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MULTIVARIATE MEASURES OF COMMUNITY STRESS AND THEIR APPLICATION TO MARINE POLLUTION STUDIES IN THE EAST ASIAN REGION

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ABSTRACT

Three recently developed measures of stress on marine communities, which utilise species-dependent multivariate methods, are described. The first is a "meta-analysis" of benthic macrofaunal community data from NE Atlantic shelf stations with known community responses to pollution or disturbance at the phyletic level. These can be used as a training data set against which new data can be evaluated. The method has the advantages that it overcomes taxonomic difficulties (requiring phylum-level identification only) and offers the possibility of evaluating the severity of disturbance on a more globally comparative scale. Two other methods measure the increase in variability of multivariate community structure and the breakdown of zonation (or seriation) patterns as a result of disturbance. These latter methodologies are valuable because they can be used to assess environmental impacts on those types of communities which are of primary conservation value in the East Asian region (notably coral communities and their associated biota) and which are therefore a major focus for environmental concern.

INTRODUCTION

Multivariate methods of data analysis are very sensitive for detecting differences in community structure between samples in space, or changes over time (Gray *et al.*, 1990; Warwick and Clarke, 1991; Dawson Shepherd *et al.*, 1992). 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, dominance 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 may also arise when, as occasionally happens, the species responsible for increased dominance 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, and the resulting conclusions will be derived from an informal hybrid of species-independent and species-dependent information (Warwick and Clarke, 1994). In this paper we describe three possible approaches to the measurement of community stress using the fully species-dependent multivariate methods, and discuss their appropriateness for application in the East Asian Seas Region. In all cases, computation utilised the software package PRIMER (Plymouth Routines in Multivariate Ecological Research) developed at the Plymouth Marine Laboratory, UK.

Tropical areas of the world have some features which differ from temperate regions, where most of the techniques for assessing the biological effects of pollution on communities have been developed. Thorson (1966) for example noted that, for the soft-bottom benthos which are most widely used in pollution impact studies, communities off the west coast of Thailand (Andaman Sea) were characterised by a large number of species each represented by only a few specimens (*i.e.* high diversity and low dominance) compared with the communities from higher latitudes which he had previously studied.

Furthermore, he was disappointed to find that there were no "parallel" communities (similar habitats characterised by the same dominating genera) which had previously been recognized in different parts of the world. Unfortunately he died before being able to make his intended new evaluation of this problem. On the other hand, Warwick and Ruswahyuni (1987) found obvious parallel communities, with similar diversity and dominance profiles to those of higher latitudes, on the north coast of Java. Perhaps the most daunting problem associated with the analysis of soft-bottom communities for pollution impact studies in many tropical areas (including East Asia) is the inadequacy of the available taxonomic literature, making the sorting of samples into species a very difficult and time-consuming exercise. The meta-analysis approach described below circumvents this problem by working at a very high taxonomic level (that of phyla) so that the identification problems are minimised. In the tropics, too, there are fragile communities of high conservation value, notably coral reefs and their associated biota (*e.g.* fish), which do not occur at higher latitudes and for which many methodologies for assessing pollution impacts have not been well evaluated. Two of the methods describe below have been applied to such communities, and one of them was specifically developed to assess the effects of increased sedimentation on coral communities in Ko Phuket.

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 "nemertines" with another at which this taxon has been divided into species (Wu, 1982).

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, disturbance effects are now known to be 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 effects of the Amoco Cadiz oil-spill in the Bay of Morlaix, France (Warwick, 1988; Warwick and Clarke, 1993a) and the effects of the Ekofisk oil-drilling platform in the North Sea (Gray *et al.*, 1990; Warwick and Clarke, 1993a).

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 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. 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:

$$P = (B/A)^{0.73} \times A \qquad \ldots\ldots\ldots\ldots(1)$$

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 (Brey, 1990). 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). The 20 phyla used are given in Table 1.

