CBM:s skriftserie 3: 131–148. Uppsala 2001.

Choice of methodology for marine pollution monitoring in intertidal soft-sediment communities

Nelly Krassulya

Krassulya, N. 2001. Choice of methodology for marine pollution monitoring in intertidal soft-sediment communities. – CBM:s Skriftserie 3: 131–148.

The changes in macrobenthic communities due to organic pollution stress were studied in Proper Bay, South Australia. I tested several choices of biological variables and associated statistical methods with the aim of identifying the most sensitive and cost-effective approach to designing marine pollution assessment and monitoring programs. Data for the study was collected at Proper and Boston Bays, South Australia, in summer 1998 and autumn 1999. Changes in soft-sediment intertidal macrobenthic communities from three impacted sites were analysed in comparison to six control sites. Results of both one-way and two-way Analysis of Variance (ANOVA) for univariate measures (number of individuals, number of species and species diversity) were inconsistent. Rank-abundance methods (log-normal distribution and k-dominance curves) applied to the summer data confirmed typical patterns of distribution of individuals of species in stressed communities, but the results for the autumn data were inconsistent. All three multivariate methods tested (Hierarchical Agglomerative Clustering, Multi-Dimensional Scaling (MDS) and Analysis of Similarities (ANOSIM)) were successful in discriminating between impacted and control sites. Analysis of variability among replicates, by examination of MDS plots and Index of Multivariate Dispersion, revealed an increased variability only for autumn data. Different taxonomic resolutions (species level, class level and species from selected taxa) were tested as possible methods of cost reduction. Results of all three multivariate methods, when applied to data using different taxonomic resolutions, were consistent and no significant information was lost in comparison with traditional species level identification. In conclusion, I recommend that analysis of community structure using multivariate methods on class level (or a selected group such as Polychaeta) is the most effective and inexpensive methodological choice.

Nelly Krassulya, Swedish Biodiversity Centre, P. O. Box 7007, SE-750 07 Uppsala, SWEDEN

Introduction

Macrobenthic communities inhabiting intertidal flats are attractive for the detection and monitoring of organic pollution as they are known to be responsive to organic enrichment and are relatively easy to sample. Properly designed methods, based on an understanding of processes occuring in communities affected by organic pollution, could give us a cheap and reliable approach for environmental impact monitoring. As different characteristics of a community behave in different ways in response to different types of disturbance, the choice of biological variables to be measured and particular methods associated with them is a crucial component in any monitoring process and is presently heavily debated (Keough and Quinn 1991, Warwic 1993).

Diversity

One of the most commonly used methods for assessing changes in communities is estimation of species diversity. This is based on the relationship between the diversity of a community and it's stability - the more diverse and complex the community, the more stable it is (though this theory is not always supported by data (ie May 1975)). Pollution, or other types of human disturbance, affect the stability of a community and this may be detected by a change in diversity. Overviews of diversity measurements are given in Magurran (1988) and Washington (1984). The most widely used parameters, species richness (S), species diversity (Shannon's Diversity Index, H') and evenness, show a clear correlation with increasing or decreasing pollution levels in many cases (Rygg 1985, Gray et al 1990). However, these parameters may respond differently to changing replication and may have different power characteristics (Bernstein and Zalinsky, 1986). In some cases, such as an impact study of mining on coral reef fish communities in the Maldives (Dawson-Shepherd et al. 1992), the mean Shannon's Index did not differ between mined and control sites where multivariate analysis showed a clear-cut difference. Additionally, it may be extremely difficult, especially in Australia (see above), to identify all species in samples. Thus, it was tested whether this method could be applied on levels higher than species, or only for selected taxa (Polychaeta for example, which are usually well represented in samples and have a high abundance).

Rank abundance methods

There are two commonly used methods for graphically presenting distribution of individuals among species: log-normal distribution and k-dominance curves (Nelson 1987, Gray 1981, Clarke 1990). Both of these methods give promising results for the detection of pollution-induced changes in communities. A good log-normal fit of distribution represents ecological equilibrium of an undisturbed community where most species rarely respond to the stochastisity of environmental and biological interactions. Disturbance in the form of pollution produces a skewed distribution, where the commoner species become more abundant and the rarer species more scarce.

Multivariate approach

Hierarchical agglomerative clustering and multi-dimensional scaling (MDS) are two of the most frequently used multivariate methods. Both of these are based on similarity coefficients calculated between every pair of samples. The first method facilitates a classification or clustering of samples into groups which are mutually similar, while the second method maps the samples (in two or three dimensions) in such a way that the distances between pairs of samples reflects their relative dissimilarity in species composition (Clarke & Warwick 1994). The less frequently used ANOSIM (analysis of similarities) is built on a simple non-parametric permutation procedure, which is applied to the similarity matrix underlying the ordination or classification of samples (Clarke, 1993). Using numerous examples, Clarke and Warwick (1994) showed that multivariate methods of data analysis were more sensitive in detecting differences in community structure between samples in space,

or changes over time, in comparison to univariate techniques. The major drawback of the multivariate approach is the necessity to identify all species in samples. However, this can be overcome if lower taxonomic resolution proves to be applicable and sensitive enough to detect changes in macrobenthic communities.

Variability

It has been shown in a variety of environmental impact studies that variability among samples collected from impacted sites is much greater than that from controls. Warwick and Clarke (1993) described an increase in variability of four different types of marine communities (meobenthos, macrobenthos, corals, reef-fish). They compared variance/mean or standard devation/mean ratio of the number of individuals of all species and diversity indices (H'). The most marked results were obtained for meobenthos and macrobenthos communities. In all cases, a pronounced increase in variability between replicate samples was revealed by analysis of MDS plots and suggested Index of Multivariate Dispertion (IMD).

One of the major difficulties researchers and environmental managers face when considering macrobenthic communities is the costly and time consuming sampling process, especially species identification. This task becomes even more complicated in areas such as Australia, where an extremely high diversity of macrobenthic fauna is insufficiently taxonomically described. Due to a lack of up-to-date taxonomic keys, identification to species level is also becoming extremely difficult and expensive. One of the approaches presently being discussed widely in the scientific press (James et al 1995, Somerfield & Clarke 1995, Ferraro&Cole 1990, Chapman 1998, Olsgard et al 1997, Vanderklift 1996) is the reduction of the level of taxonomical resolution. In other words, the identification of macrofauna to levels higher than species, family, order, class or even groups with relatively similar roles in the ecosystem. Another approach, which would allow significant savings in time and effort, is the identification and analysis of species only from selected groups or taxa, instead of a whole community. Polychaeta would be a useful group, as they are ubiquitous in virtually all marine sediments, are typically present in high numbers and are represented by many species.

