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Review Article

The Application of Multivariate Analytical Techniques to the Study of Marine Benthic Assemblages:
A Review with Special Reference to the Maltese Islands.

Renè M. Micallef and Patrick J. Schembri
Department of Biology, University of Malta, Msida MSD 06, Malta

Summary. *In recent years there have been numerous studies made on the marine benthic assemblages of Maltese coastal waters, either as elements of the seascape, or in order to gain an understanding of the nature of these assemblages and of the factors which structure them, or to assess the potential of such assemblages as indicators of environmental change, principally that due to anthropogenic activities. The massive data sets generated by such studies can realistically only be analysed objectively using an array of sophisticated statistical techniques that it has only been possible to apply now that powerful computers are readily available.*

Starting with the basics of data analysis, this paper reviews the statistical techniques currently used for the analysis of benthic assemblages, particularly those that have been found suitable for the type and character of data from the Mediterranean. Emphasis is placed on multivariate techniques, since benthic data are usually highly multivariate. A brief review of the development of these techniques and of their application to benthic ecological research is also given. The objective is to provide a guide to techniques and to the literature which local workers may find useful as a starting point when designing an experimental, data collection, or analytical protocol.

Introduction

The study of the marine benthic assemblages of Maltese coastal waters has developed along parallel lines as has the subject on a global scale, albeit with a considerable time lag. Thus, the earliest studies were made by naturalists who were primarily interested in cataloguing the biota (for example: McAndrew, 1850; Mamo in Caruana, 1867; Aradas and Benoit, 1870; Medlycott, 1870; Benoit and Gulia, 1872; Gulia, 1873; Sommier and Caruana Gatto, 1915; Despott, 1919; Caruana Gatto and Despott, 1919a,b). At most, these works included only general indications of abundance and habitat. While faunistic and floristic studies have continued to the present, starting in the mid-1960s, attention shifted to the study of the biology of individual species, mainly aspects of physiology, biochemistry, behaviour and autecology (for a compilation of the earlier work, see Bannister, 1974; see also Lythgoe and Woods, 1966), with some workers attempting to relate the biology of the species they studied to synecology (for example, Bannister, 1970; Zammit, 1972; Schembri and Jaccarini, 1978; Fenech, 1980).

The study of marine benthic assemblages as biological entities was pioneered locally by the work of Crossett and Larkum (1966), Crossett et al. (1965), Larkum et al. (1967), and Drew (1969) on algal assemblages, and of Biggs and Wilkinson (1966), Wilkinson et al. (1967) and Richards (1983) on molluscan assemblages. In the late 1980s, the marine benthic assemblages of the Maltese Islands started being systematically investigated by two research groups based at the Department of Biology of the University of Malta. Many of these studies are as yet unpublished (for abstracts see Axiak 1993, 1994, 1995, and Dandria 1996, 1997).

This work has taken two directions: (1) the description of assemblages as elements of the seascape - what may be termed the 'geographical approach'; and (2) a more biological approach in which the focus is community structure and function. The geographical approach has been necessitated by the need to map and characterise the marine environment in connection with the assessment of the environmental impact of coastal development projects and the identification and designation of marine protected areas (Anderson et al., 1992; Borg and Schembri, 1993; Mallia and Schembri, 1995a; Schembri, 1995; Pirotta and Schembri, 1997a,b; Borg et al., 1997a,b).

The primary objective of the biological study of local marine assemblages is to gain an understanding of the nature of these assemblages and of the factors which structure them (see abstracts in Axiak 1993, 1994, 1995, and Dandria 1996, 1997; see also Borg and Schembri, 1995a,b). A secondary, but important objective is to use biotic assemblages as indicators of environmental change, principally that due to anthropogenic activities (Borg and Schembri, 1993, 1995c; Mallia and Schembri, 1993, 1995b).

As has happened elsewhere, the trend has been to move from purely descriptive work to quantitative studies. For all but a few impoverished benthic assemblages however, such studies generate massive data sets. The ready availability of powerful computers has permitted

the application of a wide array of sophisticated statistical techniques to the analysis of such data sets, allowing greater objectivity and more reliable conclusions to be drawn. However, the statistical analysis of ecological data can be a double-edged sword - a powerful tool provided that the appropriate method is chosen and that its strengths and limitations are understood, but otherwise likely to lead to quite erroneous conclusions (James and McCulloch, 1990). This is perhaps even more so in the local situation, where numerical ecology is a fledgling field.

