29.0), 3: (29.0, 31.0), ..., 8: (34.7, 35.1), 9: >35.1 ppt.

displays the strong correlation of the zooplankton community structure with the salinity gradient.⁵ It also helps to focus attention on sites which appear slightly anomalous in this respect, and raises

questions about whether there are secondary environmental variables which might explain the biological differentiation of samples at similar salinities.

5. Note the horseshoe effect (more properly termed the arch effect), which is a common feature of ordinations from single, strong environmental gradients. Both theoretically and empirically, non-metric MDS would seem to be less susceptible to this than metric ordination methods, but without the drastic (and somewhat arbitrary) intervention in the plot that a technique like detrended correspondence analysis invokes (specifically to "cut and paste" such ordinations to a straight line), some degree of curvature is unavoidable and natural. Where samples towards opposite ends of the environmental gradient have few species in common (thus giving dissimilarities near 100%), samples which are even further apart on the gradient have little scope to increase their dissimilarity further. To some extent, non-metric MDS can compensate for this by the flexibility of its monotonic regression of distance on dissimilarity (Chapter 5), but arching of the tails of the plot is clearly inevitable after dissimilarities of 100% are reached.

EXAMPLE: Garroch Head macrofauna

The macrofauna samples from the 12 stations on the Garroch Head transect (G) lead to the MDS plot of Fig. 11.5a. For a change, this is based not on abundance but biomass values (root-transformed).⁶ Earlier in the chapter, it was seen that the contaminant gradient induced a marked response in species diversity (Fig. 11.2), and there is an even more graphic representation of steady community change in the multivariate plots as the dump centre is approached (stations 1 through to 6), with gradual reversion to the original community structure on moving away from the centre (stations 6 through to 12). The correlation of the biotic pattern with particular contaminant variables is clearly illustrated by the superimposition technique introduced above; Fig. 11.5b displays the values of % carbon in the sediment (Table 11.1) as circles of varying diameter, which confirms the main axis of the biotic MDS as one of increasing organic enrichment. Several of the metal concentrations from Table 11.1 show a similar pattern, one exception being Mn, which displays a strong gradient in the other direction (Fig. 11.5c). In fact, some of the metal and organic variables are so highly correlated with each other (e.g. compare the plot for Pb in Fig. 11.5d with 11.5b) that there is little

point in retaining all of them in the environmental data matrix. Clearly, when two abiotic variables are so strongly related (*collinear*), separate putative effects on the biotic structure could never be disentangled (their effects are said to be *confounded*).

EXAMPLE: Exe estuary nematodes

The Garroch Head data is an example of a smooth gradation in faunal structure reflected in a matching gradation in several contaminant variables. In contrast, the Exe estuary nematode communities (X), discussed extensively in Chapter 5, separate into five well-defined clusters of samples (Fig. 11.6a). For each of the 19 intertidal sites, six environmental variables were also recorded: the median particle diameter of the sediment (MPD), its percentage organic content (% Org), the depth of the water table (WT) and of the blackened hydrogen sulphide layer (H₂S), the interstitial salinity (Sal) and the height of the sample on the shore, in relation to the inter-tidal range (Ht). When each of these is superimposed in turn on the biotic ordination, some instructive patterns emerge. MPD, represented appropriately by circles of differing size (Fig. 11.6b), appears to increase monotonically along the main MDS axis but cannot be responsible for the division, for example, between sites 1-4 and 7-9. On the other hand, the relation of salinity to the MDS configuration is non-monotonic (Fig. 11.6c), with larger values for the "middle" groups, but now providing a contrast between the 1-4 and 7-9 clusters. Other variables, such as the height up the shore,

6. Chapter 14 argues that, where it is available, biomass can sometimes be more biologically relevant than abundance, though in practice MDS plots from both will be broadly similar, especially under heavy transformation as the data tends towards presence/absence (Chapter 9).

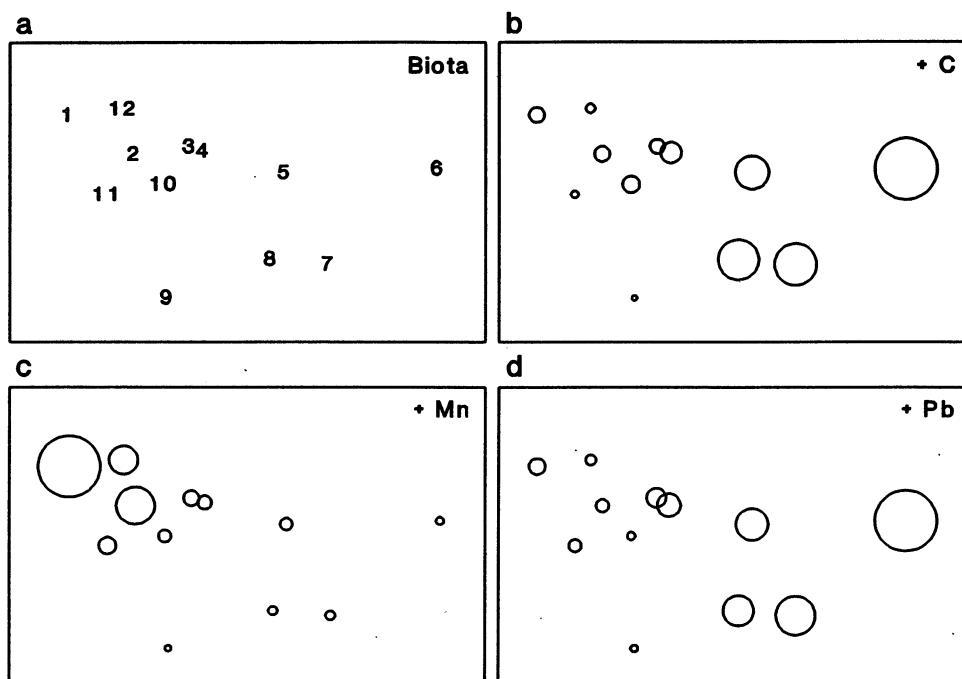


Fig. 11.5. Garroch Head macrofauna (G). a) MDS of Bray-Curtis similarities from $\sqrt{\text{}}$ -transformed species biomass data at the 12 stations (Fig. 8.3); b)-d) the same MDS but with superimposed circles of increasing size with increasing sediment concentration of C, Mn and Pb, from Table 11.1. (Stress = 0.05).

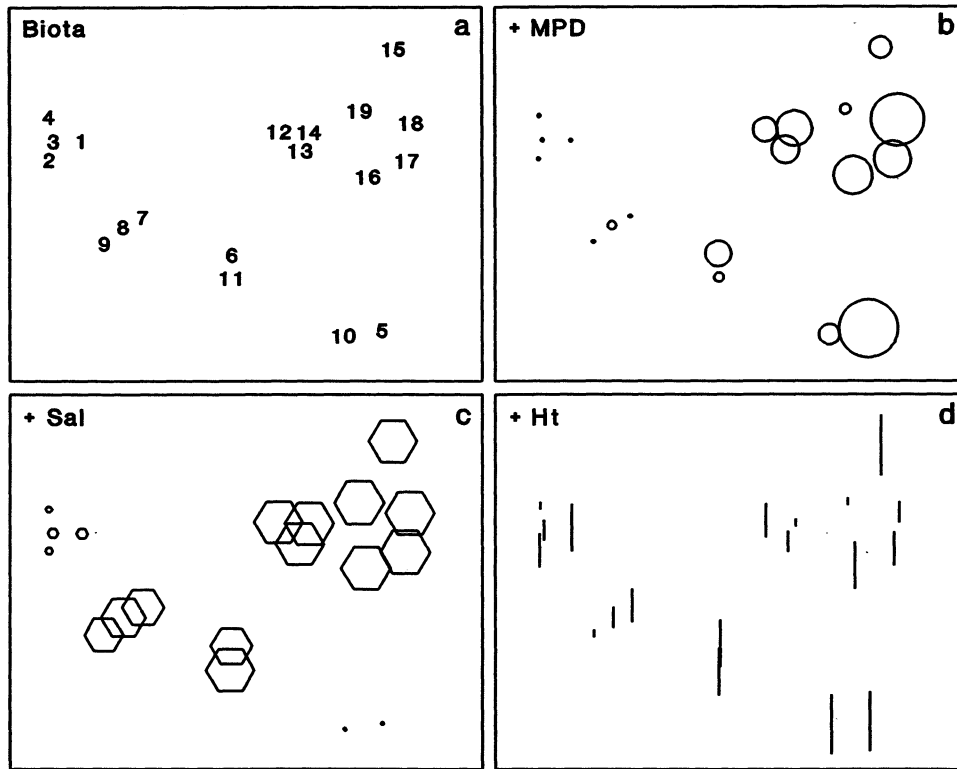


Fig. 11.6. Exe estuary nematodes (X). a) MDS of species abundances at the 19 sites, as in Fig. 5.1; b)–d) the same MDS but with superimposed symbols representing, respectively, median particle diameter of the sediment, its interstitial salinity and height up the shore of the sampling locations. (Stress = 0.05).

represented more appropriately by lines than circles (Fig. 11.6d), appear to bear little relation to the biotic structure, in that samples within the same faunal groups are frequently at opposite extremes of the intertidal range.

These plots, however, make clear the limitations in relating the community structure to a single environmental variable at a time: there is no basis for answering questions such as "how well does the full set of abiotic data jointly explain the observed biotic pattern" and "is there a subset of the environmental variables that explains the pattern equally well, or better?" These questions are answered in classical multivariate statistics by techniques such as canonical correlation (e.g. Mardia *et al.*, 1979) but, as discussed in earlier chapters, this requires assumptions which are unrealistic for species abundance or biomass data (correlation and Euclidean distance as measures of similarity for biotic data, linear relationships between abundance and environmental gradients etc.). Instead, the need is to relate community structure to multivariate descriptions of the abiotic variables, using the type of non-parametric, similarity-based methods of previous chapters.

LINKING BIOTA TO MULTIVARIATE ENVIRONMENTAL PATTERNS

The intuitive premise adopted here is that if the suite of environmental variables responsible for structuring the community were known, then samples having rather similar values for these variables would be expected to have rather similar species composition, and an ordination based on this abiotic information would group sites in the same way as for the biotic plot. If key environmental variables are omitted, the match between the two plots will deteriorate. By the same token, the match will also worsen if abiotic data which are irrelevant to the community structure are included.

The Exe estuary nematode data (X) again provides an appropriate example. Fig. 11.7a repeats the species MDS for the 19 sites seen in Fig. 11.6a. The remaining plots in Fig. 11.7 are of specific combinations of the six sediment variables: H₂S, Sal, MPD, % Org, WT and Ht, as defined above. For consistency of presentation, these plots are also MDS ordinations but based on an appropriate dissimilarity matrix (Euclidean distance on the normalised abiotic variables). In practice, since the number of variables is small, and the distance measures the same, the MDS plots will be largely indistinguishable from PCA configurations (note that

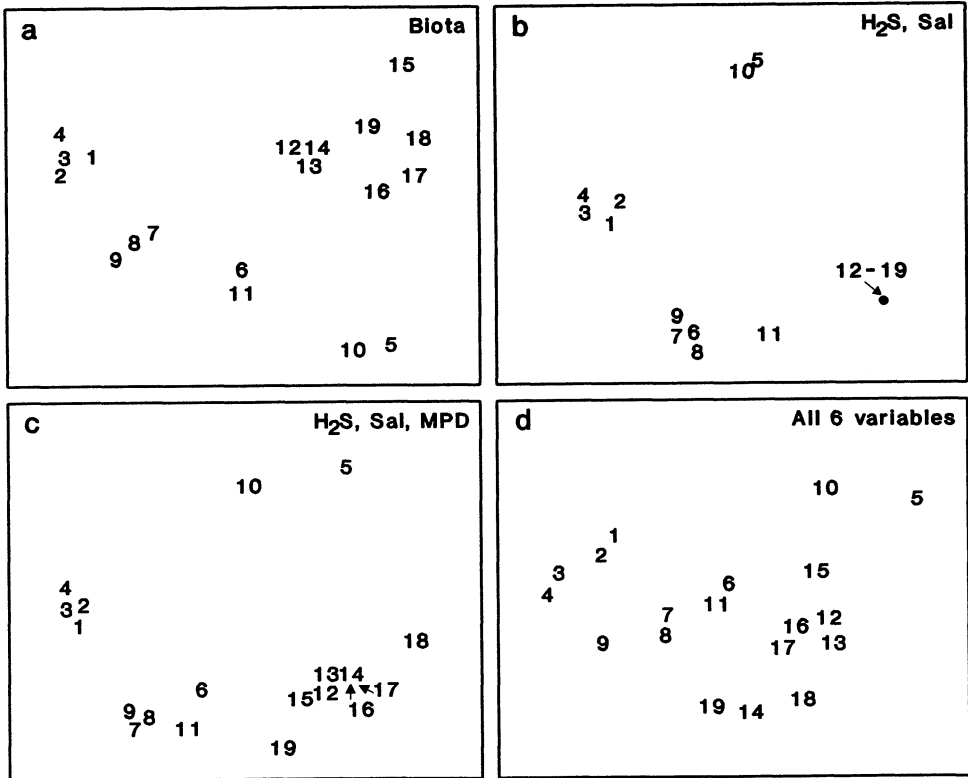


Fig. 11.7. *Exe estuary nematodes (X)*. MDS ordinations of the 19 sites, based on: a) species abundances, as in Fig. 5.1; b) two sediment variables, depth of the H₂S layer and interstitial salinity; c) the environmental combination "best matching" the biotic pattern: H₂S, salinity and median particle diameter; d) all six abiotic variables. (Stress = 0.05, 0, 0.04, 0.06).

Fig. 11.7b is effectively just a scatter plot, since it involves only two variables).

The point to notice here is the remarkable degree of concordance between biotic and abiotic plots, particularly Figs. 11.7a and c; both group the samples in very similar fashion. Leaving out MPD (Fig. 11.7b), the (7-9) group is less clearly distinguished from (6, 11) and one also loses some matching structure in the (12-19) group. Adding variables such as depth of the water table and height up the shore (Fig. 11.7d), the (1-4) group becomes more widely spaced than is in keeping with the biotic plot, sample 9 is separated from 7 and 8, sample 14 split from 12 and 13 etc., and the fit again deteriorates. In fact, Fig. 11.7c represents the *best fitting* environmental combination, in the sense defined below, and therefore best "explains" the community pattern.

Measuring agreement in pattern

Quantifying the match between any two plots could be accomplished by a Procrustes analysis (Gower, 1971), in which one plot is rotated, scaled or reflected to fit the other, in such a way as to minimize a sum of squared distances between the superimposed configurations. This is not wholly consistent, however, with the approach in earlier chapters; for exactly the same reasons as advanced in deriving the ANOSIM statistic in Chapter 6, the "best match" should not be

dependent on the dimensionality one happens to choose to view the two patterns. The more fundamental constructs are, as usual, the similarity matrices underlying both biotic and abiotic ordinations.⁷ These are chosen differently to match the respective form of the data (e.g. Bray-Curtis for biota, Euclidean distance for environmental variables) and will not be scaled in the same way. Their ranks, however, can be compared through a rank correlation coefficient, a very natural measure to adopt bearing in mind that a successful MDS is a function only of the similarity ranks.

The procedure is summarised schematically in Fig. 11.8, and Clarke and Ainsworth (1993) describe the approach in detail. Two possible matching coefficients are defined between the (unravell'd) elements of the respective rank similarity matrices ($r_i; i = 1, \dots, N$) and ($s_i; i = 1, \dots, N$), where $N = n(n-1)/2$ and n is the number of samples. These are the simple *Spearman coefficient* (e.g. Kendall, 1970):

7. For example, in spite of the very low stress in Fig. 11.7, a 2-d Procrustes fit of 11.7a and c will be rather poor, since the (5, 10) and (12-19) groups are interchanged between the plots. Yet, the interpretation of the two analyses is fundamentally the same (five clusters, with the (5, 10) group out on a limb etc.), and this will be fully expressed, without arbitrary dimensionality constraints, in the underlying similarity matrices.

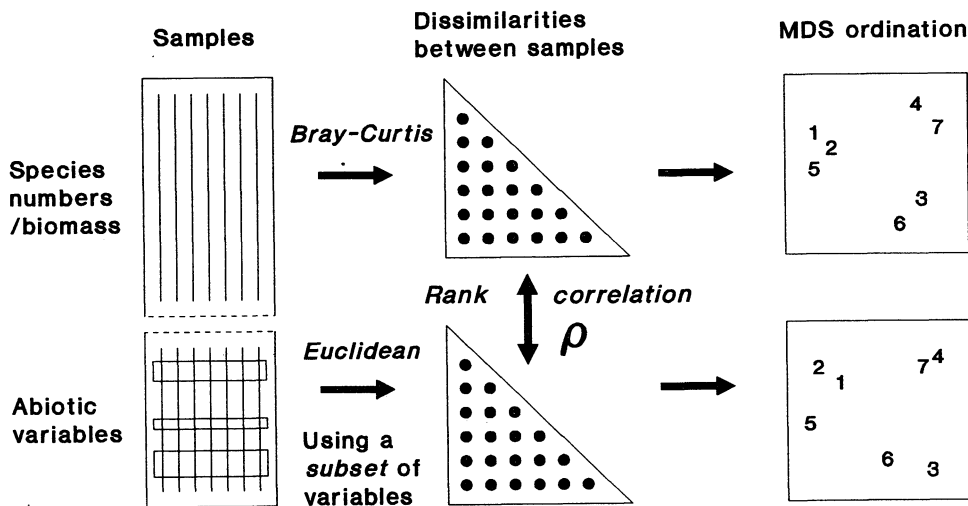


Fig. 11.8. Schematic diagram of the BIO-ENV procedure: selection of abiotic variable subset maximising rank correlation (ρ) between biotic and abiotic (dis)similarity matrices.

$$\rho_s = 1 - \frac{6}{N(N^2-1)} \sum_{i=1}^N (r_i - s_i)^2 \quad (11.3)$$

and a modified form of it, the *weighted Spearman* (or *harmonic*⁸) rank correlation:

$$\rho_w = 1 - \frac{6}{N(N-1)} \sum_{i=1}^N \frac{(r_i - s_i)^2}{r_i + s_i} \quad (11.4)$$

The constant terms are defined such that, in both cases, ρ lies in the range $(-1, 1)$, with the extremes of $\rho = -1$ and $+1$ corresponding to the cases where the two sets of ranks are in complete opposition or complete agreement, though the former is unlikely to be attainable in practice because of the constraints inherent in a similarity matrix. Values of ρ around zero correspond to the absence of *any* match between the two patterns, but typically ρ will be positive. It is tempting, but wholly wrong, to refer ρ_s to standard statistical tables of Spearman's rank correlation, to assess whether two patterns are "significantly" matched ($\rho > 0$). This is invalid because the ranks $\{r_i\}$ (or $\{s_i\}$) are not mutually independent variables, since they are based on a large number (N) of strongly interdependent similarity calculations.

In itself, this does not compromise the use of ρ_s as an index of agreement of the two triangular matrices. However, it could be less than ideal because few of the equally-weighted difference terms in equation (11.3)

involve "nearby" samples. In contrast, the premise at the beginning of this section makes it clear that we are seeking a combination of environmental variables which attains a good match of the *high* similarities (low ranks) in the biotic and abiotic matrices. The value of ρ_s , when computed from triangular similarity matrices, will tend to be swamped by the larger number of terms involving "distant" pairs of samples, contributing large squared differences in (11.3). This motivates the down-weighting denominator term in (11.4), which on limited evidence (Clarke and Ainsworth, 1993) does appear to have the desired practical effect.

The BIO-ENV procedure

The matching of biotic to environmental patterns can now take place⁹, as outlined schematically in Fig. 11.8. Combinations of environmental variables are considered at steadily increasing levels of complexity, i.e. k variables at a time ($k = 1, 2, 3, \dots, v$). Table 11.2 displays the outcome for the Exe estuary nematodes. The *single* abiotic variable which best groups the sites, in a manner consistent with the faunal patterns, is the depth of the H_2S layer ($\rho_w = 0.62$); next best is the organic content ($\rho_w = 0.54$), etc. Of course, since the faunal ordination is not essentially 1-dimensional (Fig. 11.7a), one would not expect a single environmental variable to provide a very successful match, though knowledge of the H_2S variable alone does distinguish points to the left and right of Fig. 11.7a (samples 1 to 4

8. This is so defined by Clarke and Ainsworth (1993) because it is algebraically related to the average of the harmonic mean of each (r_i, s_i) pair. The denominator term, $r_i + s_i$, down-weights the contribution of large ranks; note that these are the low similarities, the highest similarity corresponding to the lowest value of rank similarity (1), as usual.

9. This is implemented in the PRIMER program BIOENV, which allows either a full search over all abiotic variable combinations or over specified subsets, e.g. all combinations containing certain variables or containing a fixed number of variables.

and 6 to 9 have lower values than for samples 5, 10 and 12 to 19, with sample 11 intermediate).

The best 2-variable combination also involves depth of the H₂S layer but adds the interstitial salinity. The correlation ($\rho_w = 0.76$) is markedly better than for any other 2-variable subset, and this is the combination shown in Fig. 11.7b. The best 3-variable combination retains these two but adds the median particle diameter, and gives the overall optimum value for ρ_w of 0.80 (Fig. 11.7c); ρ_w drops slightly to 0.79 for the best 4- and higher-way combinations. The results in Table 11.2 do therefore seem to accord with the visual impressions in Fig. 11.7.¹⁰ In this case, the first column of Table 11.2 has a hierarchical structure: the best combination at one level is always a subset of the best combination on the line below. This is not guaranteed (although it seems to happen surprisingly often) since all combinations have been evaluated and simply ranked.