The main purpose of this study was to 1) estimate the changes in macrobenthic communities under organic pollution stress in Proper Bay, Port Lincoln and 2) identify the most cost-effective and sensitive choice of biological variables and associated methods.

The following biological variables and associated methods were considered with respect to their sensitivity and applicability to routine environmental monitoring.

Study area and methods

Study area

Samples were collected from nine stations, three impacted and six controls (Figure 1), at the end of December 1998. Three stations (impacted site P3, controls C1 and C2) were sampled again in April 1999. A description of the sites, with sources of contaminants, is given in Table 1. The first impacted site was located at an outlet from an area of the intertidal flat that has been separated from the sea by a railway embankment for several decades. This area, called "the wetland", has accumulated run-off from an emu farm and a dirt road for many years. A limited exchange of water between the wetland and the sea has resulted in an accumulation of black, sulfur-enriched sediments covered with a shallow layer of extremely saline water (65-70 %). The second and third impacted sites were near outfall pipes from a fish-processing factory. The sediment structure (dead shells, sand, gravel and mud) was similar at all three impacted locations. At all impacted sites, algae cover indicative of organic pollution was observed. Control sites in Proper Bay were chosen at a distance from impacted sites (the closest site, C1, was approximately 10 km away from site P3) and were chosen regardless of gradient and without visible signs of contamination (ie algae growth).

We also decided to use two control sites

outside of Proper Bay to avoid possible confusion, but it was impossible to find physically similar sites. Thus, control sites 5 and 6 have a quite different sediment structure in comparison with the other locations.

Sampling methods

At each site, a total of eight cores in the summer and six cores in the autumn were taken, giving a total of 72 samples for December and 18 for April. Replicates at each site were collected randomly along a 50m transect parallel to the shore (50m from the highest tide mark). For impacted sites, transects were centered on the outfall and extended 25m in either direction. This sampling design was chosen to avoid any confusing zonation effect on the distribution of the intertidal macrofauna. Samples were taken at low tide using a 10cm diameter hand core. All samples were then sieved on a 500(mesh screen and fixed in 5% formalin.

Individuals were identified to the lowest possible level (species or morphospecies) except for Anthozoans, Nemerteans, Sipuncules and Oligochaetes. With the exception of Oligochaetes, all these taxa were comparatively rare.

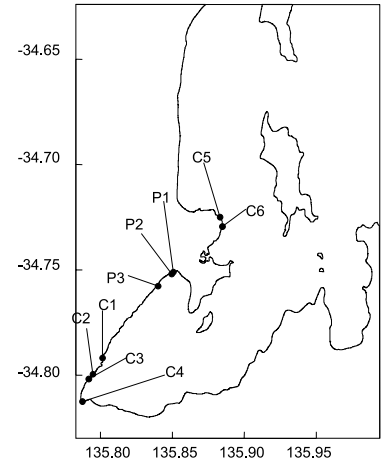

Figure 1. Sampling site locations

Statistical analysis

Univariate approaches

In addition to total number of individuals and total number of species, two further diversity indices were also calculated: Hill's N1 (the exponential of the Shannon-Wiener function H') and Hill's N2 (Simpson's reciprocal D). Hill's N1 was chosen as an index most sensitive to changes

Table 1. Description of sampling sites

Site	Location	Sediment structure*)	Sample date (No. of replicates)	Source of impact
Impacted 1 (P1)	Proper Bay	Medium sand, silt	December 1998 (8)	"Wetland" outfall (see text)
Impacted 2 (P2)	Proper Bay	Medium sand, silt	December 1998 (8)	Fish-processor outfall
Impacted 3 (P3) April 1999 (6)	Proper Bay Fish-processor outfall	Medium sand, silt	December 1998 (8)	
Control 1 (C1)	Proper Bay	Medium sand, silt	December 1998 (8) April 1999 (6)	
Control 2 (C2)	Proper Bay	Medium sand, silt	December 1998 (8)	
Control 3 (C3)	Proper Bay	Medium sand, silt	December 1998 (8)	
Control 4 (C4)	Proper Bay	Medium sand, silt	December 1998 (8) April 1999 (6)	
Control 5 (C5)	Boston Bay	Fine sand	December 1998 (8)	
Control 6 (C6)	Boston Bay	Fine sand	December 1998 (8)	

*)by Holme&McIntre, 1971

in rarer species, while Hill's N2 was used as most sensitive to changes in dominant species (Peet 1974). With these univariate measures as response variables, a simple one-way ANOVA model was used to test for differences between impacted and control sites for the December samples. For the April samples, and a matched subset of the December samples, a two-way ANOVA to test was also used for time effects and any site-time interactions. If significant effects were detected, a Post Hoc Test (Student-Newman-Keuls) was applied to all variances used.

Homogeneity of variance was tested for using Leven's Test of Equality of Error Variance. The data was not homogeneous both before and after transformations (log (x+1), fourth root). Furthermore, mostly untransformed data were used (fourth root transformation was used in some cases for multivariate analysis).

Rank Abundance methods

Two commonly used methods for graphical presentation of distribution of individuals among species were applied:

1. Plots of x 2 geometric species abundance classes against number of species,
2. K-dominance curves (x-logged, y-cumulative % dominance).

Graphs were produced for each site for both summer and autumn data. To show general trends, all control sites were pooled into one group and impacted sites into another and the two curves compared for the summer data.

Multivariate approaches

Hierarchical agglomerative clustering, multidimensional scaling (MDS) and ANOSIM (Analysis of Similarities) were used in separate analyses for the summer and autumn data. Variability among samples was analysed using Multi-Dimensional Scaling ordination of replicates and calculation of Index of Multivariate Dispersion (IMD) (as described in Warwick & Clarke 1993). PRIMER (Plymouth Marine Laboratory, 1996) software was used to test for statistically significant differences between sites.

Taxonomic resolution

To test how taxonomic resolution affects the sensitivity of multivariate methods for detecting changes in benthic communities, multivariate statistical analyses (Hierarhical clustering, MDS and ANOSIM) were applied to three sets of data:

1) All individuals identified to species/morphospecies level,
2) Individuals only classified to class level,
3) Only species/morphospecies of Polychaeta.

Results

Number of individuals, number of species and species diversity

A total of 12675 individuals were collected from all sites, comprising 99 species / morphospecies. In December, the highest abundance of benthic macrofauna was found at impacted site P1, with a slightly lower abundance at impacted sites P2 and P3 and at approximately the same level at the controls C1 and C4 (Table 2). The remaining controls were characterized by uniformly low abundance.

ANOVA performed on the number of individuals revealed a significant difference between sites (Table 3). A Post Hoc Test (Table 4) identified two distinct groups of stations: C2, C3, C5, C6 (low abundance) and P1, P2, P3, C1, C4 (high abundance).