Table 1. Joint NE Atlantic shelf-studies ("meta analysis"). List of phyla used in the species aggrigation (* = phylum not encountered in this study).

1. Porifera	8. Pogonophora	15. Brachiopoda
2. Cnidaria	9. Sipuncula	16. Bryozoa
3. Platyhelminthes	10. Ehiura	17. phoronida
4. Nemertea	11. Annelida	18. Echinodermata
5. Nematoda	12. Chelicerata	19. Hemichordata
6. Priapulida	13. Crustacea	20. Chordata
7. Entoprocta	14. Mollusca	

Although only 14 of these were encountered in the examples used, all remaining phyla which are likely to occur in the macrobenthos are included for the purpose of incorporating new data (see below). The abundance and biomass matrices were then combined to form a production matrix using the above formula. All data sets were then merged into a single production matrix and a non-metric Multi-dimensional Scaling Ordination, MDS (Kruskal and Wish, 1978) performed on the standardised, 4th root-transformed data using the Bray Curtis similarity measure (Clarke 1993 discusses the rationale and guidelines for practical use of these techniques in community ecology studies). All macrobenthic studies from a single region (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 across a sewage sludge dump-ground at Garroch Head, Firth of Clyde, Scotland. Stations in the middle of the transect show clear signs of gross pollution (Pearson, 1987; Warwick *et al.*, 1987).

2). A time series of samples from 1963-1973 at stations in two West Scottish sea-lochs, L. Linnhe and L. Eil, covering the period of commissioning of a pulp-mill (Pearson, 1975). 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 (Pearson, 1975: Warwick, 1986).

3). Samples collected at six stations in Frierfjord (Oslofjord), Norway (Gray *et al.*, 1988). The stations 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, France (Dauvin, 1984). Samples taken at station "Pierre Noire" on 21 occasions at roughly 3 mo intervals between April 1977 and February 1982, spanning the period of the oil spill some 50 km west of the station which occurred in March 1978. In view of the large number of observations, the 21 sampling occasions have been aggregated into 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 (Josefson, 1981). 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, N.E. England (Buchanan and Warwick, 1974).

7). An undisturbed station in Carmarthen Bay, S. Wales (Warwick *et al.*, 1978).

8). An undisturbed station in Kiel Bay; mean of 22 sets of samples (Arntz and Rumohr, 1982).

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. 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. 2, with the remaining samples represented as dots.

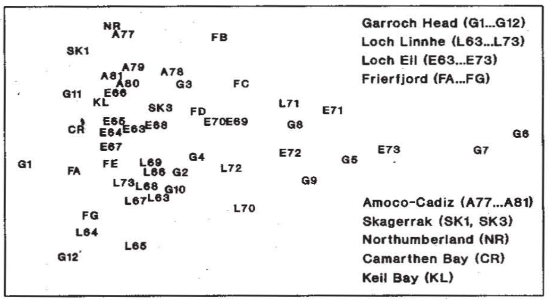

Figure 1. Joint NE Atlantic shelf studies ("meta-analysis"). Two dimensional MDS ordination of phylum level "production" data (stress = 0.16).

1) ***Garroch Head (Clyde) sewage-sludge dump-ground.*** Samples taken along this transect span the full scale of the long axis of the configuration (Fig. 2a). 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 and 7) are at the extreme right.

2) ***Loch Linnhe and Loch Eil.*** In the early years (1963-68) both stations are situated at the unpolluted left-hand end of the configuration (Fig. 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 (Oslofjord).*** The left to right sequence of stations in the meta-analysis is A-G-E-D-B-C (Fig. 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).*** 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. 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. 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 (see Field *et al.*, 1982 for methodology). 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"