Table 11.2 *Exe estuary nematodes (X). Combinations of the 6 environmental variables, taken k at a time, yielding the best matches of biotic and abiotic similarity matrices for each k, as measured by weighted Spearman rank correlation ρ_w ; bold type indicates overall optimum. See earlier text for variable abbreviations.*

| k | Best variable combinations (ρ_w) |
|---|---|
| 1 | H ₂ S %Org Sal ... (.62) (.54) (.53) |
| 2 | H ₂ S, Sal H ₂ S, MPD H ₂ S, %Org Sal, %Org ... (.76) (.67) (.65) (.61) |
| 3 | H ₂ S, Sal, MPD H ₂ S, Sal, %Org H ₂ S, Sal, WT ... (.80) (.75) (.73) |
| 4 | H ₂ S, Sal, MPD, %Org H ₂ S, Sal, MPD, Ht ... (.79) (.78) |
| 5 | H ₂ S, Sal, MPD, %Org, Ht ... (.79) |
| 6 | H ₂ S, Sal, MPD, %Org, Ht, WT (.77) |

10. This will not always be the case if the 2-d faunal ordination has non-negligible stress. It is the matching of the similarity matrices which is definitive, although it would usually be a good idea to plot the abiotic ordination for the best combination at each value of k, in order to gauge the effect of a small change in ρ_w on the interpretation. Experience so far suggests that combinations giving the same value of ρ_w to two decimal places do not give rise to ordinations which are distinguishable in any practically important way, thus it is recommended that ρ_w is quoted only to this accuracy, as in Table 11.2.

An exhaustive search over v variables involves

$$\sum_{k=1}^v \frac{v!}{k!(v-k)!} = 2^v - 1 \quad (11.5)$$

combinations, i.e. 63 for the Exe estuary study, though this number quickly becomes prohibitive when v is larger than 11 or 12. Above that level, one could consider stepwise (and related) procedures which search in a more hierarchical fashion, adding and deleting variables one at a time. These are not guaranteed to find the global minimum of ρ , and run the significant risk of focussing attention on a single "best" combination when, in reality, there may be very many combinations giving an essentially similar match to the biota. In practice, it may be desirable to limit the scale of the search initially, for a number of reasons, e.g. always to include a variable known from previous experience or external information to be potentially causal. Alternatively, as discussed earlier, scatter plots of the environmental variables may demonstrate that some are highly inter-correlated and nothing in the way of improved "explanation" could be achieved by entering them all into the analysis.

An example is given by the Garroch Head macrofauna study (G), for which the 11 abiotic variables of Table 11.1 are first transformed, to validate the use of Euclidean distances and standard product-moment correlations (page 11-2), and then examined for evidence of collinearity (page 11-5). A possible rule-of-thumb would be to reduce all subsets of (transformed) variables which have mutual correlations averaging more than about 0.95 (say) to a single representative. Here, this leaves 8 abiotic variables in the full BIO-ENV search, which results in an optimal match of the biotic pattern with C, N and Cd ($\rho_w = 0.78$). The corresponding ordination plots are seen in Fig. 11.9. The biotic MDS of Fig. 11.9a, though structured mainly by a single strong gradient towards the dump centre (e.g. the organic enrichment gradient seen in Fig. 11.9b), is not wholly 1-dimensional. Additional information, on a heavy metal, appears to improve the "explanation".

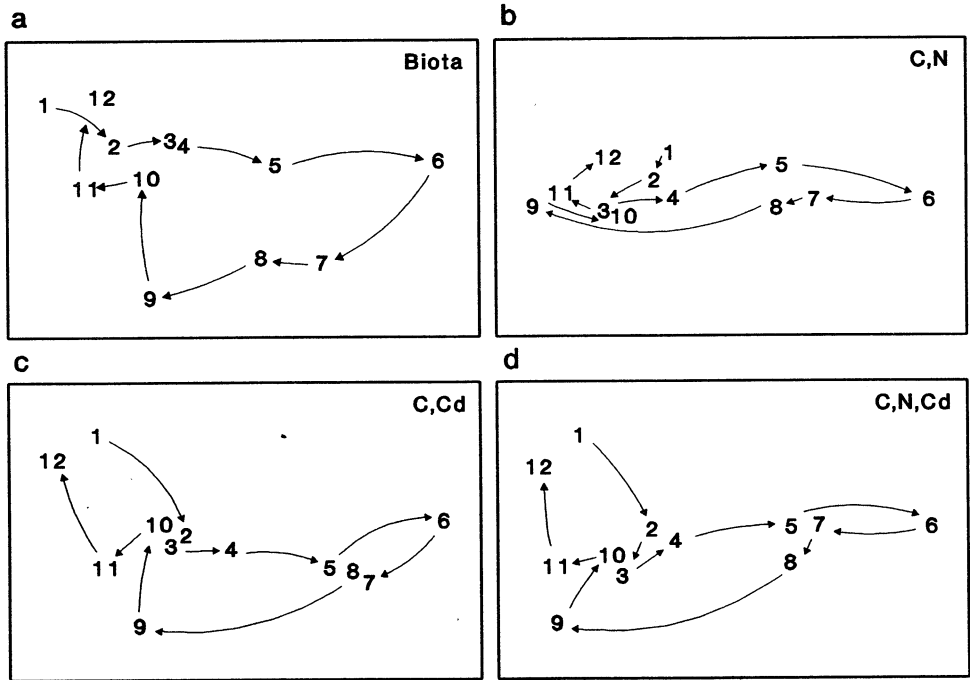


Fig. 11.9. Garroch Head macrofauna (G). MDS plots for the 12 sampling stations across the sewage-sludge dump site (Fig. 8.3), based on: a) species biomass, as in Fig. 11.5a; b)-d) three combinations of carbon, nitrogen and cadmium concentrations (log transformed) in the sediments, the optimum match with the biota over all combinations of the 8 variables being for C, N and Cd ($\rho_w = 0.78$). (Stress = 0.05, 0, 0.01, 0.01).

CONCLUDING REMARKS

Testing

Further examples of the BIO-ENV procedure are given in Clarke and Ainsworth (1993), Clarke (1993), Somerfield *et al.* (1994) and others, though its practical utility and theoretical applicability have not yet been extensively investigated.¹¹ One question which naturally arises is the extent to which the conclusions can be supported by significance tests. This is problematic given the lack of model assumptions underlying the above procedure, which can be seen as both a strength (generality, ease of understanding, simplicity of interpretation) and a weakness (lack of a structure for formal statistical inference). One simple test available¹² is of the null hypothesis that there is *no* relationship between the biotic information and a specific abiotic pattern, i.e. that ρ_w is effectively zero. This can be examined by a simple permutation/randomisation test, of a type met previously in Chapter 6 (Fig. 6.9), in which ρ_w is recomputed for all (or a random subset of) permutations of the sample labels in one of the two underlying similarity matrices.

As usual, if the observed value of ρ_w exceeds that found in 95% of the simulations, which by definition correspond to unrelated ordinations, then the null hypothesis can be rejected at the 5% level. Note however that this is *not* a valid procedure if the abiotic set being tested against the biotic pattern is the result of optimal selection by the BIO-ENV procedure, on the *same* data. For v variables, this is implicitly equivalent to carrying out $2^v - 1$ null hypothesis tests, each of which potentially runs a 5% risk of *Type I error* (rejecting the null hypothesis when it is really true). One could attempt to allow for this by setting a much more stringent significance level for the test of the optimal combination¹³ but, in practice, this null hypothesis (RELATE) test is rather an irrelevance here: the BIO-ENV procedure is best thought of as an *exploratory* tool. A more convincing *confirmatory* strategy is to use an initial set of data to suggest an optimal combination of abiotic variables, and an *independent* data set, utilising *only* that reduced number of variables, in a RELATE test or a second BIO-ENV analysis. If there were any variables featuring marginally and arbitrarily in the first run of BIOENV, they would be unlikely to do so again on the second run.

11. And may prove illusory; see Clarke and Ainsworth (1993) for further discussion of its advantages/drawbacks in relation to model-based techniques such as canonical correlation and canonical correspondence.
12. This is implemented in the PRIMER program RELATE; see also Chapter 15.

13. Such as given by the Bonferroni correction which, at its simplest, would demand a significance level of $5/(2^v - 1)\%$ for an overall 5% level test of the optimal combination, though this is a highly conservative procedure given the interdependence of the tests.

Design

Two final points can be made about the sampling design. The general subject of experimental and field survey design is an immense one, requiring a manual of its own¹⁴. It is also a problematic area for many of the (non-parametric) multivariate techniques because the lack of formal model structures makes it difficult to define *power* of statistical procedures, such as the randomisation tests described above and in Chapters 6 and 15. In the context of linking biotic and abiotic patterns, it is intuitively clear that this has the greatest prospect of success if there are a moderately large number of sample conditions, and the closest possible matching of environmental with biological data. In the case of a number of replicates from each of a number of sites, this could imply that the biotic samples, which would be well-separated in order to represent genuine variation at a site, would each have a closely-matched environmental replicate.

Another lesson of the earlier Garroch Head example is the difficulty of drawing conclusions about causality from *any* observational study. In that case, a subset of abiotic variables were so highly correlated with each other that it was desirable to omit all but one of them from the computations. There may sometimes be good external reasons for retaining a particular number of

the set but, in general, one of them is chosen arbitrarily as a *proxy* for the rest. If that variable does appear to be linked to the biotic pattern then any member of the subset could be implicated, of course. More importantly, there cannot be a definitive *causal* implication here, since each retained variable is also a proxy for any potentially causal variable which correlates highly with it, but remains *unmeasured*. Clearly, in an environmental impact study, a design in which the main pollution gradient (e.g. chemical) is highly correlated with variations in some natural environmental measures (e.g. salinity, sediment structure), cannot be very informative, whether the latter variables are measured or not. A desirable strategy, particularly for the non-parametric multivariate analyses considered here, is to limit the influence of important natural variables by attempting to select sites which have the same environmental conditions but a range of contaminant impacts (including control sites¹⁵ of course). Even then, in a purely observational study one can never entirely escape the stricture that any apparent change in community, with changing pollution impact, could be the result of an unmeasured natural variable with which the contaminant levels happen to correlate. Such issues of causality motivate the following chapter on experimental approaches.

14. Green (1979) provides some useful guidelines, mainly in the context of univariate analyses:

15. Note the plurality; Underwood (1992) argues persuasively that impact is best established against a baseline of site-to-site variability in control conditions.

CHAPTER 12: CAUSALITY: COMMUNITY EXPERIMENTS IN THE FIELD AND LABORATORY

In Chapter 11 we have seen how both univariate and multivariate community attributes can be correlated with natural and anthropogenic environmental variables. With careful sampling design, these methods can provide strong evidence as to which environmental variables appear to affect community structure most, but they cannot actually *prove* cause and effect. In experimental situations we can investigate the effects of a single factor (the *treatment*) on community structure, while other factors are held constant or controlled, thus establishing cause and effect. There are three main categories of experiments that can be used:

- 1) '*Natural experiments*'. Nature provides the treatment: i.e. we compare places or times which differ in the intensity of the environmental factor in question.
- 2) '*Field experiments*'. The experimenter provides the treatment: i.e. environmental factors (biological, chemical or physical) are manipulated in the field.
- 3) '*Laboratory experiments*'. Environmental factors are manipulated by the experimenter in laboratory mesocosms or microcosms.

The degree of 'naturalness' (hence realism) *decreases* from 1-3, but the degree of control which can be exerted over confounding environmental variables *increases* from 1-3.

In this chapter, each class of experiments is illustrated by a single example. Unfortunately all these concern the meiobenthos, since this component of the biota is very amenable to community level experiments (see Chapter 13), whereas experiments with other components of the biota have mainly been concerned with populations of individual species, rather than communities.

In all cases care should be taken to avoid *pseudoreplication*, i.e. the *treatments* should be replicated, rather than a series of 'replicate' samples taken from a single treatment (pseudoreplicates, e.g. Hurlbert, 1984). This is because other confounding variables, often unknown, may also differ between the treatments. It is also important to run experiments long enough for community changes to occur: this favours components of the fauna with short generation times (see Chapter 13).

'NATURAL EXPERIMENTS'

It is arguable whether so called *natural experiments* are actually experiments at all, and not simply well-designed field surveys, since they make comparisons of places or times which differ in the intensity of the particular environmental factor under consideration. The obvious logical flaw with this approach is that its validity rests on the assumption that places or times differ only in the intensity of the selected environmental factor (treatment); there is no possibility of *randomly allocating treatments to experimental units*, the central tool of experimentation and one that ensures that the potential effects of unmeasured, uncontrolled variables are averaged out across the experimental groups. Design is often a problem, but statistical techniques such as two-way ANOVA, e.g. Sokal and Rohlf (1981), or two-way ANOSIM (Chapter 6), may enable us to examine the treatment effect allowing for differences between sites, for example. This is illustrated in the first example below.

In some cases natural experiments may be the only possible approach for hypothesis testing in community ecology, because the attribute of community structure under consideration may result from *evolutionary mechanisms* rather than *ecological mechanisms*, and we obviously cannot conduct manipulative field or laboratory experiments over evolutionary time. One example of a community attribute which may be determined by evolutionary mechanisms relates to size spectra in marine benthic communities. Several hypotheses, some complementary and some contradictory, have been invoked to explain biomass size spectra and species size distributions in the metazoan benthos, both of which have bimodal patterns in shallow temperate shelf seas. Ecological explanations involve physical constraints of the sedimentary environment, animals needing to be small enough to move between the particles (i.e. interstitial) or big enough to burrow, with an intermediate size range capable of neither (Schwinghamer, 1981). Evolutionary explanations invoke the optimisation of two size-related sets of reproductive and feeding traits: for example small animals (meiobenthos) have direct benthic development and can be dispersed as adults, large animals (macrobenthos) have planktonic larval development and

dispersal, there being no room for compromise (Warwick, 1984). To test these hypotheses we can compare situations where the causal mechanisms differ and therefore give rise to different predictions about pattern. For example, the reproductive dichotomy noted above between macrobenthos and meiobenthos breaks down in the deep-sea, in polar latitudes and in fresh water, although the physical sediment constraints in these situations will be the same as in temperate shelf seas. The evolutionary hypothesis therefore predicts that there should be a unimodal pattern in these situations, whereas the ecological hypothesis predicts that it should remain bimodal. Using these situations as natural experiments, we can therefore falsify one or the other (or both) of these hypotheses.

However, natural experiments of this kind extend outside the purpose of this manual, and our chosen example concerns the ecological effects of disturbance on community structure.

The effects of disturbance by soldier crabs (*Mictyris platycheles*) on meiobenthic community structure (T)

On a sheltered intertidal sandflat at Eaglehawk Neck, on the Tasman Peninsula in S.E. Tasmania, the burrowing and feeding activities of the soldier crab *Mictyris platycheles* are evidenced as intensely disturbed areas of sediment which form discrete patches interdispersed with smooth undisturbed areas. The

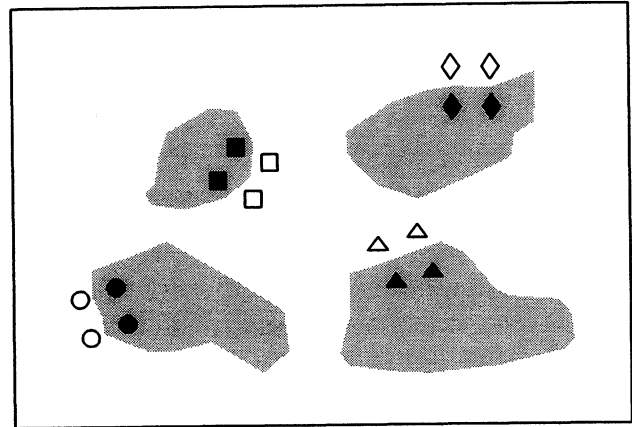


Fig. 12.1. Tasmania, Eaglehawk Neck (T). Sketch showing the type of sample design. Sample positions (same symbols as in Fig. 12.3) in relation to disturbed sediment patches (shaded).

crabs feed by manipulating sand grains in their mandibles and removing fine particulate material, but they are not predators on the meiofauna, although their feeding and burrowing activity results in intense sediment disturbance. This situation was used as a 'natural experiment' on the effects of disturbance on meiobenthic community structure. Meiofauna samples were collected in a spatially blocked design, such that each block comprised two disturbed and two undisturbed samples, each 5m apart (Fig. 12.1).

Univariate indices. The significance of differences between disturbed and undisturbed samples (treatments) was tested with two-way ANOVA (blocks/treatments), Table 12.1.

Table 12.1. Tasmania, Eaglehawk Neck (T). Mean values per core sample of univariate measures for nematodes, copepods and total meiofauna (nematodes + copepods) in the disturbed and undisturbed areas. The significance levels for differences are from a two-way ANOVA, i.e. they allow for differences between blocks, although these were not significant at the 5% level.

| | Total individuals (N) | Total species (S) | Species richness (d) | Shannon diversity (H') | Species evenness (J') |
|------------------------|-----------------------|-------------------|--------------------------|----------------------------|---------------------------|
| Nematodes | | | | | |
| Disturbed | 205 | 14.4 | 2.6 | 1.6 | 0.58 |
| Undisturbed | 200 | 20.1 | 3.7 | 2.2 | 0.74 |
| Significance (%) | 91 | 1 | 0.3 | 0.1 | 1 |
| Copepods | | | | | |
| Disturbed | 94 | 5.4 | 1.0 | 0.96 | 0.59 |
| Undisturbed | 146 | 5.7 | 1.0 | 0.84 | 0.49 |
| Significance (%) | 11 | 52 | 99 | 52 | 38 |
| Total meiofauna | | | | | |
| Disturbed | 299 | 19.8 | 3.4 | 2.0 | 0.66 |
| Undisturbed | 346 | 25.9 | 4.4 | 2.3 | 0.69 |
| Significance (%) | 48 | 1 | 3 | 3 | 16 |

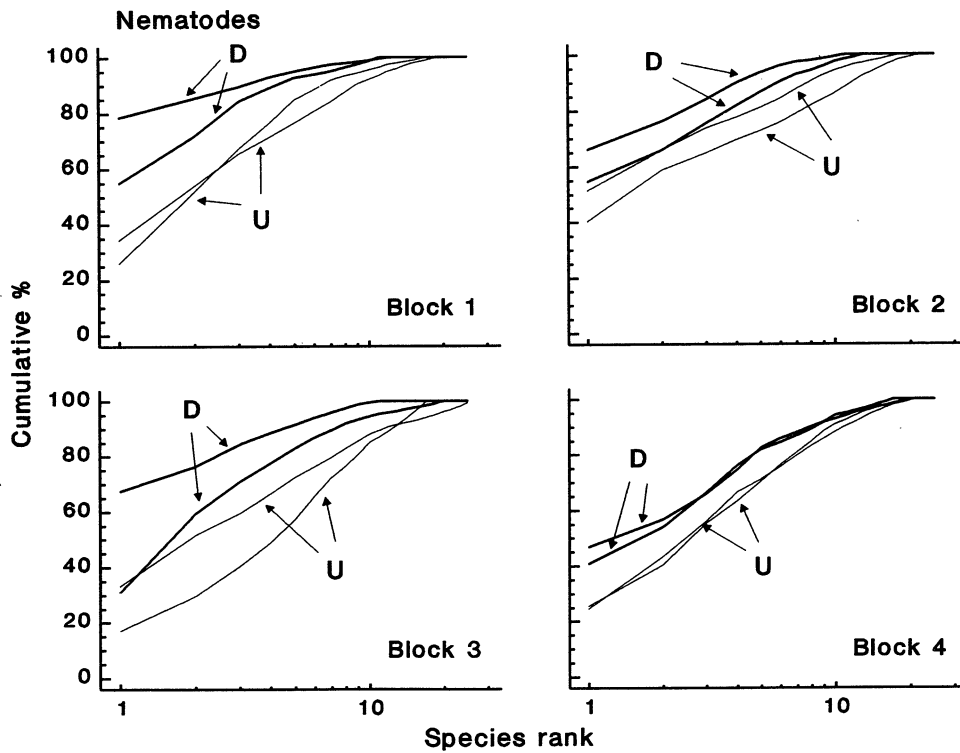


Fig. 12.2. Tasmania, Eaglehawk Neck (T). Replicate *k*-dominance curves for nematode abundance in each sampling block. D = disturbed, U = undisturbed.

For the nematodes, species richness, species diversity and evenness were significantly reduced in disturbed as opposed to undisturbed areas, although total abundance was unaffected. For the copepods, however, there were no significant differences in any of these univariate measures.

Graphical/distributional plots. *k*-dominance curves (Fig. 12.2) also revealed significant differences in the relative species abundance distributions for nematodes (using both the ANOVA and ANOSIM-based tests referred to briefly at the end of Chapter 8, and detailed in Clarke, 1990). For the copepods, however,

(plots given in Chapter 13, Fig. 13.4), *k*-dominance curves are intermingled and crossing, and there is no significant treatment effect.