Starting with the basics of data analysis, this paper reviews the statistical techniques currently used for the analysis of benthic assemblages, with particular emphasis on those that have been found suitable for the type and character of data from the Mediterranean. It also provides a brief review of the development of these techniques, and of their application to benthic ecological research in the Mediterranean. It is not our intention to review the entire field, nor the underlying statistical theory - this has already been done by far better qualified workers than ourselves (for example, Williams, 1971; Afifi and Azen, 1979; Field *et al.*, 1982; Gauch, 1982; Ludwig and Reynolds, 1988; Burd *et al.*, 1990; Everitt and Dunn, 1991; James and McCulloch, 1990; Clarke and Warwick, 1994). What we attempt to do here is to provide a guide to techniques and to the literature which local workers may find useful as a starting point when designing an experimental, data collection, or analytical protocol.

between the species, using their pattern of distribution among the samples.

Data in the sample-species matrix can be theoretically plotted as a multi-dimensional graph. In Q-Mode analysis, for two species, a two-dimensional graph is obtained, with each axis representing one species. If Species A is represented on the x-axis, and Species B on the y-axis, Sample 1, which contains 2 individuals of Species A and 5 individuals of Species B, would be the point (2,5) on the said graph (Fig.1). In R-Mode analysis, the samples are the axes and the species are the points.
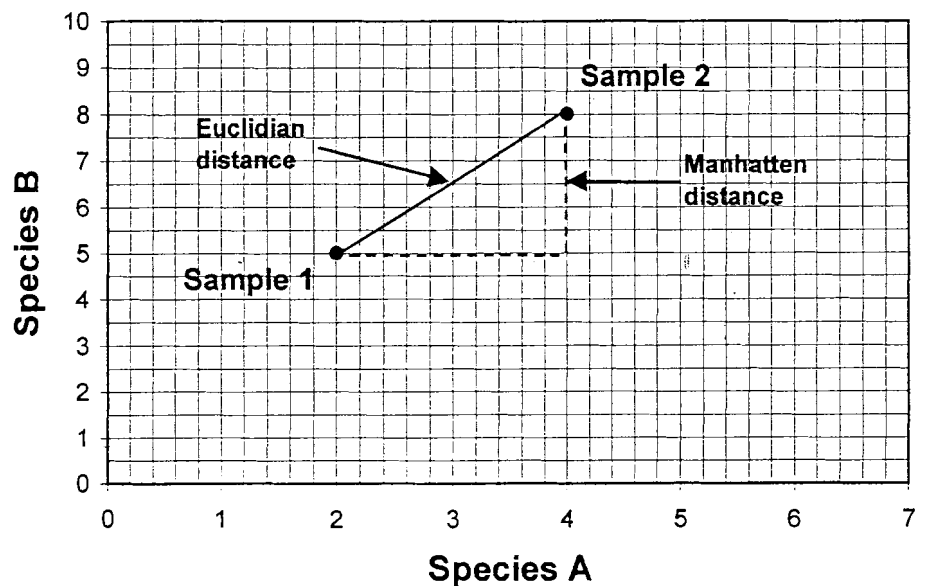

Figure 1. The multidimensionality of data sets in community ecology.

Since plots beyond the third dimension cannot be visualised (although they can be calculated), and since cases of data with less than four samples or species are seldom encountered in practice, statistical methods must be invoked to summarise the data to a two- or three-dimensional representation. Besides summarisation, which must be relatively objective and produce effectively presentable results, 'pattern analysis' techniques should also help ecologists to investigate the structure in their data (Gauch, 1982). Such a summarisation entails discarding some of the information (*dimensionality*) present in the sample-species matrix.

1. The Data Matrix
Q-Mode and R-Mode analysis
Community ecology data is usually based on an analysis of the species present in the given samples, including a measure of abundance. The standard method of presenting these data is in the form of a sample-species matrix (Table 1).