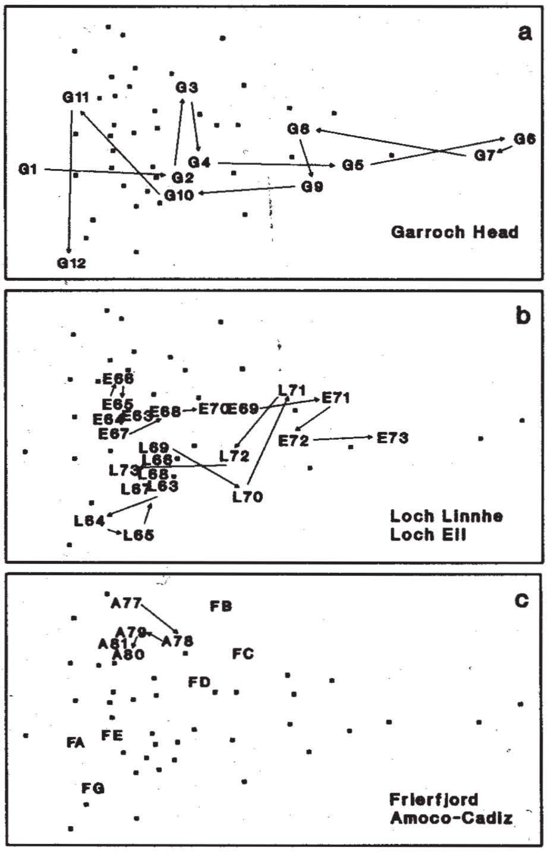

Figure 2. As Fig. 1 but with individual studies highlighted: a) Garroch Head (Clyde) dump-ground; b) Loch Linnhe and Loch Eil; c) Frierfjord and Amoco-Cadiz spill (Morlaix).

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. Crustacea are again concentrated to the left, but this time completely confined to the top part of the configuration. 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 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 specieslevel 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 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 together explain 72% of the total variation, are given in Table 2.

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 exhibiting 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 2. Joint NE Atlantic shelf studies ("meta-analysis"). 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
Nematada	+0.349	-0.127	-0.166
Priapulida	-0.019	+0.010	+0.003
Sipunculida	-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
Echinodermada	-0.693	-0.040	-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. Perhaps more importantly from the point of view of applying this technique to data from the East Asian Seas region is the potential problem that the training data are only from the NE Atlantic shelf. In view of the rather different nature of benthic communities in at least some parts of the region (see above) we need to test whether the phyletic composition of these communities in unpolluted situations is comparable to that of the training data and whether these proportions are changed in a similar way by increasing levels of disturbance. If not, amalgamation of data from the region with the training data would result in an ordination in which the new data are unhelpfully separated from the training data. However, data from another tropical locality (Trinidad, West Indies) have also been shown to conform with the same trend as the NE Atlantic data (Agard *et al.*, 1993), which augurs well for the more global applicability of this approach. For ease of application in regions where the taxonomic literature is less than adequate, this method has obvious advantages, with only phylum level identification being required.

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.*** 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 (Gee *et al.*, 1985).

2) ***Macrobenthos.*** A grab sampling survey at 39 stations around the Ekofisk oil-field centre in the North Sea (Gray *et al.*, 1990). 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.*** Changes in the structure of reef-coral communities between 1981 and 1983 along ten replicate line transects at Tikus Island in the Thousand Islands, Indonesia, resulting from the effects of the 1982-83 El Niño (Warwick *et al.*, 1990).

4) ***Reef-fish.*** The structure of fish communities at 23 coral reef-flat sites in the Maldive Islands (Dawson Shepherd *et al.*, 1992), 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. 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. 3a). For the Ekofisk macrobenthos, the Group D (most impacted) stations are much more widely spaced than those in Groups A-C (Fig. 3b). For the Tikus Island corals the 1983 replicates are widely scattered around a tight cluster of 1981 replicates (Fig. 3c), 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. 3d). 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 among replicate samples for the two groups in the two-dimensional MDS configuration. However, this configuration is usually not an exact representation of the rank orders of similarities between samples in higher dimensional space. These rank orders are contained in the triangular similarity matrix which underlies any MDS. The case for using this matrix rather than the distances is analogous to that given in Clarke and Ainsworth (1993). A possible comparative Index of Multivariate Dispersion (IMD) would therefore contrast the average rank of the similarities among impacted samples (r_t) with the average rank among control samples (r_c), having re-ranked the