Multivariate ordinations. MDS revealed significant differences in species composition for both nematodes and copepods: the effects of crab disturbance were similar within each block and similar for nematodes and copepods. Note the similarities in Fig. 12.3 between the nematode and copepod configurations: both disturbed samples within each block are above both undisturbed (except for one block for the copepods), and the blocks are arranged in sequence

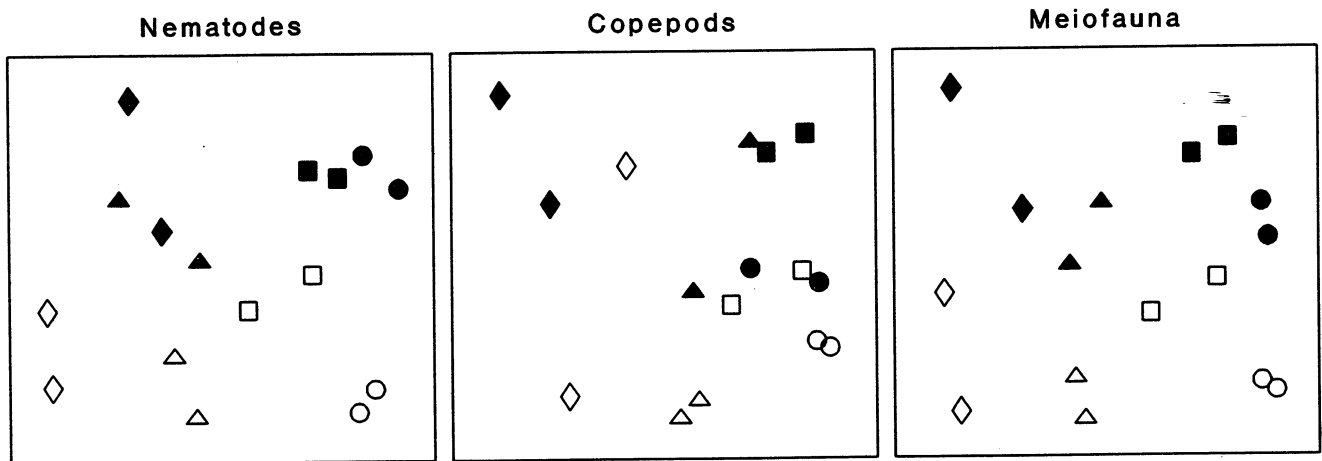


Fig. 12.3. Tasmania, Eaglehawk Neck (T). MDS configurations for nematode, copepod and 'meiofauna' (nematode + copepod) abundance (root-transformed). Different shapes represent the four blocks of samples. Open symbols = undisturbed, filled = disturbed (stress = 0.12, 0.09, 0.11 respectively).

across the plot. For both nematodes and copepods, two-way ANOSIM shows a significant effect of both treatment (disturbance) and blocks, Table 12.2, but the differences are more marked for the nematodes (with higher values of the R statistic).

Table 12.2 Tasmania, Eaglehawk Neck (T). Results of the two-way ANOSIM test for treatment (disturbance/no disturbance) and block effects.

| | Disturbance | | Blocks | |
|-----------|-------------|---------|--------|---------|
| | R | Sig.(%) | R | Sig.(%) |
| Nematodes | 1.0 | 1.2 | 0.85 | 0.0 |
| Copepods | 0.56 | 3.7 | 0.62 | 0.0 |
| Meiofauna | 0.94 | 1.2 | 0.85 | 0.0 |

Conclusions. Univariate indices and graphical/distributional plots were only significantly affected by crab disturbance for the nematodes. Multivariate analysis revealed a similar response for nematodes and copepods (i.e. it seems to be a more sensitive measure of community change). In multivariate analyses, natural variations in species composition across the beach (i.e. between blocks) were about as great as those between treatments within blocks, and the disturbance effect would not have been clearly evidenced without this block sampling design.

FIELD EXPERIMENTS

Field manipulative experiments include, for example, caging experiments to exclude or include predators, controlled pollution of experimental plots, and big-bag experiments with plankton. So far, they have mostly been used for *population* rather than *community* studies but the following example is one in which univariate, graphical and multivariate statistical analyses have been applied to meiobenthic communities.

Azoic sediment recolonisation experiment with predator exclusion (Z)

Olafsson and Moore (1992) studied meiofaunal colonisation of azoic sediment in a variety of cages designed to exclude epibenthic macrofauna to varying degrees: A – 1 mm mesh cages designed to exclude all macrofauna; B – 1 mm control cages with two ends left open; C – 10 mm mesh cages to exclude only larger macrofauna; D – 10 mm control cages with two ends left open; E – open unmeshed cages; F – uncaged

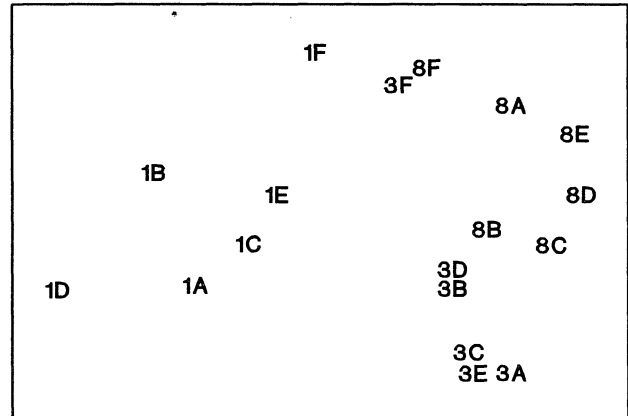


Fig. 12.4. Azoic sediment recolonisation experiment (Z). MDS configuration for harpacticoid copepods (4th root transformed abundances) after 1, 3 and 8 months, with 6 different treatments (A–F), see text (stress = 0.07).

background controls. Three replicates of each treatment were sampled after 1 month, 3 months and 8 months and analysed for nematode and harpacticoid copepod species composition.

Univariate indices. The presence of cages had a more pronounced impact on copepod diversity than nematode diversity. For example, after 8 months, H' and J' (but not S) for copepods had significantly higher values inside the exclusion cages than in the control cages with the ends left open, but for the nematodes, differences in H' were of borderline significance ($p = 5.3\%$).

Graphical/distributional plots. No significant treatment effect for either nematodes or copepods could be detected between k -dominance curves for all sampling dates, using the ANOSIM test referred to on page 8–12.

Multivariate analysis. For the harpacticoid copepods there was a clear successional pattern of change in community composition over time (Fig. 12.4) but no such pattern was obvious for the nematodes. Fig. 12.4 uses data from Table 2 in Olafsson and Moore's paper, which are for the 15 most abundant harpacticoid species in all treatments and for the mean abundances of all replicates within a treatment on each sampling date. On the basis of these data, there is no significant treatment effect using the 2-way ANOSIM test for one replicate per cell¹ (see page 6–8), but the fuller replicated data may have been more revealing.

1. This is the PRIMER ANOSIM2 test, which will be uninformative in the presence of sizeable treatment/time interactions, a likely possibility here.

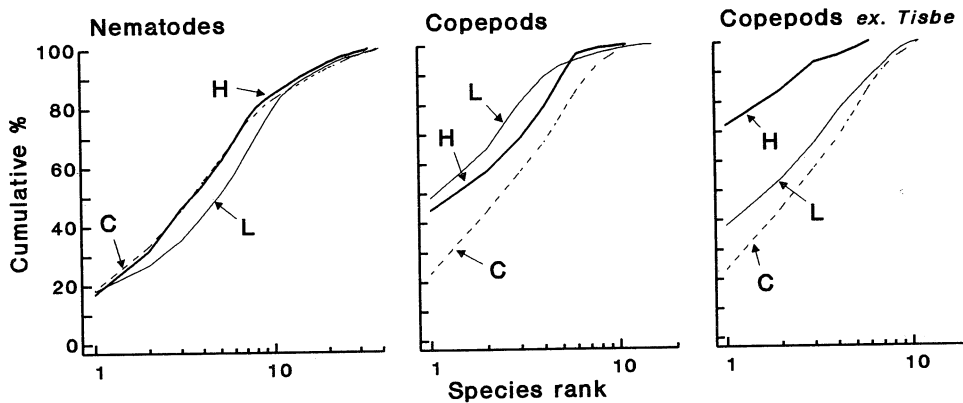


Fig. 12.5. Nutrient enrichment experiment (N). *k*-dominance curves for nematodes, total copepods and copepods omitting the 'weed' species of *Tisbe*, for summed replicates of each treatment. C = control, L = low and H = high dose.

LABORATORY EXPERIMENTS

More or less natural communities of some components of the biota can be maintained in laboratory (and also outdoor) experimental containers and subjected to a variety of manipulations. Many types of experimental systems have been used for marine studies, ranging from microcosms (containers less than 1 m³) to mesocosms (1–1000 m³). Macrocosms (larger than 10³ m³), usually involving the artificial enclosure of natural areas in the field, have also been used, but so far mainly for research on fish.

Effects of organic enrichment on meiofaunal community structure (N)

Gee *et al.* (1985) collected undisturbed box cores of sublittoral sediment and transferred them to the experimental mesocosms established at Solbergsstrand, Oslofjord, Norway. They effected organic enrichment by the addition of powdered *Ascophyllum nodosum* in quantities equivalent to 50 g C m⁻² (four replicate boxes) and 200 g C m⁻² (four replicate boxes), with four undosed boxes as controls, in a randomised design within one of the large mesocosm basins. After 56 days, five small core samples of sediment were taken from each box and combined to give one sample. The structure of the meiofaunal communities in these samples was then compared.

Univariate indices. Table 12.3 shows that, for the *nematodes*, there were no significant differences in species richness or Shannon diversity between treatments, but evenness was significantly higher in enriched boxes than controls. For the *copepods*, there were significant differences in species richness and evenness between treatments, but not in diversity.

Graphical/distributional plots. Fig. 12.5 shows the average *k*-dominance curves over all four boxes in each treatment. For the *nematodes* these are closely coincident, suggesting no obvious treatment effect.

For the copepods, however, there are apparent differences between the curves. A feature of the copepod assemblages in the enriched boxes was the presence, in highly variable numbers, of several species of the large epibenthic harpacticoid *Tisbe*, which are 'weed' species often found in old aquaria and associated with organic enrichment. If this genus is omitted from the analysis, a clear sequence of increasing elevation of the *k*-dominance curves is evident from control to high dose boxes.

Table 12.3. Nutrient-enrichment experiment (N). Univariate measures for all replicates at the end of the experiment, with the *F*-ratio and significance levels from one-way ANOVA.

| | Species richness (<i>d</i>) | Shannon diversity (<i>H'</i>) | Species evenness (<i>J'</i>) |
|---------------------------|-------------------------------|---------------------------------|--------------------------------|
| Nematodes | | | |
| Control | 3.02 | 2.25 | 0.750 |
| | 3.74 | 2.39 | 0.774 |
| | 3.36 | 2.47 | 0.824 |
| | 4.59 | 2.76 | 0.747 |
| Low dose | 4.39 | 2.86 | 0.877 |
| | 2.65 | 2.47 | 0.840 |
| | 4.67 | 2.89 | 0.875 |
| | 2.33 | 2.27 | 0.860 |
| High dose | 2.86 | 2.17 | 0.782 |
| | 2.82 | 2.39 | 0.843 |
| | 4.30 | 2.40 | 0.829 |
| | 4.09 | 2.47 | 0.853 |
| <i>F</i> ratio | 0.04 | 1.39 | 5.13 |
| Significance (<i>p</i>) | ns | ns | <5% |
| Copepods | | | |
| Control | 2.53 | 1.93 | 0.927 |
| | 1.92 | 1.56 | 0.969 |
| | 2.50 | 1.77 | 0.908 |
| | 2.47 | 1.94 | 0.931 |
| Low dose | 1.80 | 1.60 | 0.643 |
| | 1.66 | 1.28 | 0.532 |
| | 1.66 | 1.16 | 0.484 |
| | 1.79 | 1.54 | 0.640 |
| High dose | 1.75 | 1.59 | 0.767 |
| | 0.97 | 1.00 | 0.620 |
| | 1.03 | 0.30 | 0.165 |
| | 1.18 | 1.70 | 0.872 |
| <i>F</i> ratio | 17.72 | 2.65 | 4.56 |
| Significance (<i>p</i>) | <0.1% | ns | <5% |

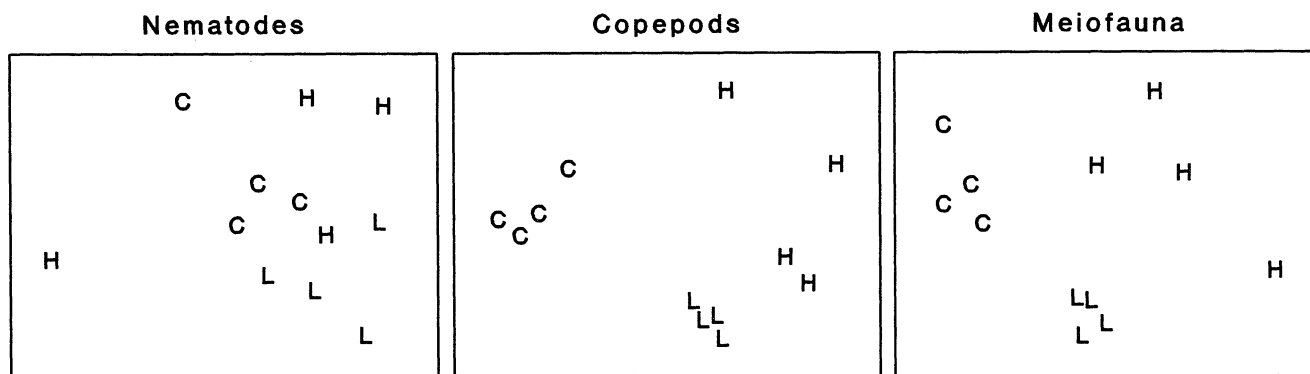


Fig. 12.6. Nutrient enrichment experiment (N). MDS of $\sqrt{\sqrt{}}$ -transformed abundances of nematodes, copepods and total meiofauna (nematodes + copepods). C = control, L = low dose, H = high dose (stress = 0.18, 0.09, 0.12).

Table 12.4. Nutrient enrichment experiment (N). Values of the R statistic from the ANOSIM test, in pairwise comparisons between treatments, together with significance levels. C = control, L = low dose, H = high dose.

| Treatment | Statistic value (R) | % Sig level |
|------------------|---------------------|-------------|
| Nematodes | | |
| (L, C) | 0.27 | 2.9 |
| (H, C) | 0.22 | 5.7 |
| (H, L) | 0.28 | 8.6 |
| Copepods | | |
| (L, C) | 1.00 | 2.9 |
| (H, C) | 0.97 | 2.9 |
| (H, L) | 0.59 | 2.9 |

Multivariate analysis. Fig. 12.6 shows that, in an MDS of $\sqrt{\sqrt{}}$ -transformed species abundance data, there is no obvious discrimination between treatments for the nematodes. In the ANOSIM test (Table 12.4) the values of the R statistic in pairwise comparisons between treatments are low (0.2 – 0.3), but there is a significant difference between the low dose treatment and the control, at the 5% level. For the copepods, there is a clear separation of treatments on the MDS, the R statistic values are much higher (0.6–1.0), and there are

significant differences in community structure between all treatments.

Conclusions. The univariate and graphical/distributional techniques show lowered diversity with increasing dose for copepods, but no effect on nematodes. The multivariate techniques clearly discriminate between treatments for copepods, and still have some discriminating power for nematodes. Clearly the copepods have been much more strongly affected by the treatments in all these analyses, but changes in the nematode community may not have been detectable because of the great variability in abundance of nematodes in the high dose boxes. The responses observed in the mesocosm were similar to those sometimes observed in the field where organic enrichment occurs. For example, there was an increase in abundance of epibenthic copepods (particularly *Tisbe* spp.) resulting in a decrease in the nematode/copepod ratio. In this experiment, however, the causal link is closer to being established, though the possible constraints and artefacts inherent in any laboratory mesocosm study should always be borne in mind (see, for example, the discussion in Underwood and Peterson, 1988).

CHAPTER 13: DATA REQUIREMENTS FOR BIOLOGICAL EFFECTS STUDIES: WHICH COMPONENTS AND ATTRIBUTES OF THE BIOTA TO EXAMINE?

COMPONENTS

The biological effects of pollutants can be studied on assemblages of a wide variety of organisms:

Pelagos

- plankton (both phytoplankton and zooplankton)
- fish (pelagic and demersal)

Benthos (soft-bottom)

- macrobenthos
- meiobenthos
- (microbenthos, not much used for community studies)

Benthos (hard-bottom)

- epifauna (encrusting forms, eg. corals)
- motile fauna (both macrofauna and meiofauna in e.g. algae, holdfasts and epifauna)

These various components of the biota each have certain practical and conceptual advantages and disadvantages for use in biological effects studies. These are discussed in this chapter, and an example is given for each of the components (although not all of these examples are directly concerned with pollution effects).

PLANKTON

The *advantages* of plankton are that:

- a) Long tows over relatively large distances result in community samples which reflect integrated ecological conditions over large areas. They are therefore useful in monitoring more global changes.
- b) Identification of macro-planktonic organisms is moderately easy, because of the ready availability of appropriate literature.

The *disadvantage* of plankton is that, because the water masses in which they are suspended are continually mobile, they are not useful for monitoring the local effects of a particular pollutant source.

Example: Continuous Plankton Recorder

Plankton samples have been collected from 'ships of opportunity' plying their usual commercial routes across the NE Atlantic since the late 1940s (Colebrook,

1986). The plankton recorders collect samples through a small aperture, and these are trapped on a continuously winding roll of silk so that each section of silk contains an integrated sample from a relatively large area. This has enabled long term trends in plankton abundance to be assessed: there has been a gradual decline in both zooplankton and phytoplankton since the early 1950s, with an upturn in the 1980s (Fig. 13.1).

FISH

The *advantages* of fish are that:

- a) Because of their mobility they are again more useful for studying general rather than local effects, but some demersal fish communities may show site fidelity, such as the coral-reef fish in the example below.
- b) The taxonomy of fish is relatively easy, at least in Europe and N. America.

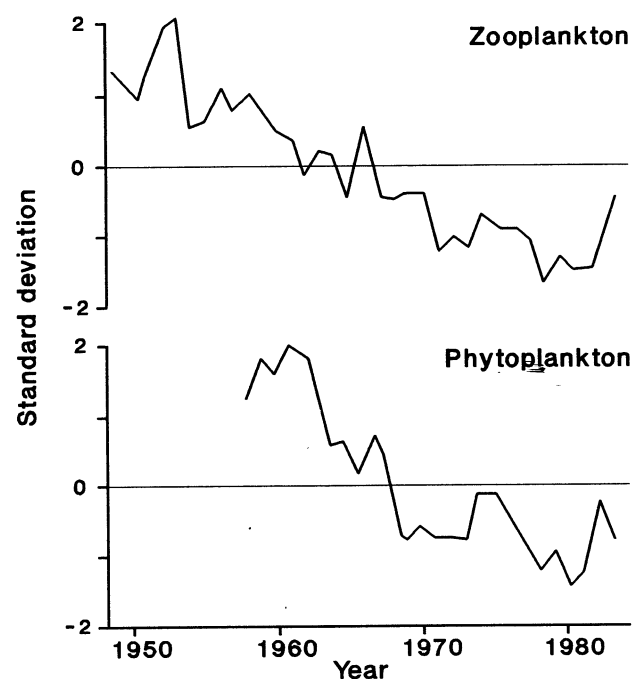


Fig. 13.1. Continuous Plankton Recorder Survey of the NE Atlantic (P). First principal components for zooplankton and phytoplankton, over the years of the survey (from Colebrook, 1986). Graphs scaled to zero mean and unit variance.

- c) Fish are of immediate commercial and public interest, and so studies of fish communities are more directly related to the needs of environmental managers than, for example, the meiobenthos (despite the fact, of course, that the latter are vitally important to the early life-stages of fish!).

The *disadvantages* of fish are that:

- a) Strictly quantitative sampling which is equally representative of all the species in the community is difficult. The overall catching efficiency of nets, traps etc. is often unknown, as are the differing abilities of species to evade capture or their susceptibility to be attracted to traps. Visual census methods are also not free from bias, since some species will be more conspicuous in colouration or behaviour than other dull secretive species.
- b) Uncertainty about site fidelity is usually, but not always, a problem.

Example: Maldives coral reef-fish

In the Maldivian islands, Dawson-Shepherd *et al.* (1992) used visual census methods to compare reef-fish assemblages at 23 coral reef-flat sites, 11 of which had been subjected to coral mining for the construction industry and 12 were non-mined controls. The MDS (Fig. 13.2) clearly distinguished mined from non-mined sites.

MACROBENTHOS

The *advantages* of soft-bottom macrobenthos are that:

- a) They are relatively non-mobile and are therefore useful for studying the local effects of pollutants.

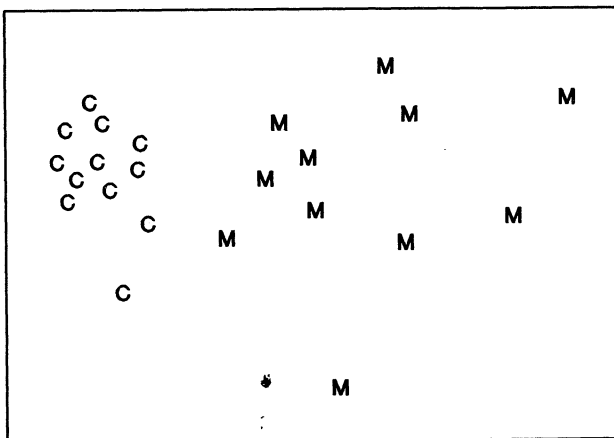


Fig. 13.2. Maldivian Islands, coral-reef fish [M]. MDS ordination of fish species abundance data from mined (M) and control (C) reef-flats (stress = 0.08).

- b) Their taxonomy is relatively easy.
- c) Quantitative sampling is relatively easy
- d) There is an extensive research literature on the effects of pollution, particularly organic enrichment, on macrobenthic communities, against which particular case-histories can be evaluated.

This combination of advantages has resulted in the soft-bottom macrobenthos being probably the most widely used component of the marine biota in environmental impact studies. Despite this, they do have several *disadvantages*:

- a) Relatively large-volume sediment samples must be collected, so that sampling requires relatively large research ships.
- b) Because it is generally not practicable to bring large volumes of sediment back to the laboratory for processing, sieving must be done at sea and is rather labour intensive and time consuming (therefore expensive).
- c) The potential response time of the macrobenthos to a pollution event is slow. Their generation times are measured in years, so that although losses of species due to pollution may take immediate effect, the colonisation of new species which may take advantage of the changed conditions is slow. Thus, the full establishment of a community characterising the new environmental conditions may take several years.
- d) The macrobenthos are generally unsuitable for causality experiments in mesocosms, because such experiments can rarely be run long enough for fully representative community changes to occur, and recruitment of species to mesocosm systems is often a problem because of their planktonic larval stages (see Chapter 12).