The highest species richness during the summer was found at the control sites C2 and C3. The number of species was also comparatively high at the impacted sites P1 and P3, whereas impacted site P2 and controls C4 and C6 were characterised by comparatively low abundances (Table 2). One-way ANOVA recognized significant difference between sites (Table 3), but the grouping of stations by a Post Hoc Test was confusing (Table 4), whilst impacted and control sites were mixed without any visible pattern.

Generally, both diversity indices were lower at impacted sites in comparison with controls in both seasons (Table 2, Figure 2), with the exception of control C4 in the summer, where diversity was the lowest because of the highly

dominant gastropod S. solidus. One-way ANOVA performed on summer data revealed a statistically significant difference between sites for both indices N1 and N2 (Table 2) and Post Hoc Tests recognised four significantly different groups of stations for N1 and five groups for N2 (Table 3). With the exception of the first group, where impacted site P2 was merged with controls, for both indices three (shaded) different impacted sites were in the same groups. A Post Hoc Test failed to clearly separate them from controls.

Generally, results of one-way ANOVA tests performed on different variables (number of individuals, species richness and species diversity indices Hill's N1 and N2) were inconsistent. Though in most cases a significant difference was revealed between sites, Post Hoc Tests failed to clearly separate impacted and control sites.

A lower abundance of benthic macrofauna was found in autumn at all three sampled stations compared to in summer, with the lowest abundance at impacted site P3. Two-way ANOVA performed on the number of individuals at sites P3, C1 and C4, and between the two seasons, revealed a significant ($p<0.05$) difference between seasons but not between sites (Table 4).

Species richness was also lower in the autumn, with the lowest number of species at control C4. Two-way Analysis of Variance for the number of species distinguished ($p<0.05$) between sites and seasons (Table 4), but a Post Hoc Test failed to separate impacted sites and controls.

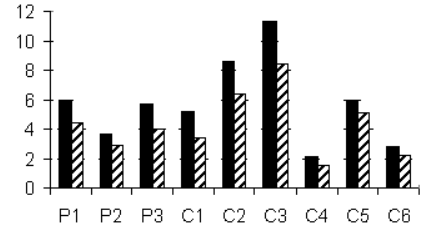

Figure 2. Values of Hill's N1(solid) and N2

Values of both diversity indices were low in the autumn at all sites and slightly higher at the controls in comparison with impacted sites. Two-way ANOVA and a Post Hoc Test performed on diversity indices for autumn data revealed significant differences between sites and seasons for Hill's N1, but failed to distinguish between seasons in the case of Hill's N2. Student-Newman-Keuls Test failed to separate the impacted sites from the controls (Table 4).

Generally, results of two-way ANOVA for different univariate measures (number of individuals, species richness, two diversity indices) were confusing and inconsistent. Even though

Table 2. Univariate Indices Values of the stations in summer and autumn.

Site	Summer								Autumn							
	No. of individuals		No. of species		Hill's N1		Hill's N2		No. of individuals		No. of species		Hill's N1		Hill's N2	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
P1	351.5	112.2	13.7	0.7	6.0	0.7	4.4	0.5								
P2	226.8	42.2	8.1	0.7	3.7	0.3	2.9	0.3								
P3	280.8	39.7	14.6	1.1	5.7	0.3	4.0	0.4	94.6	52.2	7.1	1.5	3.1	0.3	2.3	0.3
C1	267.0	24.1	15.2	0.7	5.2	0.4	3.4	0.3	234.0	11.3	9.1	0.4	3.4	0.2	2.5	0.2
C2	47.5	19.7	12.8	2.1	11.3	0.9	8.4	0.9								
C3	53.5	7.0	16.6	1.2	8.6	0.8	6.4	0.6								
C4	285.6	54.6	10.0	1.1	2.1	0.2	1.5	0.1	211.6	13.5	8.1	0.4	3.2	0.3	2.5	0.2
C5	16.5	5.1	7.3	1.5	5.9	1.1	5.1	0.9								
C6	15.0	8.3	4.0	1.0	2.8	0.4	2.2	0.3								

Table 3. One-way ANOVA for summer data

	Number of individuals					Number of species				
Source	Type III SS	df	Mean Square	F	Sig.	Type III SS	df	Mean Square	F	Sig.
SITE	1179129.694	8	147391.212	8.424	.000	1150.944	8	143.868	11.920	.000
Error	1102281.625	63	17496.534			760.375	63	12.069		
Subsets grouped by S-N-K test	P1 P2 P3 C1 C4 C2 C3 C5 C6					P3 C3 C1 C2 P1 C4 P2 C5 C6				
	Hill's N1					Hill's N2				
Source	Type III SS	df	Mean Square	F	Sig.	Type III SS	df	Mean Square	F	Sig.
SITE	525.331	8	65.666	17.397	.000	299.811	8	37.476	13.855	.000
Error	237.794	63	3.775			170.412	63	2.705		
Subsets grouped by S-N-K test	P1 P3 C1 C5 P2 C4 C6 C2 C3					P1 P3 P2 C1 C6 C4 C2 C5 C3				

*In all cases significance of S-N-K test <0.05

in most cases seasonal changes were revealed, the test failed to clearly differentiate impacted and control sites.

Rank abundance

Plots of geometric abundance classes for all sites are given in Figure 3. Curves are steep at all control sites, indicating that many species are only represented by single individuals and very few abundance classes are represented. Curves for impacted sites have a completely different shape due to a low abundance of rare species and being extended over more abundance classes. Figure 4 shows k-dominance curves for the same data. Curves plotted separately for each site look confusing, particularly for controls C1 and C4, but the graph for pooled data reveals a clear pattern, with the curve for impacted sites elevated, indicating lower diversity in comparison to controls.

The pattern of distribution of individuals among species in the autumn is similar for sites P3 and C1, but less obvious in the case of control C4 (Figure 3 (c)). K-dominance curves for autumn data look even more confusing, with curves for control sites slightly above those for polluted sites (Figure 4(c)).

Generally, results of rank abundance methods applied to the data confirmed typical patterns of distribution of individuals among species in disturbed macrobenthic communities. All three impacted sites sampled in the summer were characterized by a lower number of rare species and more abundant common species in

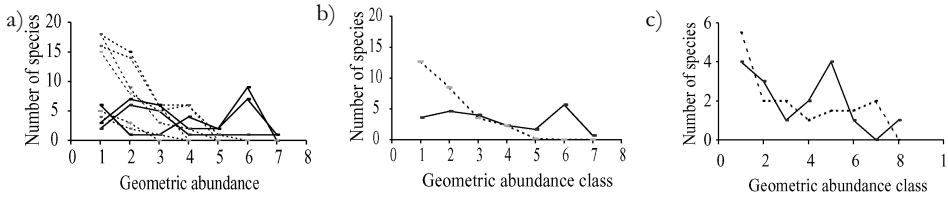

Figure 3. Plots of x2 geometric species abundance classes (solid lines – impacted sites, broken lines – controls): (a) all sites in summer; (b) all sites pooled into two groups: impacted and controls in summer; (c) sites pooled into two groups: impacted and controls in autumn.

comparison to control sites. However, results for the autumn data were confusing, which could be explained by an insufficient number of samples containing low numbers of individuals and species.