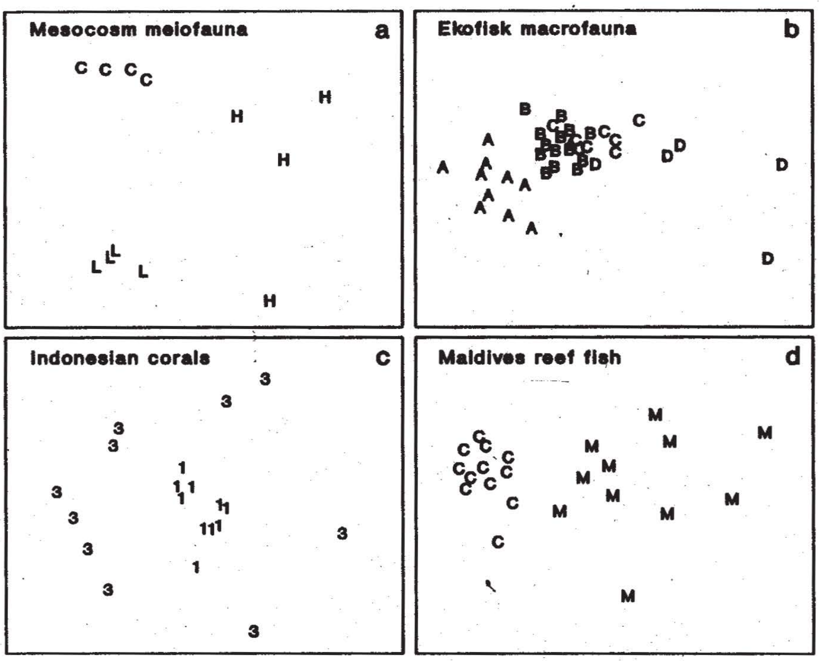

Figure 3. Variability study. Two-dimensional configurations for MDS ordinations of the four data sets. Treatment codes: a) H = High dose, L = Low dose, C = Controls; b) A-D are the station groupings by pollution load; c) 81 = 1981, 83 = 1983; d) M = Mined, C = Controls (stress: a) 0.08, b) 0.12, c) 0.11, d) 0.08)

full triangular matrix *ignoring* all between-treatment similarities. Noting that high similarity corresponds to low *rank* similarity, a suitable statistic, appropriately standardised, is:

$$\mathbf{IMD} = 2(r_t - r_c) / (N_t + N_c) \qquad \text{........}(2)$$

where

$$N_c = n_c(n_c - 1)/2, N_t = n_t(n_t - 1)/2 \qquad \text{.......}(3)$$

and n_c, n_t are the number of samples in the control and treatment groups respectively. The chosen denominator ensures that IMD has maximum value of +1 when *all* similarities among impacted samples are lower than *any* similarities among control samples. The converse case gives a minimum for IMD of -1, and values near zero imply no difference between treatment groups.

In Table 3, IMD values are compared between each pair of treatments or conditions for the four examples. For the mesocosm meiobenthos, comparisons between the high dose and control treatments and the high dose and low dose treatments give the most extreme IMD value of +1, whereas there is little difference between the low dose and controls. 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 spatial differences within groups to dilute the dispersive effects.

Table 3. Index of Multivariate Dispersion (IMD) between all pairs of conditions.