Example: Amoco Cadiz oil-spill

The sensitivity of macrobenthic community structure to pollution events, when using multivariate methods of data analysis, is discussed in Chapter 14. The response of the macrobenthos in the Bay of Morlaix to the Amoco Cadiz oil-spill some 40 km away, described in Chapter 10, is a good example of this (Fig. 13.3).

MEIOBENTHOS

Apart from sharing the advantage of non-mobility, and therefore usefulness for local effects studies, the relative advantages and disadvantages of the meiobenthos are exactly the reverse of the macrobenthos. Their *advantages* are:

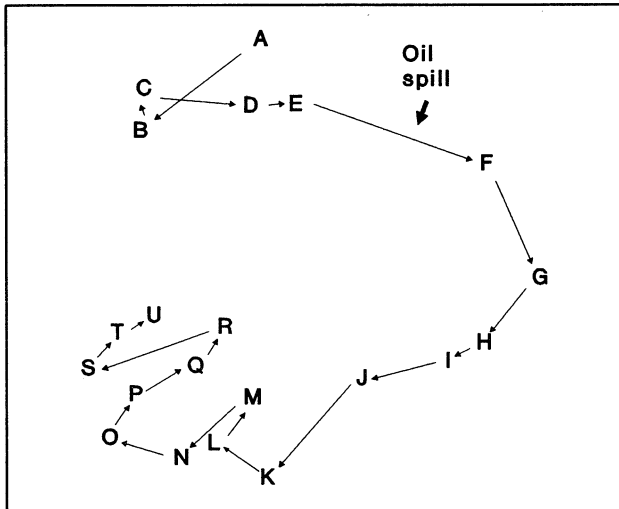


Fig. 13.3. Amoco-Cadiz oil spill, Bay of Morlaix (A). MDS for macrobenthos at station "Pierre Noire", at approximately 3-monthly sampling intervals (stress = 0.09).

- a) Because of their small size and high density in marine sediments, quantitative sampling of the meiobenthos is easy from small ships, open boats etc.
- b) The small volume of the samples means that they can easily be transported to the laboratory, and need not be processed on board ship.
- c) Their generation times are usually measured in months rather than years, so that their potential response time to pollution events is much faster than that of the macrobenthos.
- d) Because of this fast response time, and direct benthic rather than planktonic development, the meiobenthos are good candidates for causality experiments in experimental microcosms and mesocosms.

The *disadvantages* of meiobenthos are that:

- a) Their taxonomy is considered difficult. Identification of almost all the meiobenthic taxa to species level presents difficulties even in Europe and North America, and in many parts of the world the fauna is almost completely unknown. However, three factors mitigate to a considerable degree against this problem:
 - i. The robustness of community analyses to the use of taxonomic levels higher than species (see Chapter 10).
 - ii. The cosmopolitan nature of most meiobenthic genera.
 - iii. The increasing availability of easily used keys to meiobenthic genera. For example, the pictorial

keys to marine nematodes of Platt and Warwick (1988) have been used successfully worldwide.

- b) Community responses of the meiobenthos to pollution are not well documented, so that there is not an extensive body of information in the literature against which particular case-histories can be evaluated.

Example: Soldier crab disturbance of nematode assemblages, Tasmania

This natural field experiment was described in Chapter 12. It will be remembered that the nematode diversity profiles were affected by the crab disturbance (Fig. 12.2), whereas no significant effect was noted for copepods (Fig. 13.4). Many nematode species are more sedentary in habit than copepods, often adhering to sand-grains by secretions from their caudal glands, and some species prefer conditions of low oxygen concentration or are obligate anaerobes. The so called 'thiobiotic' meiofaunal community contains many nematode species, but apparently no copepods. Non-bioturbated sediments will have a vertical gradient in physical and chemical conditions ranging from wave-disturbed sediments with an oxiphilic meiofauna community near the surface to a stable sediment with a thiobiotic community deeper down. Dramatic disturbance by crabs, of the kind found at this site, will inevitably destroy this gradient, so that the whole sediment column will be well aerated and unstable. This reduction in habitat complexity is probably the most parsimonious explanation for the reduction in nematode species diversity.

The differential response of these two components of the meiobenthos has been elaborated here in order to demonstrate how a knowledge of the biology of these components can aid in the interpretation of community responses to perturbation. The macrobenthos and meiobenthos may also respond differently to different kinds of perturbation (e.g. physical disturbance, "pollution") so that a comparative study of both may be indicative of the cause.

Example: Macrobenthos and meiobenthos in Hamilton Harbour, Bermuda

Fig. 13.5 shows the average *k*-dominance curves for the macrobenthos and the nematode component of the meiobenthos at six stations in Hamilton Harbour. For the macrobenthos, the curves at three of the stations (H3, H4 & H6) are much more elevated than the other three, suggesting some kind of perturbation at these sites. For the nematodes, however, all curves are closely coincident. There must therefore be some form

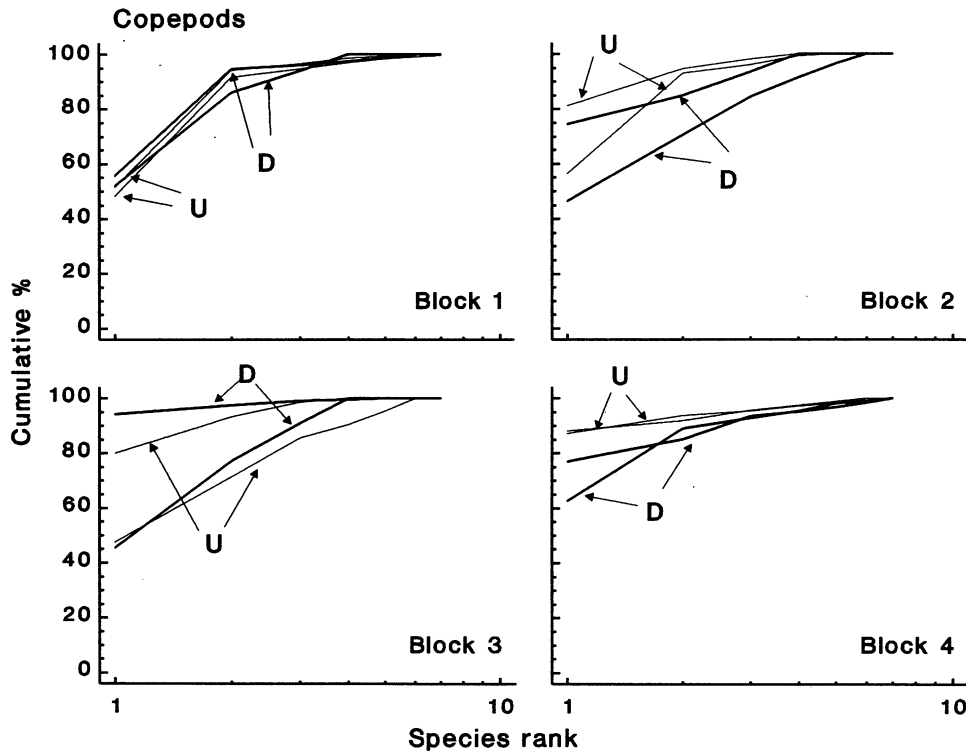


Fig. 13.4. Tasmania, Eaglehawk Neck (T). k-dominance curves for disturbed (D) and undisturbed (U) copepod samples in 4 separate sampling blocks.

of perturbation affecting the macrobenthos but not the meiobenthos, and it was suggested by Warwick *et al.* (1990c) that this is more likely to be physical disturbance of the sediment resulting from the regular passage of large cruise liners in the harbour, rather than pollution. This is because the macrobenthos are much more dependent on sediment stability to maintain diversity than are the meiobenthos.

HARD-BOTTOM EPIFAUNA

The *advantages* of using hard-bottom encrusting faunas, reef-corals etc. are:

- a) They are immobile and therefore good for local effects studies.

- b) A major advantage over sedimentary faunas is that non-destructive (visual) sampling is possible.

The *disadvantages* are:

- a) Remote sampling is difficult. Intertidal or shallow subtidal sites can be surveyed (the latter by divers), but remote cameras are expensive.
- b) Enumeration of colonial organisms is difficult, so that abundance units such as number of colonies or percentage cover must be used; biomass measurements are difficult to make.

Example: Indonesian reef corals.

The example shown in Fig. 13.6, of the effects of the 1982-3 El Niño on reef coral communities at South Pari Island, was described in Chapter 10. A clear difference

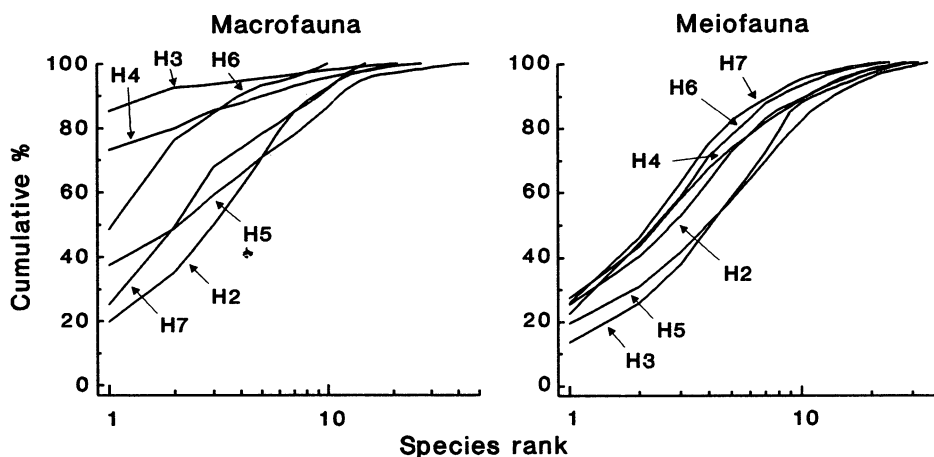


Fig. 13.5. Hamilton Harbour, Bermuda (H). k-dominance curves for macrobenthos (left) and meiobenthic nematodes (right) at six stations (H2-H7).

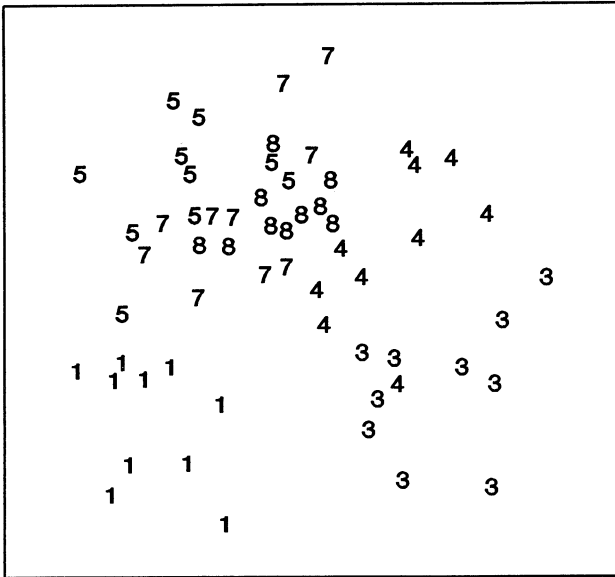


Fig. 13.6. Indonesian reef-corals (I). MDS for coral species percentage cover data for South Pari Island (10 replicate transects in each year). 1=1981, 3=1983 etc. (stress = 0.25).

is seen in community composition between 1981 and 1983, with a more steady pattern of change thereafter, though without full reversion to the initial state.

HARD-BOTTOM MOTILE FAUNA

Hard-bottom motile fauna

The motile fauna living on rocky substrates and associated with algae, holdfasts, hydroids etc. has rarely been used in pollution impact studies because of its many *disadvantages*:

- a) Remote sampling is difficult.

- b) Quantitative extraction from the substrate, and comparative quantification of abundances between different substrate types, are difficult.
- c) Responses to perturbation are largely unknown.
- d) A suitable habitat (e.g. algae) is not always available. A solution to this problem, and also problem (b), might be to deploy standardised artificial substrates, e.g. plastic mesh pan-scrubbers, along suspected pollution gradients in the field, allowing these to become colonised.

Example: Metazoan fauna of intertidal seaweed samples from the Isles of Scilly

The entire metazoan fauna (macrofauna + meiofauna) was examined from five species of intertidal macro-algae (*Chondrus*, *Laurencia*, *Lomentaria*, *Cladophora*, *Polysiphonia*) each collected at eight sites near low water from rocky shores on the Isles of Scilly, U.K. (Gee and Warwick, 1994). The MDS plots for meiobenthos and macrobenthos were very similar, with the algal species showing very similar relationships to each other in terms of their meiofaunal and macrofaunal community structure (Fig. 13.7). The structure of the weed therefore clearly influenced community structure in both these components of the benthic fauna.

ATTRIBUTES

Species *abundance* data are by far the most commonly used in environmental impact studies at the community level. However, the abundance of a species is perhaps the least ecologically relevant measure of its relative importance in a community, and we have already seen in Chapter 10 that higher taxonomic

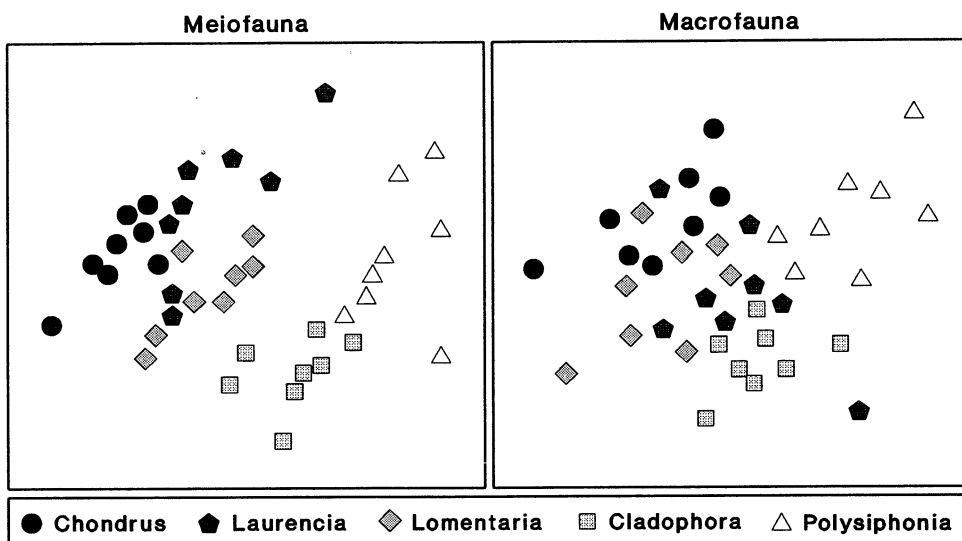


Fig. 13.7. Isles of Scilly seaweed fauna (S). MDS of standardised $\sqrt{\sqrt{\quad}}$ -transformed meiofauna and macrofauna species abundance data. The five seaweed species are indicated by different symbol and shading conventions (stress = 0.19, 0.18).

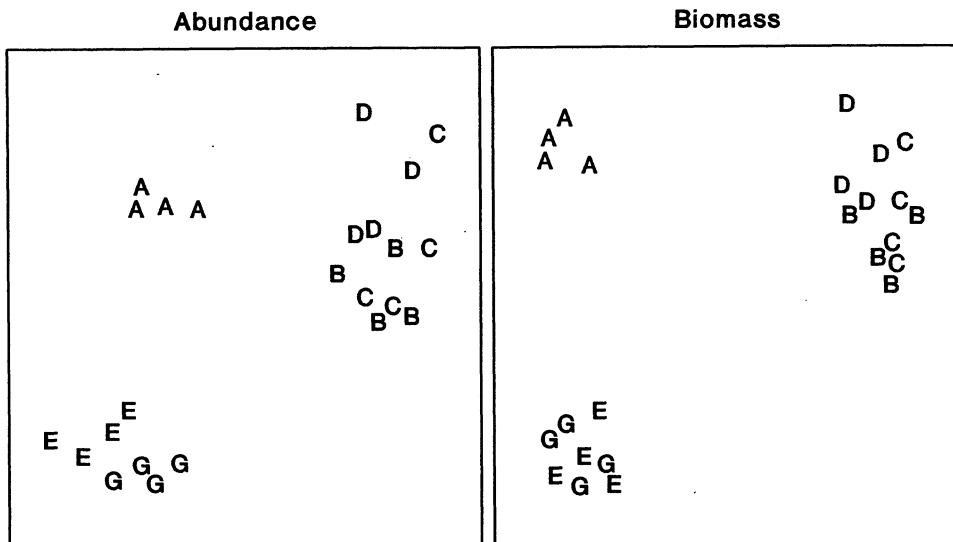


Fig. 13.8. Frierfjord macrofauna {F}. MDS ordinations for abundance and biomass of the 4 replicates at each of the 6 sites (stress = 0.10, 0.08).

levels than species may be sufficient for environmental impact analyses. So, when planning a survey, consideration should be given not only to the number of stations and number of replicates to be sampled, but also to the level of taxonomic discrimination which will be used, and which measure(s) of the relative importance of these taxa will be made.

Abundance, biomass and production

As a measure of the relative ecological importance of species, biomass is better than abundance, and production in turn is better than biomass. However, the determination of annual production of all species within a community over a number of sites or times would be so time consuming as to be completely impracticable.¹ We are therefore left with the alternatives of studying abundances, biomasses, or both. Abundances are marginally easier to measure, biomass may be a better reflection of ecological importance, and measurement of both abundance and biomass opens the possibility of comparing species-by-sites matrices based on these two different measures (e.g. by the ABC method discussed in Chapter 8). In practice, multivariate analyses of abundance and biomass data give remarkably similar results, despite the fact that the species mainly responsible for discriminating between stations are different. In Fig. 13.8, for example, the Frierfjord macrobenthos MDS configurations for abundance and

biomass are very similar but it is small polychaete species which are mainly responsible for discriminating between sites on the basis of abundance, and the large echinoid *Echinocardium cordatum* on the basis of biomass.

Species or higher taxa

We have already seen in Chapter 10 that, in many pollution-impact studies, it has been found for both graphical and multivariate analyses that there is surprisingly little loss of information when the species data are aggregated into higher taxa, e.g. genera, families or even phyla. Initial collection of data at the level of higher taxa would result in a considerable saving of time (and cost) in the analysis of samples.

RECOMMENDATIONS

It is difficult to give firm recommendations as to which components or attributes of the biota should be studied, since this depends on the problem in hand and the expertise and funds available. In general, however, the wider the variety of components and attributes studied, the easier the results will be to interpret. A broad approach at the level of higher taxa is often preferable to a painstakingly detailed analysis of species abundances. If only one component of the fauna is to be studied, then consideration should be given to working up a larger number of stations/replicates at the level of higher taxa in preference to a small number of stations at the species level. Of course, a large number of stations at which both abundance and biomass are determined at the species level is always the ideal!

1. Although relative "production" of species can be approximated using empirical relationships between biomass, abundance and production, and these "production" matrices subjected to multivariate analysis, see Chapter 15.

CHAPTER 14: RELATIVE SENSITIVITIES AND MERITS OF UNIVARIATE, GRAPHICAL/DISTRIBUTIONAL AND MULTIVARIATE TECHNIQUES

Two communities with a completely different taxonomic composition may have identical univariate or graphical/distributional structure, and conversely those comprising the same species may have very different univariate or graphical structure. This chapter compares univariate, graphical and multivariate methods of data analysis by applying them to a broad range of studies on various components of the marine biota from a variety of localities, in order to address the question of whether species dependent and species independent attributes of community structure behave the same or differently in response to environmental changes, and which are the most sensitive. Within each class of methods we have seen in previous chapters that there is a very wide variety of different techniques employed, and to make this comparative exercise more tractable we have chosen to examine only one method for each class:

Shannon–Wiener diversity index H' (see Chapter 8), k -dominance curves including ABC plots (Chapter 8), non-metric MDS ordination on a Bray–Curtis similarity matrix of appropriately transformed species abundance or biomass data (Chapter 5).

EXAMPLE 1: Macrobenthos from Frierfjord/Langesundfjord, Norway

As part of the GEEP/IOC Oslo Workshop, macrobenthos samples were collected at a series of six stations in Frierfjord/Langesundfjord (F), station A being the outermost and station G the innermost (station F was not sampled for macrobenthos). For a map of the sampling locations see Fig. 1.1.

Univariate indices

Site A had a higher species diversity and site C the lowest but the others were not significantly different (Fig. 14.1).

Graphical/distributional plots

ABC plots indicated that stations C, D and E were most stressed, B was moderately stressed, and A and G were unstressed (Fig. 14.2).

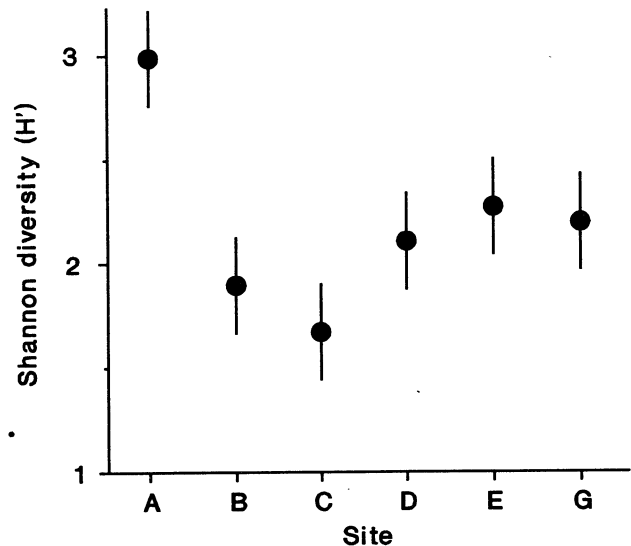


Fig. 14.1. Frierfjord macrobenthos (F). Shannon diversity (mean and 95% confidence intervals) for each station.