Community structure

With respect to broad taxonomic groups, gastropods were most abundant (35.9% of total abundance), while malacostracans, polychaetes and bivalves represented 19.8%, 14.6% and 10.5% of the sampled individuals respectively. Figure 5 shows the relative abundance of the major taxa at three impacted sites and six controls. Other taxa were comparatively rare.

In the summer, the impacted sites were

Table 4. Two-way ANOVA results for univariate values, autumn

Number of individuals						Number of species				
Source	Type III SS	df	Mean Square	F	Sig.	Type III SS	df	Mean Square	F	Sig.
SITE	37523.762	2	18761.881	1.931	.163	63.389	2	31.694	4.542	.019
TIME	57192.722	1	57192.722	5.887	.021	186.778	1	186.778	26.768	.000
SITE * TIME	31467.995	2	15733.998	1.620	.215	47.389	2	23.694	3.396	.047
Error	291429.208	30	9714.307			209.333	30	6.978		
Subsets grouped by S-N-K	P3 C1 C4					P3 C1 C4				
Hill's N1						Hill's N2				
Source	Type III SS	df	Mean Square	F	Sig.	Type III SS	df	Mean Square	F	Sig.
SITE	17.880	2	8.940	8.969	.001	6.916	2	3.458	4.880	.015
TIME	6.545	1	6.545	6.566	.016	.980	1	.980	1.383	.249
SITE * TIME	17.371	2	8.685	8.714	.001	8.996	2	4.498	6.348	.005
Error	29.902	30	.997			21.258	30	.709		
Subsets grouped by S-N-K	P3 C1 C4					P3 C1 C4				

*Statistical significance of S-N-K test in all cases <0.05

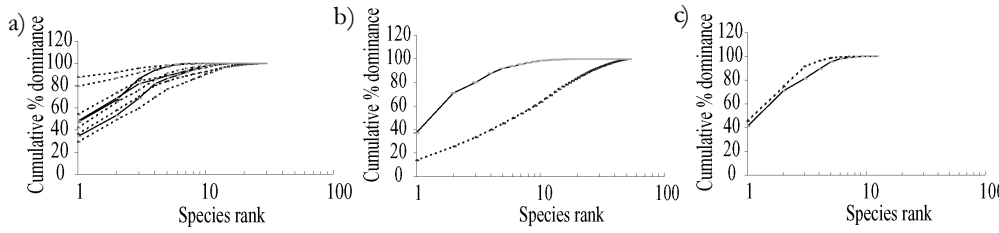

Figure 4. K-dominance curves (x-logged), (solid line-impacted sites, broken line-controls): (a) all sites in summer; b) all sites pooled into two groups: impacted and controls in summer; (c) sites pooled into two groups: impacted and controls in autumn.

characterised by the dominance of so-called opportunistic species, such as the polychaete Capitella capitata, Polydora sp., unidentified Oligochaetes and amphipods Corophium sp. The gastropods Solinator soludus, Zeocumanthus sp. and several species of bivalves, which were comparatively rare at the impacted stations, were dominant at control sites C1 and C4. Other control sites were characterised by a comparatively balanced community structure without expressed dominants.

In the autumn, species composition changed at site P3: the gastropod S. solidus became dominant, whilst the formerly abundant polychaete opportunistic species (Capitella capitata, Polydora sp.) were replaced by species of Lumbrinereidae and Orbinidae (Figure 5). The formerly dominant Amphipode Corophium sp. was also replaced by another species. In contrast, at controls C1 and C4 the community structure remained the same in comparison with the summer situation.

Multivariate approach

Figure 7(a) shows the results of a hierarchical clustering of summer data (no transformation). The three impacted sites form a distinctly separate group. Sites C1 and C4 form a second group, while the controls C2, C3 and C5, C6, despite their distant location and different sediment structure, remain more similar to each other than to the impacted sites.

The pattern for MDS ordination is less clear, with controls C1 and C4 located close to impacted sites (Figure 8(a)). The group of impacted sites became much more distinct from the controls after the data were transformed (fourth root) (Figure 8(b)). ANOSIM test performed on two groups of replicates from impacted and controls sites resulted in global R=0.276 with a significance level of 0.001%. Results of a pairwise test (Appendix 1) suggest that impacted sites are more similar to each other than to controls (sign level <0.001%), with the exception of the pair of sites P3 and C6.

When data collected in April from stations P3, C1 and C4 were added to the initial summer matrix, site P3 formed a group with samples from C4-summer. Samples from C1 in both seasons formed a separate group, closely attached to C4-autumn (Figure 7(b)).

MDS ordination of the 36 replicates from the three sites sampled in the summer and the autumn (Figure 9(b)), shows a clear separation of site P3-summer and half of the replicates from P3- autumn, whilst replicates from both controls in both seasons occupy positions close to each other. MDS plot for fourth root transformed data revealed a more distinctive pattern. Replicates from site P3 in both seasons formed a separate group with the exception of two autumn replicates positioned far from all the others. Samples from controls formed three distinctive groups: C1-summer, C4-summer and both sites in the autumn largely in one group.

Analysis of similarities performed on two groups of samples, impacted site P3 in both seasons and controls C1 and C4 in both seasons, resulted in global R=0,671 and a significance level of 0.0%. Results of a pairwise test for three sites

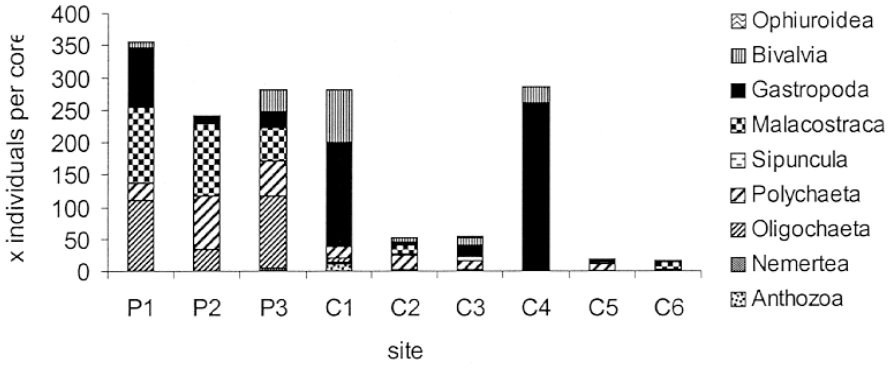

Figure 5. Abundance of macrofauna at impacted and control sites in December 1998

(P3, C1, C4) in both seasons were not significant. Once again, as was shown by the rank-abundance method, the difference between impacted and control sites in the autumn was less obvious in comparison to the summer situation.