Statistical analysis proceeds from this table and can be performed in two modes, **Q-Mode** (Normal) and **R-Mode** (Inverse). Q-Mode analysis seeks to determine relationships between samples, based on a comparison of the distribution of species within each sample. R-mode analysis, on the other hand, focuses on relationships

The simplest techniques reduce the dimensionality of the data to a minimum - a single variable for each species or sample - hence losing a considerable amount of information. Such techniques are known as univariate techniques. More complex methods (distributional and multivariate techniques) take more of the dimensionality

Table 1. Sample-species matrix

	Sample 1	Sample 2	Sample 3	Sample 4
Species A	Abundance 1A	abundance 2A	abundance 3A	abundance 4A
Species B	Abundance 1B	abundance 2B	abundance 3B	abundance 4B
Species C	Abundance 1C	abundance 2C	abundance 3C	abundance 4C

into account. Data sets in community ecology are multivariate (multidimensional), hence theoretically best analysed by multivariate methods, and the discussion of such analyses will constitute the bulk of this paper. However, we shall begin by taking a brief look at univariate techniques.

2. Univariate analytical methods

There are two main approaches to univariate data analysis. One reduces the data for a sample to an index (a 'diversity index', for example, Shannon-Wiener's, Simpson's, and many more; see below) and, using 'traditional' statistics (analysis of variance, the chi-squared test, and others), compares the indices. The other approach is to select an indicator species and perform these same tests on its abundance in different samples.

2.1 Diversity and similarity measures
2.1.1 Alpha, beta, and gamma diversity

A typical modern 'textbook' definition of alpha, beta, and gamma diversities would be as follows (Lincoln *et al.*, 1998):

α-diversity: The diversity or richness of a species within a particular habitat, community, local area or individual sample.

β-diversity: The richness of a species in a specified geographical region; the rate and extent of change in species along a gradient from one habitat to others.

γ-diversity: The richness of a species across a range of habitats within a geographical area or in widely separated areas.

Diversity, however, is one of those concepts in ecology that have proven to be very elusive to mathematical definition. Robert H. Whittaker introduced the idea of different levels of diversity in the 1960s and this continued to develop through the years, with much active participation on his part (Colinvaux, 1993). The original idea sparked from the dichotomy between 'within habitat diversity' and 'between habitat diversity' which Whittaker called alpha and beta diversity, respectively. Extending the concept, we get, on one side, point diversity, that is, diversity found in very small samples, and on the other side, gamma diversity, that is, diversity between whole regions. The inherent problem with such a classification is the subjectivity of scale, that is, what one understands by 'small sample', 'habitat', and 'region'.

In 1972, Whittaker redefined beta diversity as "*a measure of the rate and extent of change in species along a gradient, from one habitat to others*" (Southwood, 1978). Mathematically, Whittaker first expressed his second version of β-diversity as the ratio of α-diversity to γ-diversity, and eventually refined this to consider it equal to the mean similarity among sites. The most significant point to note, however, is that β-diversity is a vector quantity (ignoring Whittaker's initial and forgotten definition) while α- and γ-diversities are scalar, although both kinds are very elusive to quantification.

As all natural ecosystems exhibit some degree of **dominance**, whereby a few species are much more abundant than the rest, it is clear that a good quantification of α- or γ-diversity must include, besides number of species (**species richness**), some measure of **evenness**, that is, the relative proportions of individuals contributed by each species (for mathematical approaches to the phenomenon of dominance see Cassie, 1962; Whittaker, 1965; McNaughton and Wolf, 1970; Tokeshi, 1990). A habitat with 100 individuals and 10 distinct species is considered to be more diverse if each species contributes 10 individuals than if one species contributed 91 of the individuals present. The weight one gives to each of these two ingredients (richness and evenness) is a subjective issue, and the amount of recipes available, more officially termed diversity indices, is immense. As Southwood (1978) puts it, "*there is no universal 'best-buy', although there are rich opportunities for inappropriate usages*", and one must select an index according to the purpose of one's research. A typical index is the Shannon diversity index[1] (see Fig. 2).