Multivariate analysis

An MDS of all 24 samples (4 replicates at each station), supported by the ANOSIM test, showed that only stations B and C were not significantly different from each other (Fig. 14.3). Gray *et al.* (1988) show that the clusters correlate with water depth rather than with measured levels of anthropogenic variables such as hydrocarbons or metals.

Conclusions

The MDS was much better at discriminating between stations than the diversity measure, but perhaps more importantly, sites with similar univariate or graphical/distributional community structure did not cluster together on the MDS. For example, diversity at E was not significantly different from D but they are furthest apart on the MDS; conversely, E and G had different ABC plots but clustered together. However, B, C and D all have low diversity and the ABC plots indicate disturbance at these stations. The most likely explanation is that these deep-water stations are affected by seasonal anoxia, rather than anthropogenic pollution.

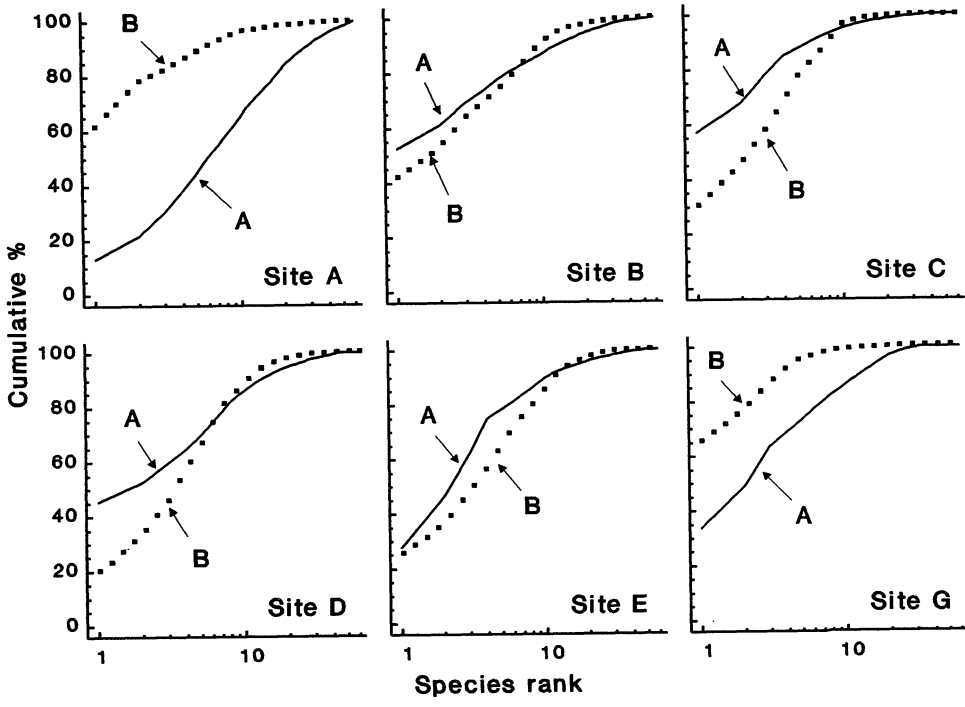


Fig. 14.2. Frierfjord macrobenthos (F). ABC plots based on the totals from 4 replicates at each of the 6 sites. Solid lines, abundances; dotted lines, biomass.

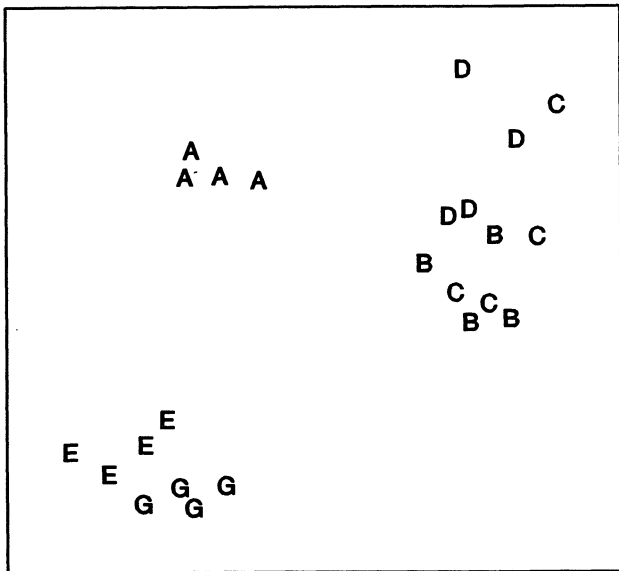


Fig. 14.3. Frierfjord macrobenthos (F). MDS of 4 replicates at each of sites A-E, G, from Bray-Curtis similarities on 4th root-transformed counts (stress = 0.10).

EXAMPLE 2: Macrobenthos from the Ekofisk oilfield, N. Sea

Changes in community structure of the soft-bottom benthic macrofauna in relation to oil drilling activity at the Ekofisk platform in the North Sea (E) have been described by Gray *et al.* (1990). The positions of the sampling stations around the rig are coded by shading and symbol conventions in Fig. 14.4a, according to

their distance from the currently active centre of drilling activity.

Univariate indices

It can be seen from Fig. 14.4b that species diversity was only significantly reduced in the zone closer than 250 m from the rig, and that the three outer zones did not differ from each other in terms of species diversity.

Graphical/distributional plots

The *k*-dominance curves (Fig. 14.4c) also only indicate a significant effect within the inner zone, the curves for the three outer zones being closely coincident.

Multivariate analysis

In the MDS analysis (Fig. 14.4d) community composition in all of the zones was distinct, and there was a clear gradation of change from the inner to outer zones. Formal significance testing (using ANOSIM) confirmed statistically the differences between all zones. It will be recalled from Chapter 10 that there was also a clear distinction between all zones at higher taxonomic levels than species, even at the phylum level.

Conclusions

Univariate and graphical methods of data analysis suggest that the effects on the benthic fauna are rather localised. The MDS is clearly more sensitive, and can detect differences in community structure up to 3 km away from the centre of activity.

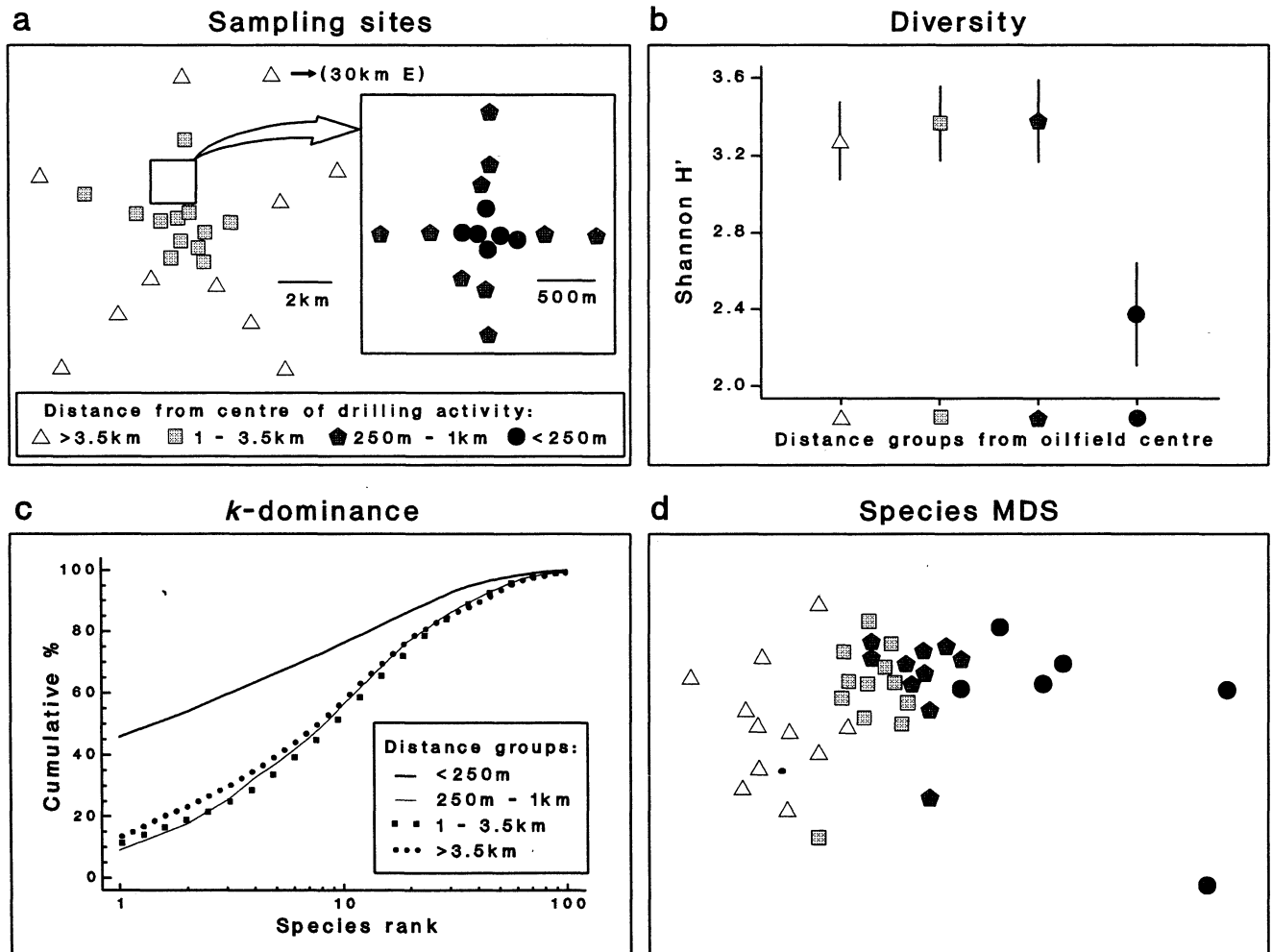


Fig. 14.4. Ekofisk macrobenthos (E). a) Map of sampling sites, represented by different symbol and shading conventions according to their distance from the 2/4K rig at the current centre of drilling activity; b) Shannon diversity (mean and 95% confidence intervals) in these distance zones; c) mean k -dominance curves; d) MDS from root-transformed species abundances (stress = 0.12).

EXAMPLE 3: Reef corals at South Pari Island, Indonesia

Warwick et al. (1990b) analysed coral community responses to the El Niño of 1982-3 at two reefs sites in the Thousand Islands, Indonesia (I), based on 10 replicate line transects for each of the years 1981, 83, 84, 85, 87 and 88.

Univariate indices

At Pari Island there was an immediate reduction in diversity in 1983, apparent full recovery by 1985, with a subsequent but not significant reduction (Fig. 14.5).

Graphical/distributional plots

The mean k -dominance curves were similar in 1981 and 1985, with the curves for 1983, 84, 87 and 88 more elevated (Fig. 14.6). Tests on the replicate curves (see the end of Chapter 8) confirmed the significance of

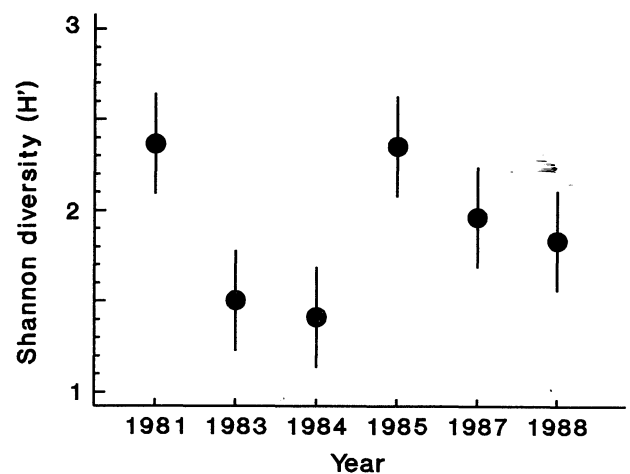


Fig. 14.5. Indonesian reef corals, Pari Island (I). Shannon diversity (means and 95% confidence intervals) of the species coral cover from 10 transects in each year.

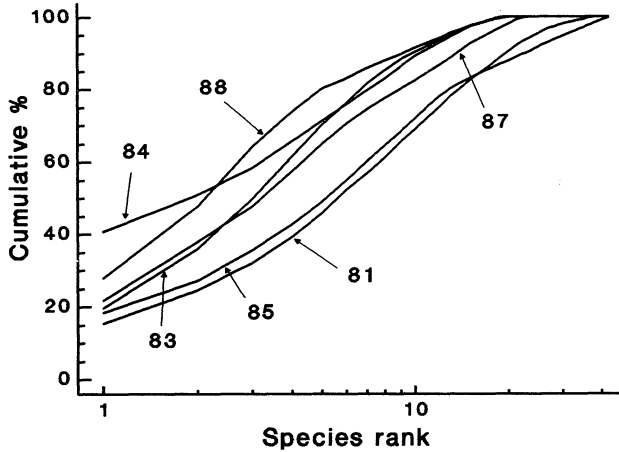


Fig. 14.6. Indonesian reef-corals, Pari Island (I). *k*-dominance curves for totals of all ten replicates in each year.

differences between 1981, 1985 and the other years, but the latter were not distinguishable from each other.

Multivariate analysis

Though the MDS has rather a high stress it nonetheless shows an immediate location shift in community composition at the ten replicate sites between 1981 and 1983, and ANOSIM indicates significant differences between all pairs of years. Recovery proceeded in the pre-El Niño direction but was not complete by 1988 (Fig. 14.7).

Conclusions

All methods of data analysis demonstrated the dramatic post El Niño decline in species, though the

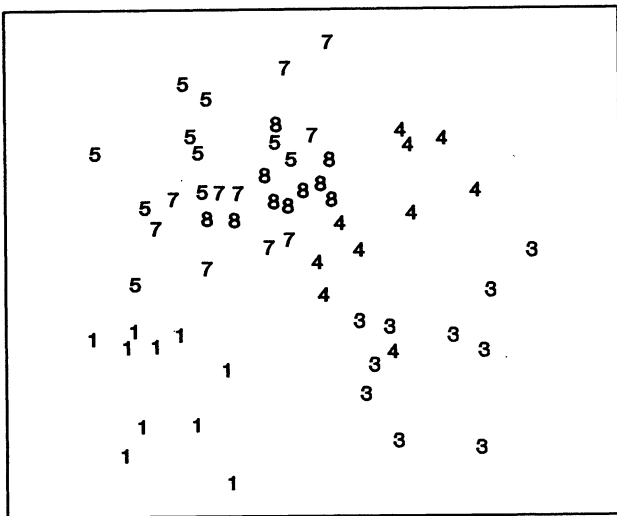


Fig. 14.7. Indonesian reef-corals, Pari Island (I). MDS for coral species percentage cover data (1 = 1981, 3 = 1983 etc.).

multivariate techniques were seen to be more sensitive in monitoring the recovery phase in later years.

EXAMPLE 4: Fish communities from coral reefs in the Maldives

In the Maldivian islands, Dawson-Shepherd *et al.* (1992) compared reef-fish assemblages at 23 coral reef-flat sites (M), 11 of which had been subjected to coral mining for the construction industry and 12 were non-mined controls. The reef-slopes adjacent to these flats were also surveyed.

Univariate indices

Using ANOVA, no significant differences in diversity (Fig. 14.8) were observed between mined and control sites, with no differences either between reef flats and slopes.

Graphical/distributional plots

No significant differences could be detected between mined and control sites, in *k*-dominance curves for either species abundance or biomass. Fig. 14.9 displays the mean curves for reef-flat data pooled across the replicates for each condition.

Multivariate analysis

The MDS (Fig. 14.10) clearly distinguished mined from control sites on the reef-flats, and also to a lesser degree even on the slopes adjacent to these flats, where ANOSIM confirmed the significance of this difference.

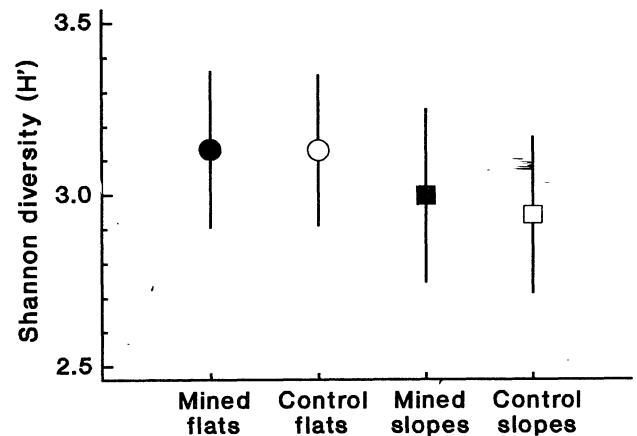


Fig. 14.8. Maldivian Islands, coral-reef fish (M). Shannon species diversity (means and 95% confidence intervals) at mined (closed symbols) and control (open symbols) sites, for both reef flats (circles) and reef slopes (squares).

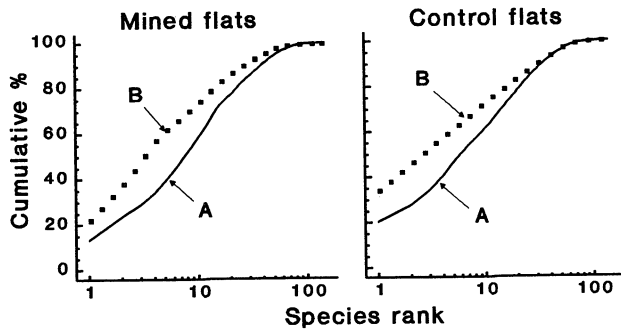


Fig. 14.9. Maldive Islands, coral-reef fish [M]. Average k -dominance curves for abundance and biomass at mined and control reef-flat sites.

Conclusions

There were clear differences in community composition due to mining activity revealed by multivariate methods, even on the reef-slopes adjacent to the mined flats, but these were not detected at all by univariate or graphical/distributional techniques, even on the flats where the separation in the MDS is so obvious.

EXAMPLE 5: Macro- and meiobenthos from Isles of Scilly seaweeds

The entire metazoan fauna (macrofauna + meiofauna) has been analysed from five species of intertidal macro-algae (*Chondrus*, *Laurencia*, *Lomentaria*, *Cladophora*, *Polysiphonia*) each collected at either sites near low water from rocky shores on the Isles of Scilly {S} (Fig. 14.11).

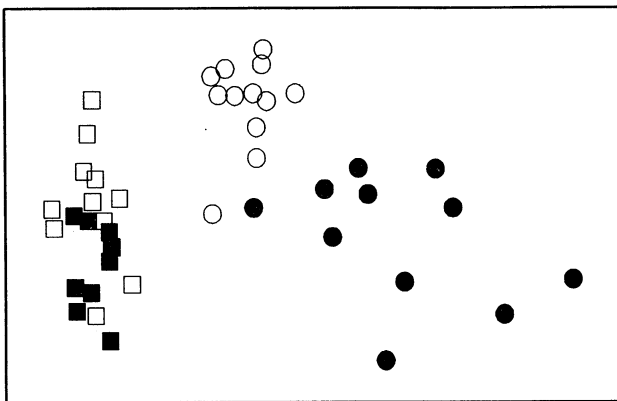


Fig. 14.10. Maldive Islands, coral-reef fish [M]. MDS of 4th root-transformed species abundance data. Symbols as in Fig. 14.8, i.e. circles = reef-flat, squares = slope, solid = mined, open = control (stress = 0.09).

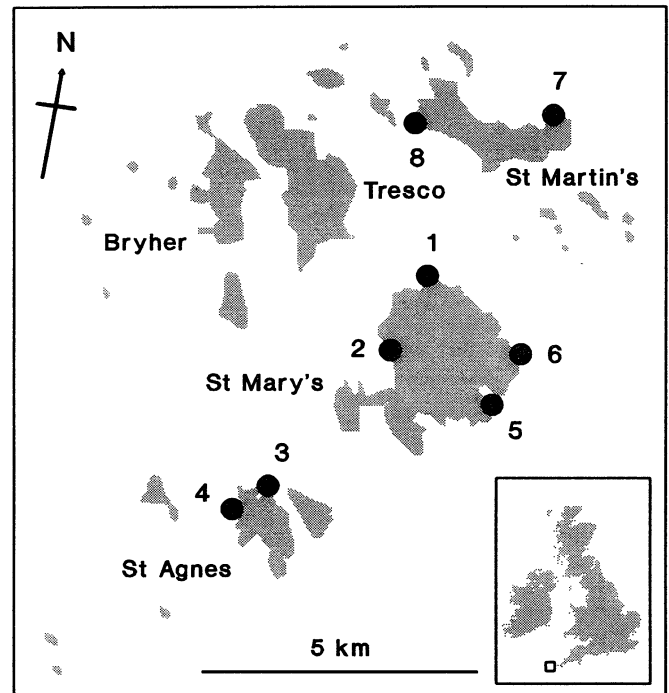


Fig. 14.11. Isles of Scilly {S}. Map of the 8 sites from each of which 5 seaweed species were collected.

Univariate indices

The meiofauna and macrofauna showed clearly different diversity patterns with respect to weed type; for the meiofauna there was a trend of increasing diversity from the coarsest (*Chondrus*) to the finest (*Polysiphonia*) weed, but for the macrofauna there was no clear trend and *Polysiphonia* had the lowest diversity (Fig. 14.12).

Graphical/distributional plots

These differences in meiofauna and macrofauna diversity profiles were also reflected in the k -dominance curves (Fig. 14.13) which had different sequencing for these two faunal components, for example the *Polysiphonia* curve was the lowest for meiofauna and highest for macrofauna.