Variability

Figure 9(a) presents results of MDS ordination of replicates for all sites in the summer. The replicates from impacted sites were not more scattered than those from the controls. Results of calculations of Index of Multivariate Dispersion are presented in Tables 5 and 6. Index of Multivariate Dispersion has a maximum value of +1 when all similarities between impacted samples are lower than any similarities between controls. The converse case gives a minimum IMD of -1 and values near zero imply no difference between treatment groups (Warwick & Clarke 1993). In this study, the average IMD values between impacted and control sites and between controls were both negative (-0.2 and -0.47 respectively). The only more or less high positive value among pairwise comparisons was between P3 and C4 (0.65) (Table 6). Average values of Relative Dispersion for impacted and control sites were 0.86 and 0.81 respectively - ie no difference was found.

Opposite to the case of the summer data, replicates from impacted site P3-autumn were much more scattered in comparison with replicates from C1-autumn and C4-autumn, which formed a very tight group. Index of Multivariate Dispersion for pairwise comparison was as follows: between P1 and C1 = 0.636, between P1 and C4 = 0.662, and between two controls = -0.076. Both comparisons between impacted and control sites gave high positive

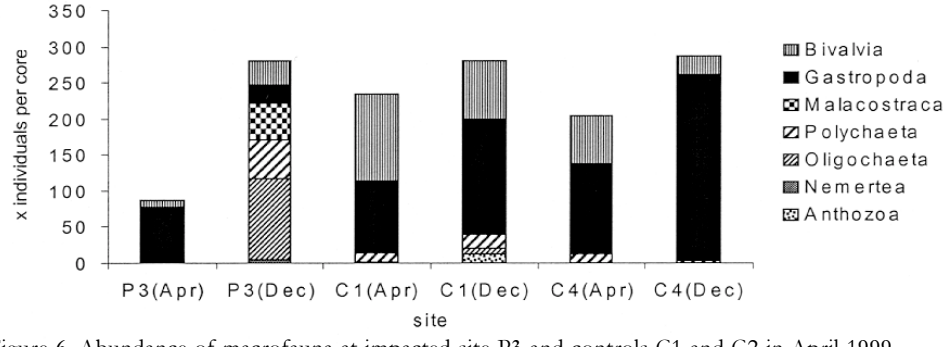

Figure 6. Abundance of macrofauna at impacted site P3 and controls C1 and C2 in April 1999

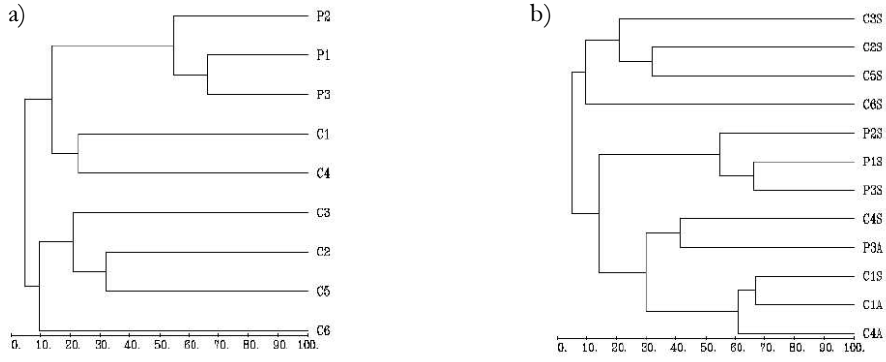

Figure 7. Dendrogram of: (a) nine stations in the summer; (b) nine stations in the summer plus three stations resampled in the autumn; both using group average clustering from Bray-Curtis similarities on untransformed abundances.

values, whereas comparisons between control sites themselves did not reveal much difference. The same pattern was confirmed by Relative Dispersion values, where dispersion for impacted sites was much higher (Table 5).

Generally, all three multivariate methods tested were much more successful in discriminating between impacted and control sites then univariate methods. An increased variability among replicate samples was only revealed for autumn data. Fourth root transformation was found helpful in clearly discriminating patterns in some cases.

Taxonomic resolution

Figure 10 shows the results of a hierarchical clustering of summer data classified to (a) class level and (b) separately for Polychaeta species (no transformation used). In both cases, the impacted sites P1, P2 and P3 are clearly separated from all controls. However, the arrangement of controls into groups differs from the case at species level (Figure 7(a)), especially for Polychaetes. The group of impacted sites remains clearly separated from the controls on both plots of MDS ordination of summer data at class level (Figure 11(a)) and for Polychaetes (Figure 12 (a)). Fourth root transformation of data for class level had little effect in revealing a more obvious pattern (Figure 11(b)). ANOSIM test applied to two groups of impacted and control sites on class level and Polychaetes resulted in global R=0.279 and 0.358 respectively with a significance level of 0.0% (which is similar to global R=0.276 in the case of species level). In both cases, a pairwise comparison revealed an insignificant difference

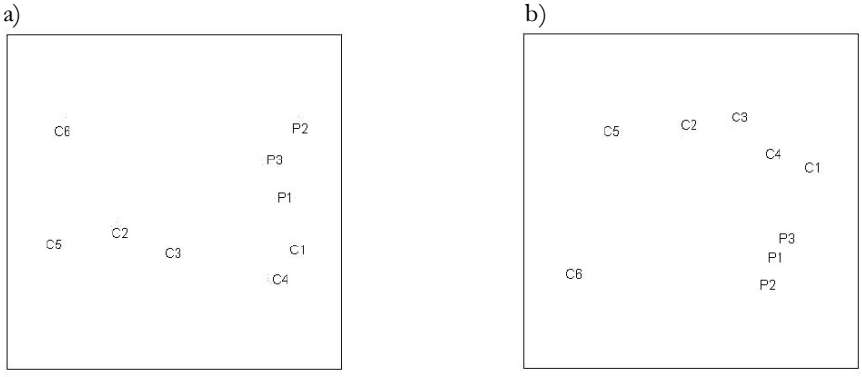

Figure 8. MDS ordination of the nine sites based on: (a) untransformed; (b) fourth root transformed abundances and Bray-Curtis similarities (stress 0.06).