Study	Condition compare	IMD
Meiobenthos	High dose / Control	+1
	High dose / Low dose	+1
	Low dose/Control	-0.33
Macrobenthos	Group D / Group C	+0.77
	Group D / Group B	+0.80
	Group D / Group A	+0.60
	Group C / Group B	-0.02
	Group C / Group A	-0.50
	Group B / Group A	-0.59
Corals	1983 / 1981	+0.84
Reef fish	Mined / Control reefs	+0.81

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, although it can be extended to several groups in straightforward fashion. Let r_i denote the mean of the $N_i = n_i(n_i - 1)/2$ rank similarities among the n_i samples within the ith group $(i = 1, \ldots, g)$, 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 $(N = S_i N_i)$. Then the dispersion sequence

$$r_1/k,\ r_2/k,\ \ldots,\ r_g/k \qquad \ldots\ldots\ldots\ldots(4)$$

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 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 4 have been computed for the four studies considered above (Table 4). This can be seen as complementary information to the IMD values; Table 3 provides the pairwise comparisons following on from the global picture in Table 4. The conclusions from the latter 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 separation, being nonetheless smaller than the increased dispersion at the central, impacted stations.

Table 4. Relative dispersion of the groups (Equation 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

Overall, this particular methodology does appear to have a degree of generality and is clearly applicable to the kinds of communities which are of especial concern in the tropics, i.e. coral reefs and their associated biota, as exemplified by the reef-coral and reef-fish studies described above.

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 (see review by Peterson, 1991). 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 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 (Connell, 1978), 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.

In 1986, a 10-month dredging operation established a deep-water port at Cape Panwa on the S.E. coast of Ko Phuket (Fig. 4). Three transects were designated across the coral reefs in this bay, Transect A being closest to the port and subject to greatest sedimentation, partly through the escape of fine clay particles through the containing wall of the port. 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.

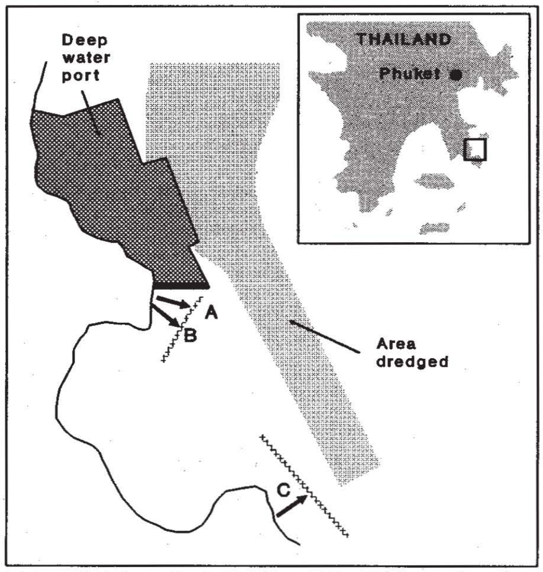

Figure 4. Map of study site at Ko Phuket, showing locations of transects A, B and C.

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. 5). 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. 5. 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. 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 (r_S, *e.g.* Kendall, 1970) computed between the corresponding elements of two triangular matrices of rank "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 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 *M* randomly selected permutations of the sample labels, if only *m* of the *M* simulated IMS values are greater than or equal to the observed IMS, the null hypothesis can be rejected at a significance level of $100(m+1)/(M+1)$%. One distinctive feature of the current test is that tied ranks will be 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 r_S, and this form is used in the analysis below. The calculations for the test were carried out using the PRIMER program RELATE.

In 1983, before the dredging operations, MDS configurations (Fig. 5) 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 clusters separated by large gaps); the community change follows a quite gradual pattern. The values of the IMS are consequently high (Table 5), ranging from 0.62 (transect C) to 0.72 (transect B).

The correlation with a linear sequence is highly significant in all 3 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 outliner 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 (Clarke, 1993). There is no similar technical need, however, to remove this sample from the IMS calculation; this was not done in Table 5, although doing so would increase the r_S value from 0.65 to 0.74 (as indicated in Fig. 5).