Multivariate analysis

The MDS plots for meiobenthos and macrobenthos were very similar, with the algal species showing very similar relationships to each other in terms of their meiofaunal and macrofaunal community structure (see Fig. 13.7, in which the shading and symbol conventions for the different weed species are the same as those in Fig. 14.12). Two-way ANOSIM (weed species/sites) showed all weed species to be significantly different from each other in the composition of both macrofauna and meiofauna.

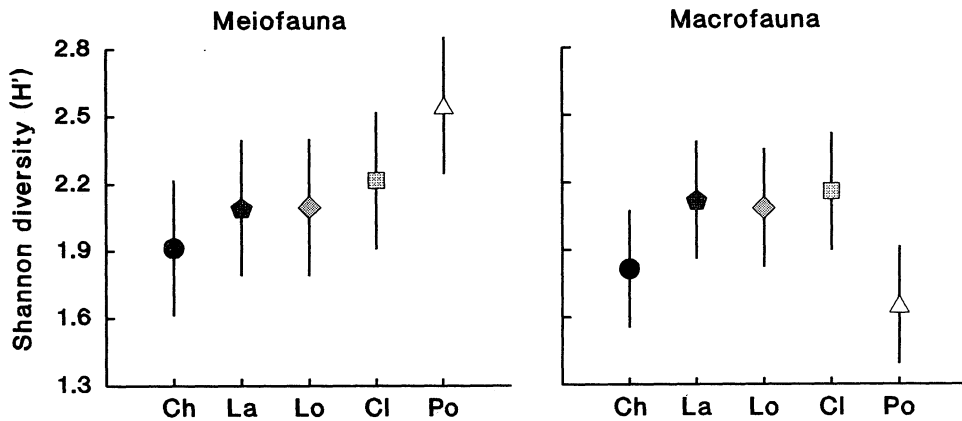


Fig. 14.12. Isles of Scilly seaweed fauna (S). Shannon diversity (mean and 95% confidence intervals) for the meiofauna and macrofauna of different weed species: Ch = Chondrus, La = Laurencia, Lo = Lomentaria, Cl = Cladophora, Po = Polysiphonia.

Conclusions

The MDS was more sensitive than the univariate or graphical method for discriminating between weed species. Univariate and graphical methods gave different results for macrobenthos and meiobenthos, whereas for the multivariate methods the results were similar for both.

EXAMPLE 6: Meiobenthos from the Tamar Estuary, S. W. England

Austen and Warwick (1989) compared the structure of the two major taxonomic components of the meiobenthos, nematodes and harpacticoid copepods, in the Tamar estuary (R). Six replicate samples were taken at a series of ten intertidal soft-sediment sites (Fig. 14.14).

Graphical/distributional plots

The average *k*-dominance curves showed no clear sequencing of sites for the nematodes, for example the curve for site 1 was closely coincident with that for site 10 (Fig. 14.15). For the copepods, however, the curves

became increasingly elevated from the mouth to the head of the estuary. However, for both nematodes and copepods, many of the curves were not distinguishable from each other.

Multivariate analysis

In the MDS, both nematodes and copepods showed a similar (arched) sequencing of sites from the mouth to the head of the estuary (Fig. 14.16). ANOSIM showed that the copepod assemblages were significantly different in all pairs of sites, and the nematodes in all pairs except 6/7 and 8/9.

Conclusions

The multivariate technique was more sensitive in discriminating between sites, and gave similar patterns for nematodes and copepods, whereas graphical methods gave different patterns for the two taxa. For nematodes, factors other than salinity seemed to be more important in determining diversity profiles, but for copepods salinity correlated well with diversity.

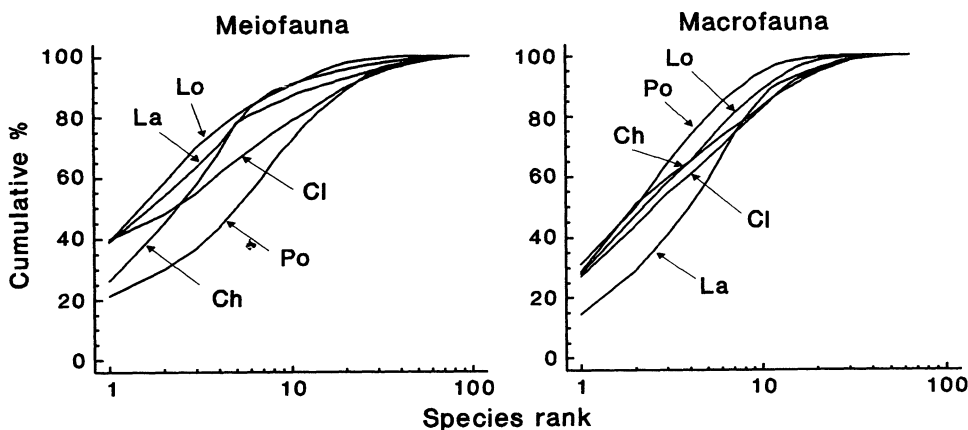


Fig. 14.13. Isles of Scilly seaweed fauna (S). *k*-dominance curves for meiofauna (left) and macrofauna (right). Ch = Chondrus, La = Laurencia, Lo = Lomentaria, Cl = Cladophora, Po = Polysiphonia.

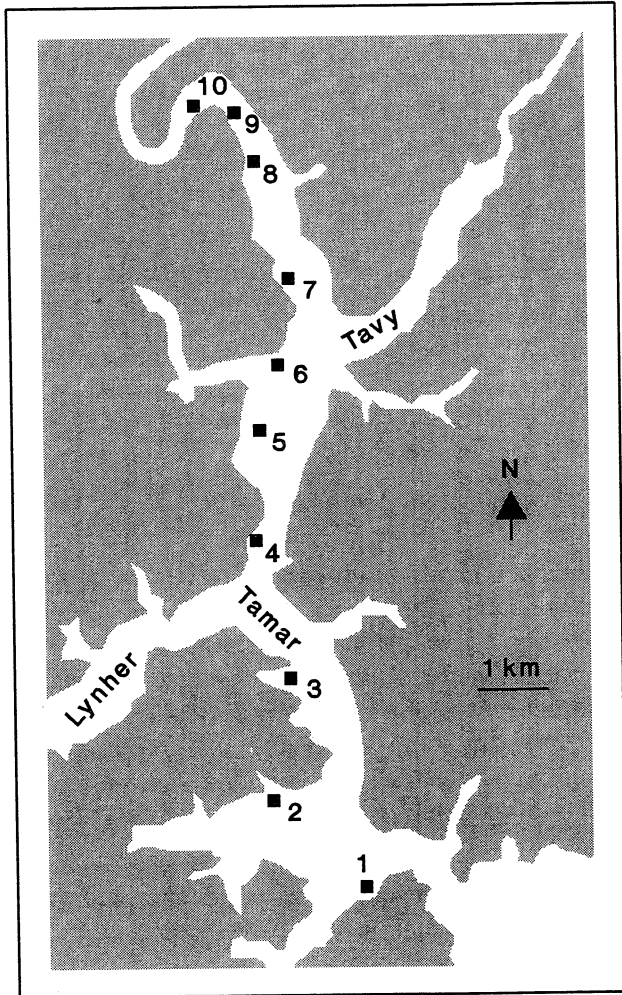


Fig. 14.14. Tamar estuary meiobenthos (R). Map showing locations of 10 intertidal mud-flat sites.

EXAMPLE 7: Meiofauna from Eaglehawk Neck sandflat, Tasmania

This example of the effect of disturbance by burrowing and feeding of soldier crabs (*T*) was dealt with in some detail in Chapter 12. For nematodes, univariate graphical and multivariate methods all distinguished disturbed from undisturbed sites. For copepods only the multivariate methods did. Univariate and graphical methods indicated different responses for nematodes and copepods, whereas the multivariate methods indicated a similar response for these two taxa.

GENERAL CONCLUSIONS

Three general conclusions emerge from these examples:

- 1) The similarity in community structure between sites or times based on their univariate or graphical/distributional attributes is different from their clustering in the multivariate analysis.
- 2) The species-dependent multivariate method is much more sensitive than the species-independent methods in discriminating between sites or times.
- 3) In examples where more than one component of the fauna has been studied, univariate and graphical methods may give different results for different components, whereas multivariate methods tend to give the same results.

The sensitive multivariate methods have hitherto only been used for detecting *differences* in community composition between sites. Although these differences can be correlated with measured levels of stressors such as pollutants, the multivariate methods so far described do not in themselves indicate

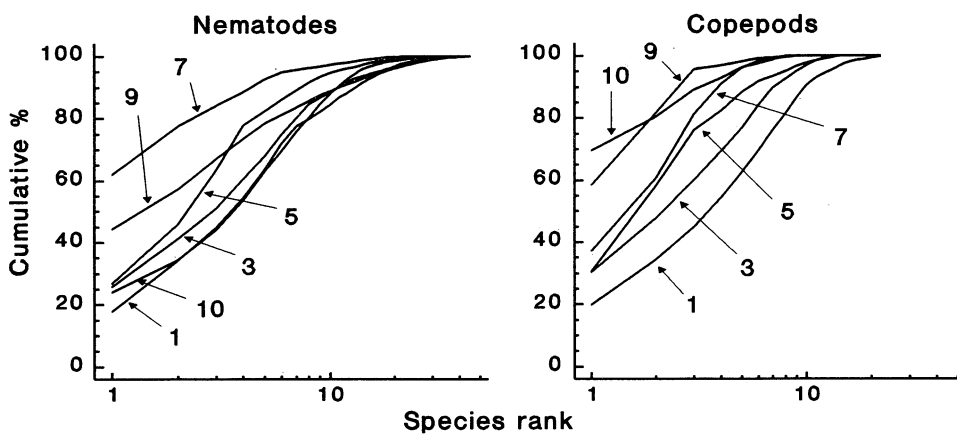


Fig. 14.15. Tamar estuary meiobenthos (R). *k*-dominance curves for amalgamated data from 6 replicate cores for nematode and copepod species abundances. For clarity of presentation, some sites have been omitted.

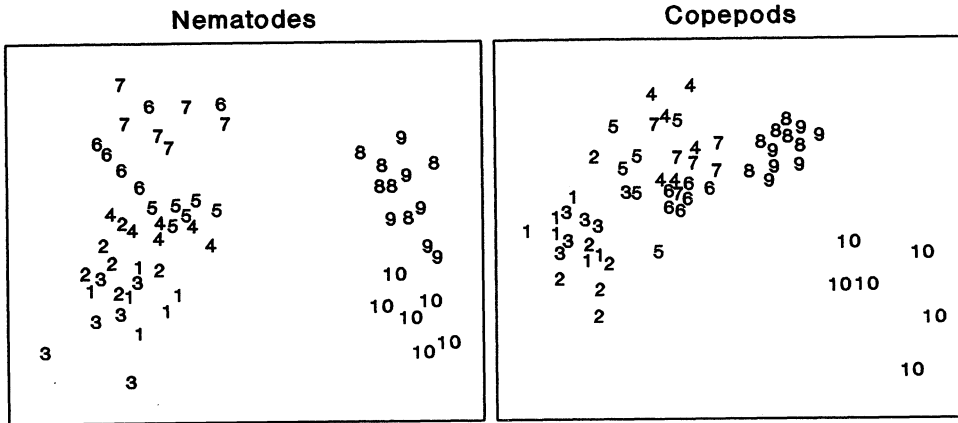


Fig. 14.16. Tamar estuary meiobenthos (R). MDS of 4th root-transformed nematode and copepod species abundance data for six replicate cores at each of 10 stations.

deleterious change which can be used in value judgements. Only the species-independent methods of data analysis lend themselves to the determination of deleterious responses although, as we have seen in Chapter 8, even the interpretation of changes in diversity is not always straightforward in these terms. There is a need to formulate sensitive techniques for determining stress which utilise the full multivariate information contained in a species/sites matrix and some recently devised possibilities form the subject of the next chapter.

RECOMMENDATIONS

At present, it is important to apply a wide variety of classes of data analysis, as each will give different information and this will aid interpretation. Sensitive multivariate methods will give an 'early warning' that community changes are occurring, but indications that these changes are deleterious are required by environmental managers, and the less sensitive species-independent methods must therefore also be used.

CHAPTER 15: MULTIVARIATE MEASURES OF COMMUNITY STRESS

We have seen in Chapter 14 that multivariate methods of data analysis are very sensitive for detecting differences in community structure between samples in space, or changes over time. Until recently, however, these methods have simply been used to detect *differences* between communities, and not in themselves as measures of *community stress* in the same sense that species-independent methods (e.g. diversity, ABC curves) have been used. Even using the relatively less sensitive species-independent methods there may be problems of interpretation in this context. Diversity does not behave consistently or predictably in response to environmental stress. Both current theory (Connell, 1978; Huston, 1979) and empirical observation (e.g. Dauvin 1984) suggest that increasing levels of disturbance may either decrease or increase diversity, and it may even remain the same. A monotonic response would be easier to interpret. False indications of disturbance using the ABC method may also arise when, as occasionally happens, the species responsible for elevated abundance curves are pollution sensitive rather than pollution tolerant species (e.g. small amphipods, *Hydrobia* etc.). Knowledge of the actual identities of the species involved will therefore aid the interpretation of ABC curves, and the resulting conclusions will be derived from an informal hybrid of species-independent and species-dependent information (Warwick and Clarke, in press). In this chapter we describe three possible approaches to the measurement of community stress using the fully species-dependent *multivariate* methods.

META-ANALYSIS OF MARINE MACROBENTHOS

This method was initially devised as a means of comparing the severity of community stress between various cases of both anthropogenic and natural disturbance. On initial consideration, measures of community degradation which are independent of the taxonomic identity of the species involved would be most appropriate for such comparative studies. Species composition varies so much from place to place depending on local environmental conditions that any general species-dependent response to stress would be masked by this variability. However, diversity measures are also sensitive to changes in natural environmental variables and an unperturbed community in one locality could easily have the same

diversity as a perturbed community in another. Also, to obtain comparative data on species diversity requires a highly skilled and painstaking analysis of species and an unusually high degree of standardisation with respect to the degree of taxonomic rigour applied to the sample analysis: e.g. it is not valid to compare diversity at one site where one taxon is designated as "nematodes" with another at which this taxon has been divided into species.

The problem of natural variability in species composition from place to place can be overcome by working at taxonomic levels higher than species. The taxonomic composition of natural communities tends to become increasingly similar at these higher levels. Although two communities may have no species in common, they will almost certainly comprise the same phyla. For soft-bottom marine benthos, we have already seen in Chapter 10 that disturbance effects are detectable with multivariate methods at the highest taxonomic levels, even in some instances where these effects are rather subtle and are not evidenced in univariate measures even at the species level, e.g. the Amoco-Cadiz {A} and Ekofisk {E} studies.

Meta-analysis is a term widely used in biomedical statistics and refers to the combined analysis of a range of individual case-studies which in themselves are of limited value but in combination provide a more global insight into the problem under investigation. Warwick and Clarke (1993a) have combined macrobenthic data aggregated to phyla from a range of case-studies {J} relating to varying types of disturbance, and also from sites which are regarded as unaffected by such perturbations. A choice was made of the most ecologically meaningful units in which to work, bearing in mind the fact that abundance is a rather poor measure of such relevance, biomass is better and production is perhaps the most relevant of all (Chapter 13). Of course, no studies have measured production (P) of all species within a community, but many studies provide both abundance (A) and biomass (B) data. Production was therefore approximated using the allometric equation:

$$P = (B/A)^{0.73} \times A \quad (15.1)$$

B/A is of course the mean body-size, and 0.73 is the average exponent of the regression of annual production on body-size for macrobenthic invertebrates. Since the data from each study are standardised (i.e. production of each phylum is expressed as a proportion of the total) the intercept of

this regression is irrelevant. For each data set the abundance and biomass data were first aggregated to phyla, following the classification of Howson (1987); 14 phyla were encountered overall (see the later Table 15.1). Abundance and biomass were then combined to form a *production matrix* using the above formula. All data sets were then merged into a single production matrix and an MDS performed on the standardised, 4th root-transformed data using the Bray Curtis similarity measure. All macrobenthic studies from a single region (the NE Atlantic shelf) for which both abundance and biomass data were available were used, as follows:

- 1) A transect of 12 stations sampled in 1983 on a west-east transect (Fig. 1.5) across a sewage sludge dump-ground at Garroch Head, Firth of Clyde, Scotland {G}. Stations in the middle of the transect show clear signs of gross pollution.
- 2) A time series of samples from 1963–1973 at stations (sites 34 and 2, Fig. 1.3) in two West Scottish sea-lochs, L. Linnhe and L. Eil {L}, covering the period of commissioning of a pulp-mill. The later years show increasing pollution effects on the macrofauna, except that in 1973 a recovery was noted in L. Linnhe following a decrease in pollution loading.
- 3) Samples collected at six stations in Frierfjord (Oslofjord), Norway {F}. The stations (Fig. 1.1) were ranked in order of increasing stress A–G–E–D–B–C, based on thirteen different criteria. The macrofauna at stations B, C and D were considered to be influenced by seasonal anoxia in the deeper basins of the fjord.
- 4) Amoco-Cadiz oil spill, Bay of Morlaix {A}. In view of the large number of observations, the 21 sampling occasions have been aggregated into five

years for the meta-analysis: 1977 = pre-spill, 1978 = immediate post-spill and 1979–81 = recovery period.

- 5) Two stations in the Skagerrak at depths of 100 and 300m. The 300m station showed signs of disturbance attributable to the dominance of the sediment-reworking bivalve *Abra nitida*.
- 6) An undisturbed station off the coast of Northumberland, NE England.
- 7) An undisturbed station in Carmarthen Bay, S Wales.
- 8) An undisturbed station in Kiel Bay; mean of 22 sets of samples.

In all, this gave a total of 50 samples, the disturbance status of which has been assessed by a variety of different methods including univariate indices, dominance plots, ABC curves, measured contaminant concentrations etc. The MDS for all samples (Fig. 15.1) takes the form of a wedge with the pointed end to the right and the wide end to the left. It is immediately apparent that the long axis of the configuration represents a scale of disturbance, with the most disturbed samples to the right and the undisturbed samples to the left. (The reason for the spread of sites on the vertical axis is less obvious). The relative positions of samples on the horizontal axis can thus be used as a measure of the relative severity of disturbance. Another gratifying feature of this plot is that in all cases increasing levels of disturbance result in a shift in the same direction, i.e. to the right. For visual clarity, the samples from individual case studies are plotted in Fig. 15.2, with the remaining samples represented as dots.

- 1) *Garroch Head (Clyde) sludge dump-ground* {G}. Samples taken along this transect span the full scale of the long axis of the configuration (Fig. 15.2a).

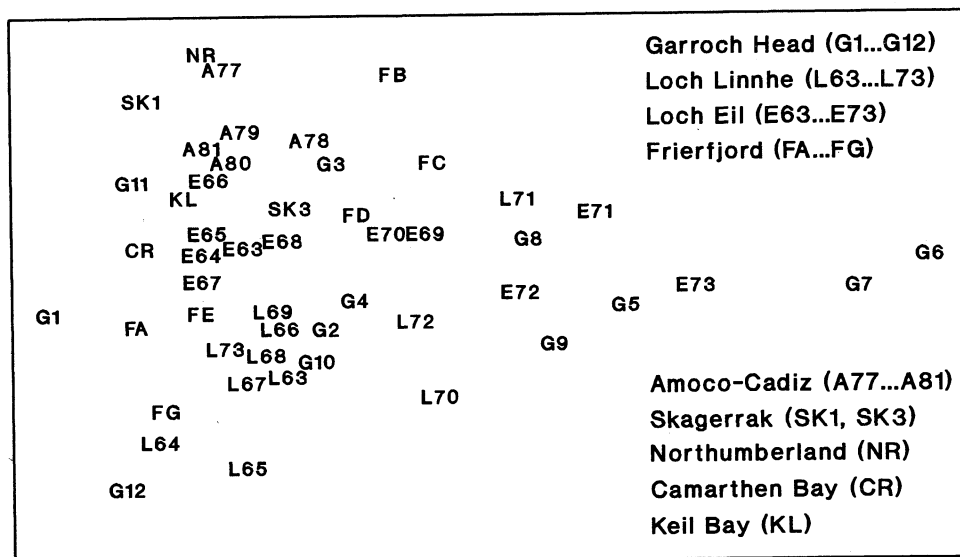


Fig. 15.1. Joint NE Atlantic shelf studies ("meta-analysis") (J). Two dimensional MDS ordination of phylum level "production" data (stress = 0.16).