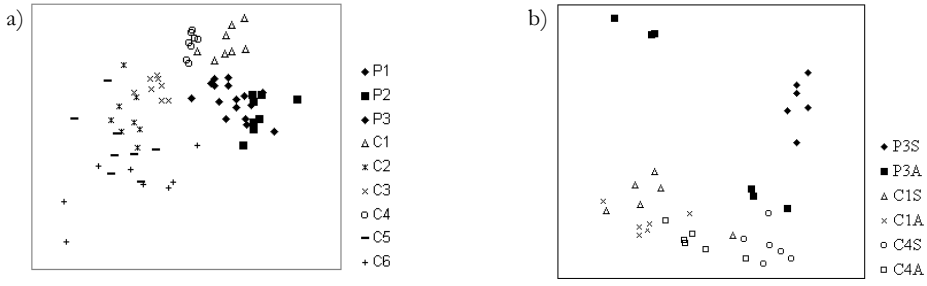

Figure 9. MDS ordination of replicates from all sites: (a) summer; (b) summer and autumn; no transformation.

between impacted sites and a significant difference between almost every pairing of impacted and control sites (Appendix 1).

MDS ordination of autumn and summer data for sites P3, C1 and C4 on class level (Figure 12(b)) revealed a pattern highly similar to that of species level. In both cases, all replicates from P3-summer and three replicates from P3-autumn were clearly separated from both controls in both seasons. ANOSIM results for a pairwise comparison were insignificant, the global R between impacted and control sites in the autumn being 0.448.

Generally, all three multivariate methods applied to data on class level and Polychaeta only succeeded in distinguishing between impacted and control sites in all cases. In this respect, no significant information was lost in comparison with an analysis at species level.

Discussion

The principle aims of this study were 1) to estimate the changes in macrobenthic communities under organic pollution stress, in Proper Bay, Port Lincoln and 2) to identify the most cost-effective and sensitive choice of biological variables and associated methods.

The study succeeded in distinguishing between impacted and control sites with a high degree of significance and compared different methods with respect to their sensitivity. Even though a decrease in species richness and an increase in the number of individuals are widely acknowledged characteristics of stressed communities, this was not observed in the present study. The main cause of this inconsistency was the great variability between control sites themselves. This supports the idea of so-called MBACI sampling design (Underwood 1994, Keough &

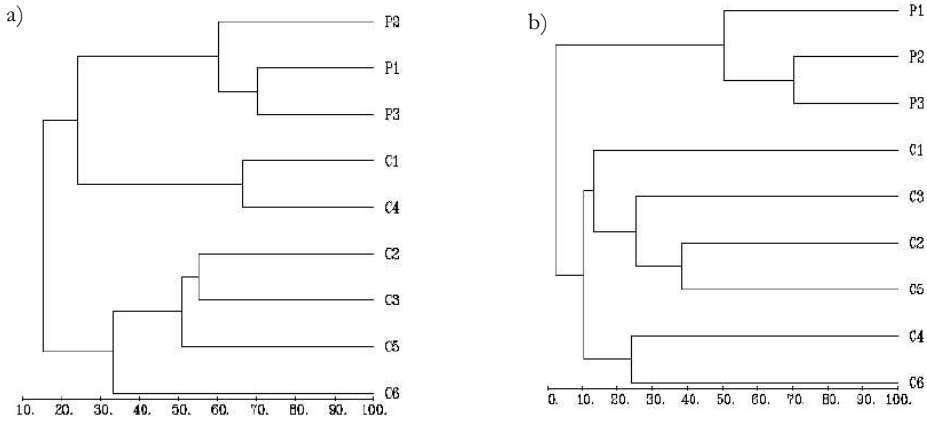

Figure 10. Hierarhical clustering of summer data classified to: (a) class level; (b) only Polychaeta species.

Table 5. Relative Dispersion of impacted and control sites in summer

Order of group	Relative Dispersion (summer)	Relative Dispersion (autumn)
C4	0.50	0.81
P3	0.68	
C1	0.71	0.77
P2	0.73	
C3	0.86	
P1	1.18	1.42
C2	1.25	
C6	1.54	
C5	1.55	

Table 6. Pairwise comparison of IMD values for impacted and control sites in summer

P1									
P2	0.47								
P3	0.51	0.06							
C1	0.46	0.03	0.00						
C2	-0.05	-0.59	-0.62	-0.54					
C3	0.36	-0.19	-0.29	-0.23	0.51				
C4	0.65	0.31	0.26	0.26	-0.75	-0.50			
C5	-0.38	-0.84	-0.87	-0.81	-0.40	-0.87	-0.91		
C6	-0.43	-0.76	-0.79	-0.76	-0.42	-0.84	-0.85	-0.13	
	P1	P2	P3	C1	C2	C3	C4	C5	C6

Quinn 1995), which suggests the use of more than one (the more the better) control site. This point was additionally illustrated by comparison of the results of diversity indices. At three control sites, diversity indices were higher than at any impacted site, whilst at two controls they were approximately on the same level, slightly lower at one. Any evidence of impact would not have been detected if sites with low diversity were used as controls and our assumptions were based only on diversity indices. Analysis of community structure revealed a well-known pattern of increased abundance of opportunistic species (*Corophium sp.*, *Capitella capitata*, *Polydora sp*, Oligochaeta) at impacted sites, but univariate methods associated with the first three variables tested did not succeed in recognising these changes.

Both rank abundance methods tested were more successful, and confirmed results from other studies showing trends in distribution of individuals among species in stressed communities (ie Gray 1981, Clarke 1990). However, patterns among control sites themselves were again highly variable and in the case of the three sites sampled in the autumn, k-dominance curves for control sites behaved in a way opposite to expected. In this respect, caution should be taken when dealing with data containing a low number of individuals and species, and a sufficient number of control sites should be used. Also, another drawback of rank abundance methods is the difficulty of expressing results in understandable and convincing values or indices.

All multivariate methods that were tested succeeded in distinguishing between impacted and control sites with a high level of confidence both in graphical and numerical representations. Analysis of variability was also found to be useful. Visual examination of MDS plots of replicates did not confirm the pattern of greater variability in stressed communities for the

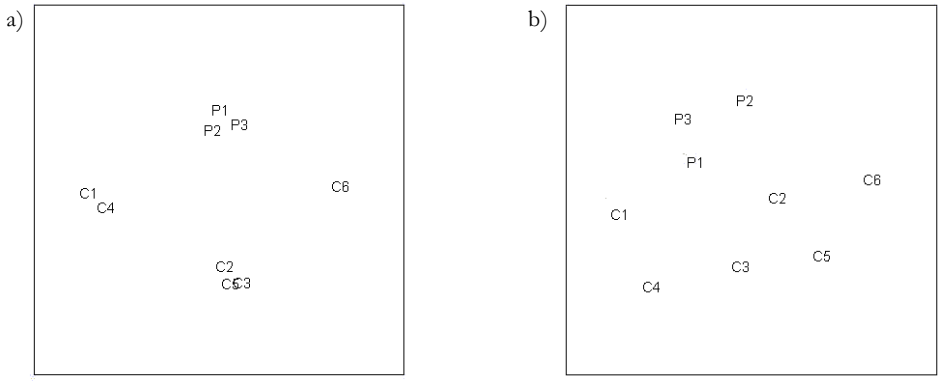

Figure 11. MDS ordination of summer data classified to class level: (a) no transformation, stress 0.01; (b) fourth root transformation, stress 0.08.