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

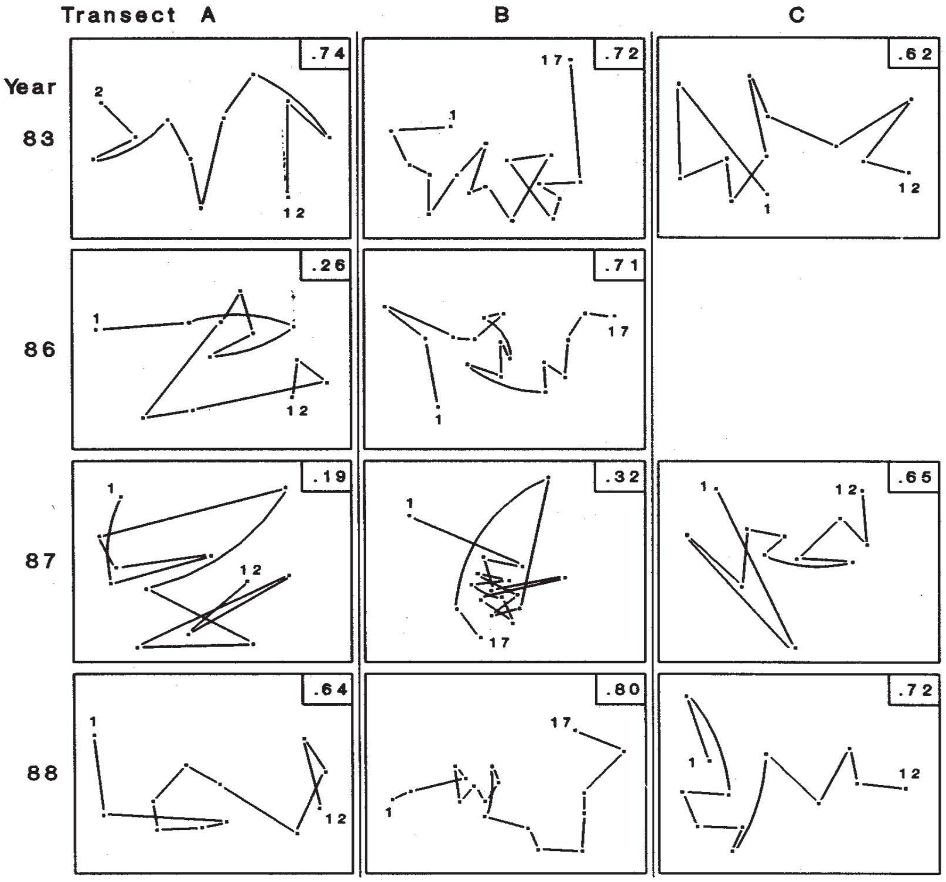

Figure 5. MDS ordination of the changing coral communities at Ko Phuket (species cover data) along three transects (A to C) at four times (1983 to 1988). The lines indicate the degree of seriation by linking successive points along a transect, from onshore (1) to offshore samples (12 or 17); corresponding 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 the rows, stress = 0.10, 0.11, 0.09; 0.10, 0.11; 0.08, 0.14, 0.11; 0.07, 0.09, 0.10).

Table 5. Index of Multivariate Seriation (IMS) along the three transects for the four sampling occasions. Figures in parenthesis are the % significance levels in a permutation test for absence of seriation (M = 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%)

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. 5 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). 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.

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, but any possible links between such relationships and environmental stress remain to be explored. Furthermore, although manifested in this single test case, the breakdown of seriation in response to perturbation has not yet been demonstrated at any other locality or for any other groups of organisms other than corals.

GENERAL DISCUSSION

At the time of writing, these three very recently devised methodologies still require more rigorous exploration, generalisation and validation before their widespread use in the East Asian Region can be recommended. Any application of them should therefore be made with these research objectives in view, rather than adopting them as routine monitoring techniques. All have potential advantages for use in the Region: the meta-analysis because it overcomes taxonomic difficulties and offeres the possibility of evaluating the severity of disturbance on a more globally comparative scale, and the variability and seriation methodologies because they can be used to assess environmental impacts on those types of communities which are of primary conservation value and therefore a major focus for environmental concern in the Region.

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