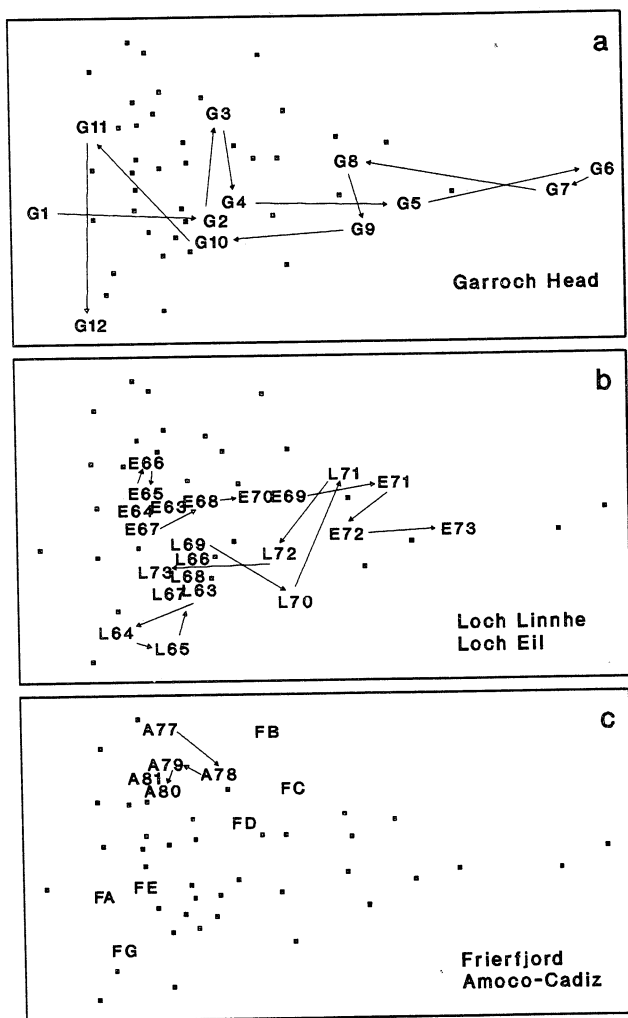


Fig. 15.2. Joint NE Atlantic shelf studies ("meta-analysis") (J). As Fig. 15.1 but with individual studies highlighted: a) Garroch Head (Clyde) dump-ground; b) Loch Linnhe and Loch Eil; c) Frierfjord and Amoco-Cadiz spill (Morlaix).

Stations at the two extremities of the transect (1 and 12) are at the extreme left of the wedge, and stations close to the dump centre (6) are at the extreme right.

- 2) *Loch Linnhe and Loch Eil* {L}. In the early years (1963-68) both stations are situated at the unpolluted left-hand end of the configuration (Fig. 15.2b). After this the L. Eil station moves towards the right, and at the end of the sampling period (1973) it is close to the right-hand end; only the sites at the centre of the Clyde dump-site are more polluted. The L. Linnhe station is rather less affected and the previously mentioned recovery in 1973 is evidenced by the return to the left-hand end of the wedge.
- 3) *Frierfjord (Osloffjord)* {F}. The left to right sequence of stations in the meta-analysis is A-G-E-D-B-C

(Fig. 15.2c), exactly matching the ranking in order of increasing stress. Note that the three stations affected by seasonal anoxia (B, C and D) are well to the right of the other three, but are not as severely disturbed as the organically enriched sites in 1) and 2) above.

- 4) *Amoco-Cadiz spill, Morlaix* {A}. Note the shift to the right between 1977 (pre-spill) and 1978 (post-spill), and the subsequent return to the left in 1979-81 (Fig. 15.2c). However, the shift is relatively small, suggesting that this is only a mild effect.
- 5) *Skagerrak*. The biologically disturbed 300m station is well to the right of the undisturbed 100m station, although the former is still quite close to the left-hand end of the wedge.
- 6-8) *Unpolluted sites*. The Northumberland, Carmarthen Bay and Keil Bay stations are all situated at the left-hand end of the wedge.

An initial premise of this method was that, at the phylum level, the taxonomic composition of communities is relatively less affected by natural environmental variables than by pollution or disturbance (Chapter 10). To test this Warwick and Clarke (1993a) superimposed symbols scaled in size according to the values of the two most important environmental variables considered to influence community structure, sediment grain size and water depth, onto the meta-analysis MDS configuration (Chapter 11). Both variables were quite randomly distributed, which supports the original assumption.

With respect to individual phyla, annelids comprise a high proportion of the total "production" at the polluted end of the wedge, with a decrease at the least polluted sites. Molluscs are also present at all sites, except the two most polluted, and have increasingly higher dominance towards the non-polluted end of the wedge. Echinoderms are even more concentrated at the non-polluted end, with some tendency for higher dominance at the bottom of the configuration (Fig. 15.3a). Crustacea are again concentrated to the left, but this time completely confined to the top part of the configuration (Fig. 15.3b). Clearly, the differences in relative proportions of crustaceans and echinoderms are largely responsible for the vertical spread of samples at this end of the wedge, but these differences cannot be explained in terms of the effects of any recorded natural environmental variables. Nematoda are clearly more important at the polluted end of the wedge, an obvious consequence of the fact that species associated with organic enrichment tend to be very large in comparison with their normal meiofaunal counterparts (e.g. Oncholaimids), and are therefore

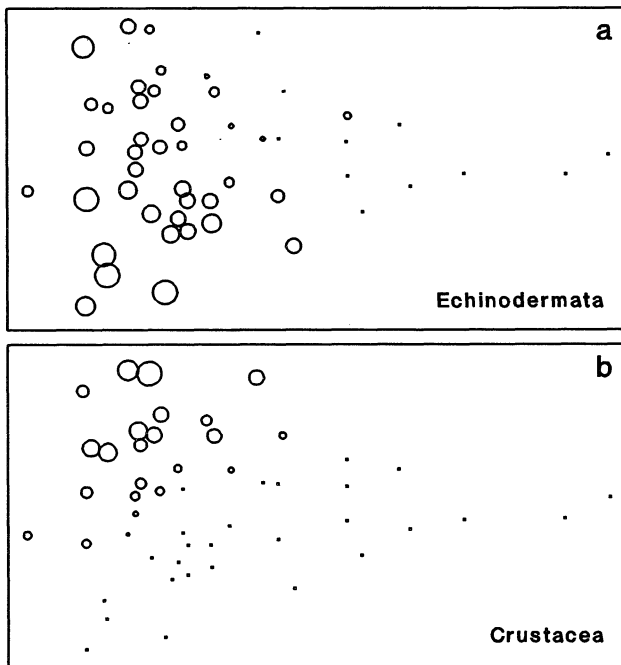


Fig. 15.3. Joint NE Atlantic shelf studies ("meta-analysis") (J). As Fig. 15.1 but highlighting the role of specific phyla in shaping the MDS; symbol size represents % production in each sample from: a) echinoderms, b) crustaceans.

retained on the macrofaunal ecologists' sieves. Other less important phyla show no clear distribution pattern, except that most are absent from the extreme right-hand samples.

This multivariate approach to the comparative scaling of benthic community responses to environmental stress seems to be more satisfactory than taxon-independent methods, having both generality and consistency of behaviour. It is difficult to assess the sensitivity of the technique because data on abundance and biomass of phyla are not available for any really low-level or subtle perturbations. However, its ability to detect the deleterious effect of the Amoco-Cadiz oil spill, where diversity was not impaired, and to rank the Frierfjord samples correctly with respect to levels of stress which had been determined by a wide variety of more time-consuming species-level techniques, suggests that this approach may retain much of the sensitivity of multivariate methods. It certainly seems, at least, that there is a high signal/noise ratio in the sense that natural environmental variation does not affect the communities at this phyletic level to an extent which masks the response to perturbation. The fact that this meta-analysis "works" presently has a rather weak theoretical basis. Why should Mollusca as a phylum be more sensitive to perturbation than Annelida, for

example? The answer to this is unlikely to be straightforward and would need to be addressed by considering a broad range of toxicological, physiological and ecological characteristics which are more consistent within than between phyla.

As presented, the application of these findings to the evaluation of data from new situations requires that both abundance and biomass data are available. The scale of perturbation is determined by the 50 samples present in the meta-analysis. These can be regarded as the training set against which the status of new samples can be judged. The best way to achieve this would be to merge the new data with the training set to generate a single production matrix for a re-run of the MDS analysis. The positions of the new data in the two dimensional configuration, especially their location on the principal axis, can then be noted. Of course the positions of the samples in the training set may then be altered relative to each other, though such re-adjustments would be expected to be small. It is also natural, at least in some cases, that each new data set should add to the body of knowledge represented in the meta-analysis, by becoming part of an expanded training set against which further data are assessed. This approach would preserve the theoretical superiority and practical robustness of applying MDS (Chapter 5) in preference to ordination methods such as PCA. However, such a strategy could be computationally demanding and there are certain circumstances in which more approximate methods might be appropriate, such as when the number of new data points is very large, no computer is available or when it is preferable to leave the training data set unmodified. Fortunately, because of the relatively low dimensionality of the multivariate space (14 phyla, of which only half are of significance), a two-dimensional PCA of the "production" data gives a plot which is rather close to the MDS solution. The eigenvectors for the first three principal components, which explain 72% of the total variation, are given in Table 15.1.

The value of the PC1 score for any existing or new sample can easily be calculated from the first column of this table, without the use of a computer. This score could, with certain caveats (see below), be interpreted as a disturbance index. This index is on a continuous scale but, on the basis of the present training data set, samples with a score of $>+1$ can be regarded as grossly disturbed, those with a value between -0.2 and $+1$ as showing some evidence of disturbance and those with values <-0.2 as not signalling disturbance with this methodology. A more robust, though less incisive, interpretation would place less reliance on the absolute

location of samples on the MDS or PCA plots and emphasise the *movement* (to the right) of putatively impacted samples relative to appropriate controls. For a new study, the spread of sample positions in the meta-analysis allows one to scale the importance of observed changes, in the context of differences between control and impacted samples for the training set.

Table 15.1. Joint NE Atlantic shelf studies ("meta-analysis") (J). Eigenvectors for first three principal components from covariance-based PCA of standardised and 4th root-transformed phylum "production" (all samples).

| Phylum | PC1 | PC2 | PC3 |
|-----------------|--------|--------|--------|
| Cnidaria | -0.039 | 0.094 | 0.039 |
| Platyhelminthes | -0.016 | 0.026 | -0.105 |
| Nemertea | 0.169 | 0.026 | 0.061 |
| Nematoda | 0.349 | -0.127 | -0.166 |
| Priapulida | -0.019 | 0.010 | 0.003 |
| Sipuncula | -0.156 | 0.217 | 0.105 |
| Annelida | 0.266 | 0.109 | -0.042 |
| Chelicerata | -0.004 | 0.013 | -0.001 |
| Crustacea | 0.265 | 0.864 | -0.289 |
| Mollusca | -0.445 | -0.007 | 0.768 |
| Phoronida | -0.009 | 0.005 | 0.008 |
| Echinodermata | -0.693 | -0.404 | -0.514 |
| Hemichordata | -0.062 | -0.067 | -0.078 |
| Chordata | -0.012 | 0.037 | -0.003 |

It is perhaps premature, however, to make a positive recommendation that new data sets should be evaluated in either of the above ways. The training data is unlikely to be fully representative of all types of perturbation that could be encountered. For example, all the grossly polluted samples presently involve organic enrichment of some kind, which is conducive to the occurrence of the large nematodes which play some part in the positioning of these samples at the extreme right of the meta-analysis MDS or PCA. This may not happen with communities subjected to toxic chemical contamination only. Also, the training data are only from the NE European shelf, although data from a tropical locality (Trinidad, West Indies) have also been shown to conform with the same trend (Agard *et al.*, 1993).

INCREASED VARIABILITY

Warwick and Clarke (1993b) noted that, in a variety of environmental impact studies, the variability among samples collected from impacted areas was much greater than that from control sites. The suggestion was that this variability in itself may be an identifiable

symptom of perturbed situations. The four examples examined were:

- 1) *Meiobenthos from a nutrient-enrichment study (N)*; a mesocosm experiment to study the effects of three levels of particulate organic enrichment (control, low dose and high dose) on meiobenthic community structure (nematodes plus copepods), using four replicate box-cores of sediment for each treatment level.
- 2) *Macrobenthos from the Ekofisk oil field, N Sea (E)*; a grab sampling survey at 39 stations around the oil field centre. To compare the variability among samples at different levels of pollution impact, the stations were divided into four groups (A-D) with approximately equal variability with respect to pollution loadings. These groups were selected from a scatter plot of the concentrations of two key pollution-related environmental variables, petroleum hydrocarbons and barium. Since the dose/response curve of organisms to pollutant concentrations is usually logarithmic, the values of these two variables were log-transformed.
- 3) *Corals from S Tikus Island, Indonesia (I)*; changes in the structure of reef-coral communities between 1981 and 1983, along ten replicate line transects, resulting from the effects of the 1982-83 El Niño.
- 4) *Reef-fish in the Maldive Islands (M)*; the structure of fish communities on reef flats at 23 coral sites, 11 of which had been subjected to mining, with the remaining 12 unmined sites acting as controls.

Data were analysed by non-metric MDS using the Bray-Curtis similarity measure and either square root (mesocosm, Ekofisk, Tikus) or fourth root (Maldives) transformed species abundance data (Fig. 15.4). While the control and low dose treatments in the meiofaunal mesocosm experiment show tight clustering of replicates, the high dose replicates are much more diffusely distributed (Fig. 15.4a). For the Ekofisk macrobenthos, the Group D (most impacted) stations are much more widely spaced than those in Groups A-C (Fig. 15.4b). For the Tikus Island corals, the 1983 replicates are widely scattered around a tight cluster of 1981 replicates (Fig. 15.4c), and for the Maldives fish the control sites are tightly clustered entirely to the left of a more diffuse cluster of replicates of mined sites (Fig. 15.4d). Thus, the increased variability in multivariate structure with increased disturbance is clearly evident in all examples.

It is possible to construct an index from the relative variability between impacted and control samples. One obvious comparative measure of dispersion would be based on the difference in average distance

For the Ekofisk macrobenthos, strongly positive values are found in comparisons between the group D (most impacted) stations and the other three groups. It should be noted however that stations in groups C, B and A are increasingly more widely spaced geographically. Whilst groups B and C have similar variability, the degree of dispersion increases between the two outermost groups B and A, probably due to natural spatial variability. However, the most impacted stations in group D, which fall within a circle of 500 m diameter around the oil-field centre, still show a greater degree of dispersion than the stations in the outer group A which are situated *outside* a circle of 7 kilometers diameter around the oil-field. Comparison of the impacted versus control conditions for both the Tikus Island corals and the Maldives reef-fish gives strongly positive IMD values. For the Maldives study, the mined sites were more closely spaced geographically than the control sites, so this is another example for which the increased dispersion resulting from the anthropogenic impact is "working against" a potential increase in variability due to wider spacing of sites. Nonetheless, for both the Ekofisk and Maldives studies the increased dispersion associated with the impact more than cancels out that induced by the differing spatial scales. For both the mesocosm meiobenthos and the Tikus Island coral studies there are no such differences in spatial layout between the treatments to dilute the observed dispersion effects.

Application of the comparative index of multivariate dispersion suffers from the lack of any obvious statistical framework within which to test hypotheses of comparable variability between groups. As proposed, it is also restricted to the comparison of only two groups, though it can be extended to several groups in straightforward fashion. Let \bar{r}_i denote the mean of the $N_i = n_i(n_i - 1)/2$ rank similarities among the n_i samples within the i th group ($i = 1, \dots, g$), having (as before) re-ranked the triangular matrix ignoring all between-group similarities, and let N denote the number of similarities involved in this ranking process ($N = \sum_i N_i$). Then the *dispersion sequence*

$$\bar{r}_1/k, \bar{r}_2/k, \dots, \bar{r}_g/k \quad (15.4)$$

defines the relative variability within each of the g groups, the larger values corresponding to greater within-group dispersion. The denominator scaling factor k is $(N + 1)/2$, i.e. simply the mean of all N ranks involved, so that a *relative dispersion* of unity corresponds to "average dispersion". (If the number of samples is the same in all groups then the values in

equation (15.4) will average unity, though this will not quite be the case if the $\{n_i\}$ are unbalanced.)

As an example, the relative dispersion values given by equation (15.4) have been computed¹ for the four studies considered above (Table 15.3). This can be seen as complementary information to the IMD values; Table 15.2 provides the pairwise comparisons following on from the global picture in Table 15.3. The conclusions from Table 15.3 are, of course, consistent with the earlier discussion, e.g. the increase in variability at the outermost sites in the Ekofisk study, because of their greater geographical spread, being nonetheless smaller than the increased dispersion at the central, impacted stations.

Table 15.3. *Variability study (N, E, I, M). Relative dispersion of the groups (equation 15.4) in each of the four studies.*

| | | |
|--------------|---------------|------|
| Meiobenthos | Control | 0.58 |
| | Low dose | 0.79 |
| | High dose | 1.63 |
| Macrobenthos | Group A | 1.34 |
| | Group B | 0.79 |
| | Group C | 0.81 |
| | Group D | 1.69 |
| Corals | 1981 | 0.58 |
| | 1983 | 1.42 |
| Reef-fish | Control reefs | 0.64 |
| | Mined reefs | 1.44 |

BREAKDOWN OF SERIATION

Clear-cut zonation patterns in the form of a serial change in community structure with increasing water depth are a striking feature of intertidal and shallow-water benthic communities on both hard and soft substrata. The causes of these zonation patterns are varied, and may differ according to circumstances, but include environmental gradients such as light or wave energy, competition and predation. None of these mechanisms, however, will necessarily give rise to discontinuous bands of different assemblages of species, which is implied by the term *zonation*, and the more general term *seriation* is perhaps more appropriate for this pattern of community change, zonation (with discontinuities) being a special case. Many of the factors which determine the pattern of

1. Both the IMD and the relative dispersion values are computed by the PRIMER program MVDISP.

seriation are likely to be modified by disturbances of various kinds. For example, dredging may affect the turbidity and sedimentation regimes and major engineering works may alter the wave climate. Elimination of a particular predator may affect patterns which are due to differential mortality of species due to that predator. Increased disturbance may also result in the relaxation of interspecific competition, which may in turn result in a breakdown of the pattern of seriation induced by this mechanism. Where a clear sequence of community change along transects is evident in the undisturbed situation, the degree of breakdown of this sequencing could provide an index of subsequent disturbance. Clarke *et al.* (1993) have described a possible index of multivariate seriation, and applied it to a study of the impact of dredging on intertidal coral reefs at Ko Phuket, Thailand (K).

In 1986, a deep-water port was constructed on the south-east coast of Ko Phuket, involving a 10-month dredging operation. Three transects were established across nearby coral reefs (Fig. 15.5), transect A being closest to the port and subject to the greatest sedimentation, partly through the escape of fine clay particles through the southern containing wall. Transect C was some 800 m away, situated on the edge of a channel where tidal currents carry sediment plumes away from the reef, and transect B was

expected to receive an intermediate degree of sedimentation.

Surveys of these three transects, perpendicular to the shore, were conducted in 1983, 86, 87 and 88. Line-samples 10m long were placed parallel to the shore at 10m intervals along the perpendicular transect from the inner reef flat to the outer reef edge; 12 lines along each of transects A and C and 17 along transect B. The same transects were relocated each year and living coral cover (m) of each species recorded. Transect C was not surveyed in 1986.

The basic data were root-transformed and Bray-Curtis similarities calculated between every pair of samples within each year/transect combination; the resulting triangular similarity matrices were then input to non-metric MDS (Fig. 15.6). By joining the points in an MDS, in the order of the samples along the offshore transect, one can visualise the degree of seriation, that is, the extent to which the community changes in a smooth and regular fashion, departing ever further from its state at the start of the transect. A measure of linearity of the resulting sequence could be constructed directly from the location of the points in the MDS. However, this could be misleading when the stress is not zero, that is, the full pattern of relationships between the samples cannot be perfectly represented in 2 dimensions; this will often be the case, as with some of the component plots in Fig. 15.6. (Even where the stress is low, the well-known *horseshoe effect*, Seber, 1984, will mitigate against a genuinely linear sequence appearing in a 2-d ordination as a straight line; see footnote 5 in Chapter 11.) Again, a more satisfactory approach is to work with the fundamental similarity matrix that underlies the MDS plots, of whatever dimension. The index of multivariate seriation (IMS) proposed is therefore defined as a Spearman correlation coefficient (ρ_s , e.g. Kendall, 1970) computed between the corresponding elements of two triangular matrices of rank n "dissimilarities". The first is that of Bray-Curtis coefficients calculated for all pairs from the n coral community samples ($n=12$ or 17 in this case). The second is formed from the inter-point distances of n points laid out, equally-spaced, along a line. If the community changes exactly match this linear sequence (for example, sample 1 is close in species composition to sample 2, samples 1 and 3 are less similar, 1 and 4 less similar still, up to 1 and 12 having the greatest dissimilarity) then the IMS takes the value 1. If, on the other hand, there is no discernible biotic pattern along the transect, or if the relationship between the community structure and distance offshore is very

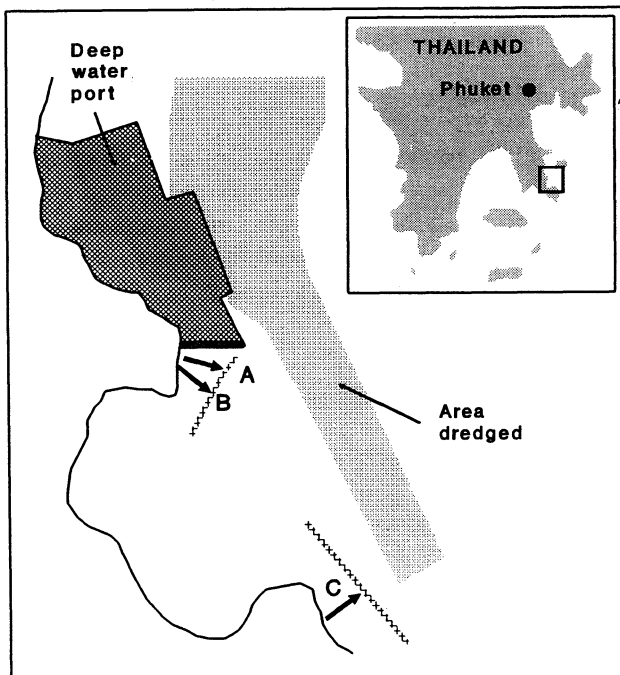


Fig. 15.5. Ko Phuket corals (K). Map of study site showing locations of transects A, B and C.