summer data, although in the autumn, replicates from impacted sites were more scattered than those from controls. Results of calculations of Index of Multivariate Dispersion were also different for summer and autumn data. In the autumn, a higher Relative Dispersion was revealed for impacted sites in comparison with controls, whilst in the summer, average Relative Dispersion was almost identical for impacted and control sites. The IMD values for comparisons between impacted and control sites in the autumn were strongly positive but negative in the summer. In other words, increased variability among replicates from impacted sites in comparison with controls was revealed for the autumn situation, but not for the summer.

Even though both rank abundance and multivariate methods proved to be sensitive to changes in communities, the major difficulty connected with them is the tedious and costly process of species identification. The two possible methods of cost reduction tested in this study (identification of all individuals to class level and use of species only from a selected group, eg. Polychaeta) both resulted in patterns highly similar to those for traditional species level and no significant information was lost.

According to the results of this study, the most cost-effective and sensitive methodological choice is an analysis of community structure using multivariate methods either at the class level or using only the Polychaete assemblage (Table 7).

Our results are consistent with the conclusions of other researchers in this area. A majority of authors (Somerfield & Clarke 1995, Vanderkliff et al. 1996) agree that identification

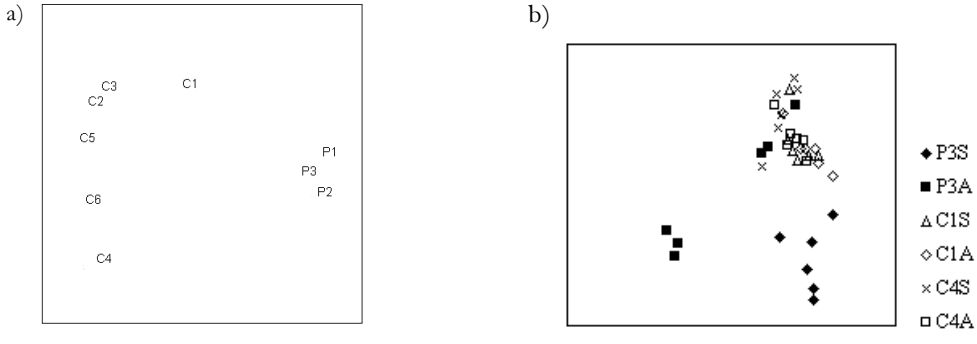

Figure 12. (a) MDS ordination for Polychaeta sampled in summer, stress 0.04, no transformation; (b) MDS ordination of replicates from three sites (P3, C1 and C4) sampled in the summer and autumn, no transformation, stress 0.09, class level.

Table 7. Advantages/disadvantages of choice of particular biological variables and sensitivity of associated methods as shown by this study

Variable	Methods associated with variable	Advantages/Disadvantages	Sensitivity to changes from the results of this study
Number of individuals	ANOVA	No species identification required	Not sensitive
Number of species	ANOVA	Species identification required	Not sensitive
Species diversity	Diversity Indices, ANOVA	Species identification required	Not sensitive
Distribution of individuals among species	Rank abundance methods	Species identification required, no simple understandable values	Sensitive, caution should be taken
Community structure (species level)	Multivariate methods	Species identification required	Sensitive
Variability in community structure among replicates (species level)	Analysis of MDS plots, Index of Multivariate Dispersion	Species identification required	Results are inconclusive
Community structure (class level)	Multivariate methods	Identification is fast and simple	Sensitive
Polychaeta assemblages	Multivariate methods	Identification takes less time than identification of all species	Sensitive

of macrofauna to family level has little or no effect, but further aggregation produces differing results in different studies, although interpretable results are possible even on the phylum level. Ferraro & Cole (1990) compared univariate measures from areas with different degrees of impact. They suggested that different taxonomic levels could be used for different situations, using a coarse resolution for harsher impacts and a finer resolution for moderate impacts and so on. They believed that biological response to stress has a hierarchically structured nature. As stress increases, the adaptability of first the individual, then the species, genus, family, etc. is exceeded. Consequently, increasing stress is manifested at higher and higher levels of biological organisation. Warwick (1988) considered that analysis of pollution effects on higher taxa minimises the confounding effects of natural variables (water depth, sediment structure and so on). This relies on the assumption that such variables usually influence the fauna more by species replacement than by changes in the proportion of major taxa present. Chapman (1998) produced positive results when studying the relationship between spatial patterns of benthic assemblages at traditional species level resolution and when all taxa were divided into nine groups according to their phylogeny and ecology.

Identification to a level higher than species drastically reduces the cost of impact assessment or monitoring. Ferraro & Cole (1995) found that

the cost of genus, family, order and phylum level identification were respectively 23%, 55%, 80% and 95% less than for species level identification. According to our estimation, identification to class level is about 80-85% less costly that to species level and does not require special training. It is agreed that identification to family level could reveal a more detailed picture, but for the purpose of rapid impact identification, class level is probably sufficient. Besides, coarser taxonomic resolution releases time and money which can be used for sampling more control sites, which, according to our results, is extremely important.

Identification of species from a selected group has not been widely discussed in the literature, and it has been suggested that such an approach could be misleading (Warwick 1993). However, our results were highly consistent and more research is now needed to ensure that changes detected in selected groups are indicative of changes in the community as a whole.

Acknowledgements – I would like to thank Prof. Stephen Hall from Flinders University of South Australia for his supervision of this study and Susan Gray for her help with sampling and identification.

References

Bellan, G. 1980. Relationship of pollution to rocky substratum polychaetes on the French Mediterranean coast. – Marine Pollution Bulletin 3: 74–77.

Bernstein, B. B., and Zalinski, J. 1986. A philosophy for effective monitoring. – IEEE Oceans'86 Conference Proceedings. (Marine Technology Society/Institute of Electrical and Electronic Engineers: Washington, DC).

Beukema, J. J. 1988. An evaluation of the ABC–method (abundance/biomass comparison) as applied to macrozoobenthic communities living on tidal flats in the Dutch Wadden. – Sea. Mar. Biol. 99: 425–433.

Burd, B. J., Nemec, A., and Brinhurst, R. O. 1990. The development and application of analytical methods in benthic marine infaunal studies. – Advances in Marine Biology 26: 169–247.

Clarke, K. R. 1990. Comparisons of dominance curves..– J. Exp. Mar. Biol. Ecol. 138: 143–157.

Clarke, K. R. 1993. Non-parametric multivariate analyses on changes in community structure. – Australian Journal of Ecology, 18: 117–143.

Dawson-Shepherd, A. R., Warwick, R. M. & Clarke, K. R. 1992. An analysis of fish community responses to coral mining in the Maldives. – Envir. Biol. Fish. 33: 367–380.

Fairweather, P. G. 1990. Sewage and the biota on seashores: assessment of impact in relation to natural variability. – Environmental Monitoring and Assessment 14: 194–210.

Fairweather, P. G. & Quinn, G. P. 1998. The State of the Marine Environment Report for Australia. Technical Annex: 1. – Web Site: http://www.erin.gov.au

Ferraro S.P. & Cole F.A. 1990. Taxonomic level and sample size sufficient for assessing pollution impacts on the Southern California Bight macrobenthos. – Marine Ecology Progress Series 67: 251–262.

Gray, J. S., Clarke, K. R., Warwick, R. M. & Hobbs, G. 1990. Detection of initial effects of pollution on marine benthos: an example from the Ekofisk and Eldfisk oilfields, North Sea. – Marine Ecology Progress Series 66: 285–299.

Holme, N.A. & McIntyre, A., 1971. Methods for the Study of Marine Benthos. – Oxford, 387 pp.

Keough, M. J. & Mapstone, B. P. 1995. Protocols for designing marine ecological monitoring programms associated with BEK mills. Technical Report Series. – National Pulp Mills Research Programm No. 11. p. 177.

Keough, M. J., Quinn G. P. 1991. Causality and the Choice of Measurement for Detecting Human Impacts in Marine Environments. – Aust. J. Mar. Freshwater Res. 42: 539–554.

Magurran, A. E. 1988. Ecological Diversity and Its Measurement.

May, R. M. 1975. Patterns of species abundance and diversity. Ecology and Evolution of Communities (eds Cody M. L. & Diamond J. M.). – Belknap Press, Harvard: 81–120.

Nelson W. G. 1987. An evaluation of deviation from the lognormal distribution among species as a pollution indicator in marine benthic communities. – J. Exp. Mar. Biol. Ecol., 113: 181–206.

Olsgard F., Somerfield P. J., Carr M. R. 1997. Relationship between taxonomic resolution and

data transformations in analyses of a macrobenthic community along an established pollution gradient. – Marine Ecology Progress Series 149: 173–181.

Peet, A. K. 1974. The measurement of species diversity. – Annual Review of Ecology and Systematics 5: 285–307.

Poore, G. C. B. & Kudenov, J. D. 1978. Benthos around an Outfall of the Werribee Sewage-treatment Farm, Port Phillip Bay, Victoria. – Aust. J. Mar. Freshwater Res., 29: 157–167.

Rygg, B. 1985. Distribution of species along pollution–induced diversity gradients in benthic communities in Norwegian fjords. – Marine Pollution Bulletin 16: 469–474.

Somerfield P. J., Clarke K. R. 1995. Taxonomic levels, in marine community studies, revisited. Marine Ecology Progress Series 127: 113–119.

Underwood, A. J. 1993. The mechanics of spatially replicated sampling programmes to detect environmental impacts in a variable world. – Australian Journal of Ecology 18: 99–116.

Underwood, A. J. 1994. On Beyond BACI: sampling design that might reliably detect environmental disturbances. – Ecol. Appl. 4: 3–15.

Vanderklift M. A., Ward T. J., Jacoby C. A. 1996. Effect of reducing taxonomical resolution on ordination to detect pollution–induced gradients in macrobenthic infaunal assemblages. – Marine Ecology Progress Series 136: 137–145.

Washington, H. G. 1984. Diversity, biotic and similarity indices: a review with special reference to aquatic ecosystems. – Water Research 18: 653–94.

Warwick, R. M. 1986. A new method for detecting pollution effects on marine macrobenthic communities. – Mar. Biol. 92: 557–62.

Warwick, R. M. 1993. Environmental impact studies on marine communities: Pragmatical considerations. – Australian Journal of Ecology 18: 63–80.

Warwick, R. M. 1988. The level of taxonomic discrimination required to detect pollution effect on marine benthic communities. – Mar. Poll. Bull. 19: 259–68.

Warwick, R. M., Clarke K. R. 1993. Increased variability as a symptom of stress in marine communities. – J.Exp. Mar. Biol. Ecol. 172: 215–226.

Appendix 1

One-way ANOSIM results for different levels of taxonomic resolution (no transformation, number of permutations = 5000)

Groups	Species R=0.770	Class R=0.753	Polychaeta R=0.551
(P1, P2)	0.505*	0.456	0.431
(P1, P3)	0.441	0.383	0.244
(P1, C1)	0.667*	0.411*	0.364
(P1, C4)	0.554*	0.403*	0.518*
(P1, C2)	0.921*	0.732*	0.729*
(P1, C3)	0.779*	0.711*	0.780
(P1, C5)	0.867*	0.888*	0.754*
(P1, C6)	0.690	0.838*	0.460*
(P2, P3)	0.318	0.323	0.142
(P2, C1)	0.997*	0.993*	0.888*
(P2, C4)	1.000*	1.000*	0.648*
(P2, C2)	0.992*	0.723	0.859*
(P2, C3)	1.000*	0.983*	0.981*
(P2, C5)	0.893*	0.902*	0.893*
(P2, C6)	0.755*	0.897*	0.585*
(P3, C1)	0.992*	0.931*	0.723
(P3, C4)	0.995*	0.979*	0.649*
(P3, C2)	0.952*	0.728	0.827*
(P3, C3)	1.000*	0.940*	0.949*
(P3, C5)	0.877*	0.877*	0.866*
(P3, C6)	0.752*	0.944*	0.593*
(C1, C4)	0.800*	0.286	0.565*
(C1, C2)	0.984*	0.927*	0.649
(C1, C3)	0.976	0.997*	0.629*
(C1, C5)	0.894*	0.969	0.744
(C1, C6)	0.762*	0.992	0.488*
(C4, C2)	0.974*	0.969*	0.400
(C4, C3)	1.000*	0.967*	0.573*
(C4, C5)	0.901*	0.991	0.408*
(C4, C6)	0.755	0.992*	0.184
(C2, C3)	0.800	0.509*	0.586*
(C2, C5)	0.200	0.110	0.139
(C2, C6)	0.632*	0.627*	0.233
(C3, C5)	0.775*	0.669*	0.583
(C3, C6)	0.744*	0.874*	0.383*
(C5, C6)	0.371	0.337	0.098

* significant (< 0.0%)