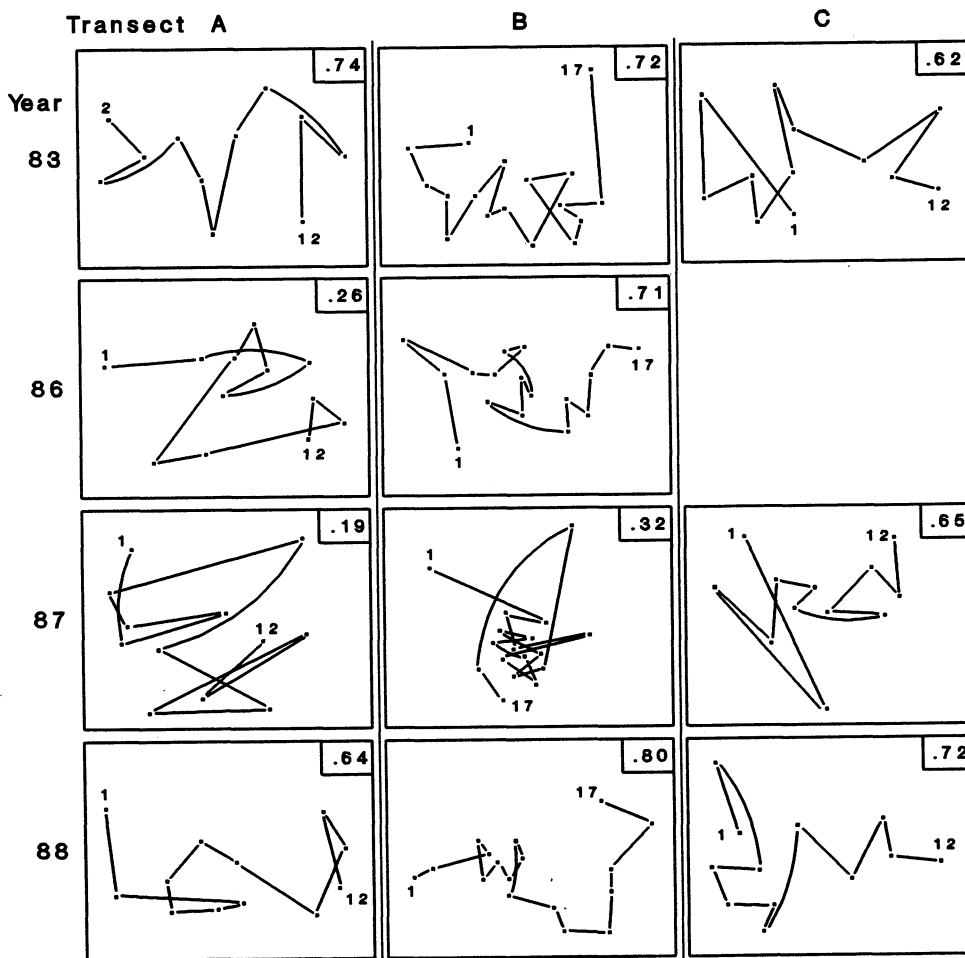


Fig. 15.6. Ko Phuket corals (K). MDS ordination of the changing coral communities (species cover data) along three transects (A to C) at four times (1983 to 1988). The lines indicate the degree of seriation by linking successive points along a transect, from onshore (1) to offshore samples (12 or 17); IMS values are at top right. Sample 1 from transect A in 1983 is omitted (see text) and no samples were taken for transect C in 1986 (reading across rows, stress = 0.10, 0.11, 0.09; 0.10, 0.11; 0.08, 0.14, 0.11; 0.07, 0.09, 0.10).

non-monotonic – with the composition being similar at opposite ends of the transect but very different in the middle – then the IMS will be close to zero. These near-zero values can be negative as well as positive but no particular significance attaches to this.

A statistical significance test would clearly be useful, to answer the question: when is the IMS sufficiently different from zero to reject the null hypothesis of a complete absence of seriation? Such a test can be derived by a *Monte Carlo permutation procedure*. If the null hypothesis is true then the labelling of samples along the transect (1,2,...,n) is entirely arbitrary, and the spread of IMS values which are consistent with the null hypothesis can be determined by recomputing it for permutations of the sample labels in one of the two similarity matrices (holding the other fixed). For T randomly selected permutations of the sample labels, if only t of the T simulated IMS values are greater than or equal to the observed IMS, the null hypothesis can be rejected at a significance level of $100(t+1)/(T+1)\%$. In structure, the test is analogous to that considered at the end of Chapter 6 (implemented in the PRIMER program ANOSIM2), and again referred to briefly in Chapter 11 in the context of the BIO-ENV procedure.

One distinctive feature of the current test is that tied ranks will be much more prevalent, particularly in the similarities computed from the linear sequence, and it is advisable to make proper allowance for this in calculating the Spearman coefficients. Kendall (1970, equation 3.7) gives an appropriate adjustment to ρ_s , and this form is used in the analysis below.²

In 1983, before the dredging operations, MDS configurations (Fig. 15.6) indicate that the points along each transect conform rather closely to a linear sequence, and there are no obvious discontinuities in the sequence of community change (i.e. no discrete

2. The calculations for the tests were carried out using the PRIMER program RELATE. The similarity-based formulation, and the associated permutation test, are also readily extendable to more complex models than a linear sequence of change along a spatial transect. In a homologous way, community change could be related to a temporal trend or cyclicity, or to the sampling positions in a 2-dimensional spatial layout. There are null hypothesis tests for all these possibilities in RELATE, in addition to a general test for lack of relationship between any two supplied similarity matrices with the same label sets (independently derived).

clusters separated by large gaps); the community change follows a quite gradual pattern. The values of the IMS are consequently high (Table 15.4), ranging from 0.62 (transect C) to 0.72 (transect B).

The correlation with a linear sequence is highly significant in all three cases. Note that in the 1983 MDS for transect A the furthest inshore sample has been omitted; it had very little coral cover and was an outlier on the plot, resulting in an unhelpfully condensed display of the remaining points. (This is to be expected in MDS analyses where one sample has a higher dissimilarity to *all* other samples than any other dissimilarity in the matrix, and the MDS needs to be replotted with this point removed.) There is no similar technical need, however, to remove this sample from the IMS calculation; this was not done in Table 15.4 though doing so would increase the ρ_s value from 0.65 to 0.74 (as indicated in Fig. 15.6).

Table 15.4. *Ko Phuket corals (K). Index of Multivariate Seriation (IMS) along the three transects, for four sampling occasions. Figures in parenthesis are the % significance levels in a permutation test for absence of seriation (T = 999 simulations).*

| Year | Transect A | Transect B | Transect C |
|------|-------------|-------------|-------------|
| 1983 | 0.65 (0.1%) | 0.72 (0.1%) | 0.62 (0.1%) |
| 1986 | 0.26 (3.8%) | 0.71 (0.1%) | – |
| 1987 | 0.19 (6.4%) | 0.32 (0.2%) | 0.65 (0.1%) |
| 1988 | 0.64 (0.1%) | 0.80 (0.1%) | 0.72 (0.1%) |

On transect A, subjected to the highest sedimentation, visual inspection of the MDS gives a clear impression of the breakdown of the linear sequence for the subsequent two sampling occasions. The IMS is dramatically reduced to 0.26 in 1986, when the dredging operations commenced, although the correlation with a linear sequence is still just significant ($p=3.8\%$). By 1987 the IMS on this transect is further reduced to 0.19 and the correlation with a linear sequence is no longer significant. On transect B, further away from the dredging activity, the loss of

seriation is not evident until 1987, when the sequencing of points on the MDS configuration breaks down and the IMS is reduced to 0.32, although the latter is still significant ($p=0.2\%$). Note that the MDS plots of Fig. 15.6 may not tell the whole story; the stress values lie between 0.07 and 0.14, indicating that the 2-dimensional pictures are not perfect representations (though unlikely seriously to mislead, see Chapter 5). The largest stress is, in fact, that for transect B in 1987, so that the seriation that is still detectable by the test is only imperfectly seen in the 2-dimensional plot. It is also true that the increased number of points (17) on transect B, in comparison with A and C (12), will lead to a more sensitive test. On transect C there is no evidence of the breakdown of seriation at all, either from the IMS values or from inspection of the MDS plot. By 1988 transects A and B had completely recovered their seriation pattern, with IMS values equal to or higher than their 1983 values, highly significant correlations with a linear sequence ($p<0.1\%$) and clear sequencing evident on the MDS plots. There was clearly a graded response, with a greater breakdown of seriation occurring earlier on the most impacted transect, some breakdown on the middle transect but no breakdown at all on the least impacted transect.

Overall, the breakdown in the pattern of seriation was due to the increase in distributional range of species which were previously confined to distinct sections of the shore. This is commensurate with the disruption of almost all the types of mechanism which have been invoked to explain patterns of seriation, and gives us no clue as to which of these is the likely cause.

RECOMMENDATIONS

At the time of writing, these three recently devised methodologies still require more rigorous exploration, generalisation and validation before their widespread use can be recommended. Any application of them should therefore be made with these objectives in view.

APPENDIX 1: INDEX OF EXAMPLE DATA

The following is a list of all (real) data sets used as examples in the text, where they are referenced by their indexing letter (A-Z). In addition to the pages on which these examples can be found, the entries give the source reference (see also Appendix 3) for the original publication of these data. Note that these are not always the appropriate references for the analyses presented in the text; the latter can generally be found in Appendix 2.

- A – Amoco-Cadiz oil spill, Bay of Morlaix, France. *Macrofauna*. (Dauvin, 1984)
p 10-4, 10-5, 13-2, 13-3, 15-2, 15-3
- B – Bristol Channel, England. *Zooplankton*. (Collins and Williams, 1982)
p 3-5, 3-6, 7-2, 7-3, 7-4, 11-3, 11-4
- C – Celtic Sea. *Zooplankton*. (Collins, pers. comm.).
p 5-9
- D – Dosing experiment, Solbergstrand mesocosm, Norway (GEEP Workshop). *Nematodes*. (Warwick *et al.*, 1988).
p 4-8, 5-8, 9-4
- E – Ekofisk oil platform, N. Sea. *Macrofauna*. (Gray *et al.*, 1990).
p 8-4, 8-5, 10-5, 10-6, 14-2, 14-3, 15-5, 15-6, 15-7
- F – Frierfjord, Norway (GEEP Workshop). *Macrofauna*. (Gray *et al.*, 1988).
p 1-3, 1-4, 1-9, 1-10, 3-1, 3-2, 6-1, 6-2, 6-3, 6-4, 8-9, 9-1, 10-1, 10-2, 13-6, 14-1, 14-2, 15-2, 15-3
- G – Garroch Head, Scotland. *Macrofauna*. (Pearson and Blackstock, 1984).
p 1-6, 1-7, 1-8, 1-11, 1-12, 8-4, 8-5, 8-6, 8-8, 8-11, 11-1, 11-2, 11-3, 11-5, 11-9, 11-10, 15-2, 15-3
- H – Hamilton Harbour, Bermuda (GEEP Workshop). *Macrofauna, nematodes*. (Warwick *et al.*, 1990c).
p 8-2, 8-3, 8-11, 13-3, 13-4
- I – Indonesian reef corals, S. Pari and S. Tikus Islands. *Coral % cover*. (Warwick *et al.*, 1990b).
p 6-5, 6-6, 8-2, 10-4, 10-5, 10-7, 13-4, 13-5, 14-3, 14-4, 15-5, 15-6, 15-7
- J – Joint NE Atlantic shelf studies ("meta-analysis"). *Macrofauna "production"*. (Warwick and Clarke, 1993a)
p 15-2, 15-3, 15-4, 15-5
- K – Ko Phuket coral reefs, Thailand. *Coral species cover*. (Clarke *et al.*, 1993).
p 15-8, 15-9, 15-10
- L – Loch Linnhe and Loch Eil, Scotland. *Macrofauna*. (Pearson, 1975).
p 1-6, 1-7, 1-10, 4-4, 4-5, 8-6, 8-7, 8-10, 10-3, 10-4, 10-6, 10-7, 15-2, 15-3
- M – Maldive Islands. *Coral reef fish*. (Dawson-Shepherd *et al.*, 1992).
p 13-2, 14-4, 14-5, 15-5, 15-6, 15-7
- N – Nutrient-enrichment experiment, Solbergstrand mesocosm, Norway. *Nematodes, copepods*. (Gee *et al.*, 1985).
p 1-12, 1-13, 10-3, 10-4, 12-5, 12-6, 15-5, 15-6, 15-7
- P – Plankton survey (Continuous Plankton Recorder), N.E. Atlantic. *Zooplankton, phytoplankton*. (Colebrook, 1986).
p 13-1
- R – Tamar estuary mud-flat, S.W. England. *Nematodes, copepods*. (Austen and Warwick, 1989).
p 14-6, 14-7, 14-8
- S – Scilly Isles, U.K. *Seaweed metazoa*. (Gee and Warwick, 1994).
p 13-5, 14-5, 14-6
- T – Tasmania, Eaglehawk Neck. *Nematodes, copepods*. (Warwick *et al.*, 1990a).
p 6-7, 6-8, 12-2, 12-3, 12-4, 13-3, 13-4, 14-7
- W – Westerschelde estuary cores, Netherlands; mesocosm experiment on food supply. *Nematodes*. (Austen and Warwick, in press)
p 6-8, 6-9, 6-11
- X – Exe estuary, England. *Nematodes*. (Warwick, 1971).
p 5-3, 5-4, 5-7, 6-11, 6-12, 7-1, 7-2, 11-5, 11-6, 11-7, 11-9
- Y – Clyde, Scotland. *Nematodes*. (Lamshead, 1986)
p 6-6, 6-7
- Z – Azoic sediment recolonization experiment. *Copepods*. (Olafsson and Moore, 1992).
p 12-4

APPENDIX 2: PRINCIPAL LITERATURE SOURCES AND FURTHER READING

This manual chiefly reflects an approach to multivariate and other graphical community analyses that has been adopted and developed at the Plymouth Marine Laboratory (PML) over the last decade, and has been the subject of assessment and training at several IOC and FAO/UNEP workshops (e.g. papers in Bayne *et al.* 1988, Addison and Clarke 1990). Methodological papers involving work at PML include: Field *et al.* (1982), Warwick (1986), Clarke and Green (1988), Clarke (1990), Warwick and Clarke (1993a & b), Clarke and Ainsworth (1993), and Clarke and Warwick (1994). Clarke (1993) and Warwick (1993) review these methods, and a number of PML papers exemplify their use through the PRIMER package: see for example the papers listed under Warwick in Appendix 3.

Of course, the exposition here draws on a wider body of statistical and descriptive techniques, and there follows a brief listing of the main papers and books that can be consulted for further details of the methods and analyses of each Chapter.

Chapter 1: Framework. The categorisation here is an extension of that given by Warwick (1988a). The Frierfjord macrofauna data and analyses (Tables 1.2 & 1.6 and Figs. 1.1, 1.2 & 1.7) are extracted and redrawn from Bayne *et al.* (1988), Gray *et al.* (1988) and Clarke and Green (1988), the Loch Linnhe macrofauna data (Table 1.4 and Fig. 1.3) from Pearson (1975), and the ABC curves from Warwick (1986). The species abundance distribution for Garroch Head macrofauna (Fig. 1.6) is first found in Pearson *et al.* (1983), and the multivariate linking to environmental variables (Fig. 1.9) in Clarke and Ainsworth (1993). The Solbergstrand mesocosm data and analysis (Table 1.7 and Fig. 1.10) are extracted and redrawn from Gee *et al.* (1985).

Chapters 2 and 3: Similarity and Clustering. These methods originated in the 1950's and 60's (e.g. Florek *et al.*, 1951; Sneath, 1957; Lance and Williams, 1967). The description here is a widening of the discussion in Field *et al.* (1982), with some points taken from the recommended general texts of Everitt (1980) and Cormack (1971). The dendrogram of Frierfjord macrofaunal samples (Fig. 3.1) is redrawn from Gray *et al.* (1988), and the zooplankton example (Figs. 3.2 & 3.3) from Collins and Williams (1982).

Chapter 4: Ordination by PCA. This is one of the founding techniques of multivariate statistics; standard modern texts include Chatfield and Collins (1980) and Everitt (1978). The concluding example (Fig. 4.2) is from Warwick *et al.* (1988).

Chapter 5: Ordination by MDS. Non-metric MDS was introduced by Shepard (1962) and Kruskal (1964); standard texts are Kruskal and Wish (1978) and Schiffman *et al.* (1981). Here, the exposition parallels that in Field *et al.* (1982) and Clarke (1993); the Exe nematode graphs (Figs. 5.1–5.4) are redrawn from the former. The dosing experiment (Fig. 5.5) is discussed in Warwick *et al.* (1988).

Chapter 6: Testing. The basic permutation test and simulation of significance levels can be traced to Mantel (1967) and Hope (1968), respectively. In this context (e.g. Figs. 6.2 & 6.3 and equation 6.1) it is described by Clarke and Green (1988). A fuller discussion of the extension to 2-way nested and crossed ANOSIM tests (including Figs. 6.4 & 6.6) is in Clarke (1993) (with some asymptotic results in Clarke, 1988); the coral analysis (Fig. 6.5) is discussed in Warwick *et al.* (1990b), and the Tasmanian meiofaunal MDS (Fig. 6.7) is in Warwick *et al.* (1990a). The 2-way crossed design without replication (Figs. 6.8–6.12) is tackled in Clarke and Warwick (1994); see also Austen and Warwick (in press).

Chapter 7: Species analyses. Clustering and ordination of species similarities is as illustrated in Field *et al.* (1982), for the Exe nematode data (Figs. 7.1 & 7.2, redrawn); see also Clifford and Stephenson (1975). The SIMPER ("similarity percentages") procedure is described in Clarke (1993).

Chapter 8: Univariate/graphical analyses. Pielou (1975), Heip *et al.* (1988) and Magurran (1991) are useful texts, summarising a vast literature on a variety of diversity indices and ranked species abundance plots. The diversity examples here (Figs. 8.1 & 8.2) are discussed by Warwick *et al.* (1990c, 1990b respectively) and the Caswell *V* computations (Table 8.1) are from Warwick *et al.* (1990c). The Garroch Head species abundance distributions (Fig. 8.4) are first found in Pearson *et al.* (1983); Fig. 8.3 is redrawn from Pearson and Blackstock (1984). Warwick (1986) introduced Abundance–Biomass Comparison curves, and the Loch Linnhe and Garroch Head illustrations (Figs. 8.7

& 8.8) are redrawn from Warwick (1986) and Warwick *et al.* (1987). The transformed scale and "partial dominance" curves of Figs. 8.9–8.11 were suggested by Clarke (1990), which paper also tackles issues of summary statistics (Fig. 8.12, and as employed in Fig. 8.13) and significance tests for dominance curves.

Chapter 9: Transformations. This chapter is an expansion of the discussion in Clarke and Green (1988); Fig. 9.1 is recomputed from Warwick *et al.* (1988).

Chapter 10: Aggregation. This description of the effects of changing taxonomic level is based on Warwick (1988b), from which Figs. 10.2–10.4 and 10.7 are redrawn. Fig. 10.1 is discussed in Gray *et al.* (1988), Fig. 10.5 and 10.8 in Warwick *et al.* (1990b) and Fig. 10.6 in Gray *et al.* (1990) (or Warwick and Clarke, 1993a, in this categorisation).

Chapter 11: Linking to environment. For wider reading on this type of "canonical" problem, see Chapter 5 of Jongman *et al.* (1987), including ter Braak's (1986) method of canonical correspondence analysis. The approach here of performing environmental and biotic analyses separately, and then comparing them, is a combination of that advocated by Field *et al.* (1982: superimposing variables on the biotic MDS) and by Clarke and Ainsworth (1993: the BIO-ENV program). The data in Table 11.1 is from Pearson and Blackstock (1984). Fig. 11.3 is redrawn from Collins and Williams (1982) and Fig. 11.6 from Field *et al.* (1982); Figs. 11.7–11.9 and Table 11.2 are from Clarke and Ainsworth (1993).

Chapter 12: Community experiments. Influential papers on field experiments in general include

Connell (1974) and Hurlbert (1984); Underwood and Peterson (1988) give some thoughts on mesocosm experiments. Figs. 12.2 and 12.3 are redrawn from Warwick *et al.* (1990a) and Figs. 12.5 and 12.6 from Gee *et al.* (1985).

Chapter 13: Data requirements. The exposition parallels that in Warwick (1993) but with additional examples. Figs. 13.1–13.3 and 13.8 are redrawn from Warwick (1993), and earlier found in Colebrook (1986), Dawson-Shepherd *et al.* (1992), Warwick (1988b) and Gray *et al.* (1988) respectively. Fig. 13.4 is redrawn from Warwick *et al.* (1990a), Fig. 13.5 from Warwick *et al.* (1990c), Fig. 13.6 from Warwick *et al.* (1990b) and Fig. 13.7 from Warwick and Clarke (1991).

Chapter 14: Relative sensitivities. This parallels the earlier sections of Warwick and Clarke (1991), from which all these figures (except Figs. 14.11 & 14.14) have been redrawn. Primary source versions of the figures can be found as follows: Figs. 14.1–14.3, Gray *et al.* (1988); Figs. 14.5–14.7, Warwick *et al.* (1990b); Figs. 14.9–14.10, Dawson-Shepherd *et al.* (1992); Figs. 14.11–14.12, Gee and Warwick (1994); Figs. 14.14–14.16, Austen and Warwick (1989).

Chapter 15: Multivariate measures of disturbance. This is an amalgamation of ideas from three papers: Warwick and Clarke (1993a) on "meta-analysis" of NE Atlantic macrobenthic studies, Warwick and Clarke (1993b) on the increase in multivariate dispersion under disturbance, and Clarke *et al.* (1993) on the breakdown of multivariate seriation patterns. Figs. 15.1–15.3 and Table 15.1 are redrawn and extracted from the first, Fig. 15.4 and Table 15.2 from the second and Figs. 15.5 & 15.6 and Table 15.4 from the third.

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