2.1.2 Similarity/distance measures

Given this situation, it is virtually impossible there can ever be a universally accepted absolute measure of diversity, and much of the current effort
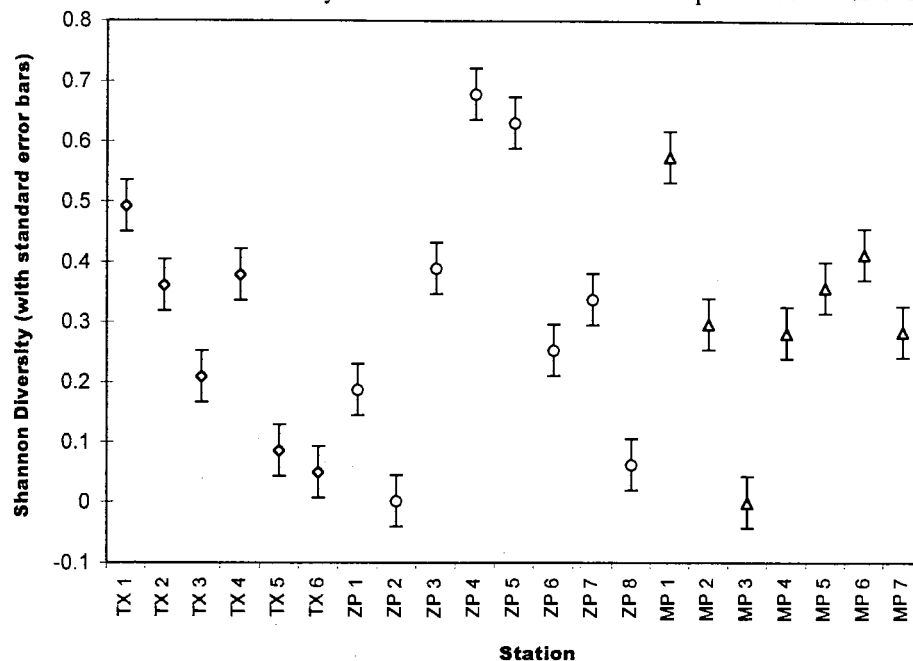

Figure 2. Univariate measures: Shannon-Wiener diversity with standard error bars for a local benthic data set (see Micallef, 1997 and Appendix B). TX (diamonds) = Ta' Xbiex site; ZP (circles) = Zonqor Point site; MP (triangles) = Mignuna Point site. For each site, the stations are arranged on the x-axis in order of increasing distance from the shore. Note that the three sites have different patterns of diversity change with depth, reflecting the level of pollution at each site.

[1] More properly known as the Shannon-Wiener index, although it is also incorrectly referred to as the Shannon-Weaver index - see Colinvaux (1986) footnote on p.651.

goes into the field of β-diversity, that is, relative measures of the change in diversity from habitat to habitat. Here too there are numerous indices, representing similarity (or distance, its inverse) between habitats. However, from mathematical examination and practical use, a handful of measures have proven to be very robust, and are becoming established as standards in all fields of ecology; notable amongst these is the Bray-Curtis coefficient (see Faith *et al.*, 1987).

It is significant to note at this point that similarity (or distance) measures, although considered as measures of β-diversity, can actually distinguish clearly between two habitats with the same species richness and dominance pattern, but with different species composition. This broadens their scope beyond studies of anthropogenic effects on ecosystems, wherein changes of diversity with pollution levels are well defined, to the study of how community composition varies along more subtle environmental gradients. The idea of comparative measures also lends itself to better mathematical treatment, hence, comparing *N* habitats, or in practice, samples, we do not end up with 10 values of absolute diversity, but, taking the similarity (or distance) between each and every habitat or sample, we obtain *N(N-1)/2* values (assuming the measure taken between habitats/ samples *j* and *k* is the same as that between *k* and *j*). This means that each habitat or sample contributes to *N-1* of the variables, and so the data for each habitat or sample, which is multivariate in nature, is not tied up into one variable, as in the former case. Analysis of this type of data, although more computationally demanding, is thus desirable, as it contains more information than for the univariate case, and is not over-summarised on the onset.

2.2.3 Diversity indices and indicator species

As we have seen, diversity is difficult to quantify, and the choice of a suitable diversity index can be quite demanding. On the other hand, selecting an indicator species is also problematic. In typical ecological data sets, a few dominant species are present to some extent in most, if not all, of the samples. It is clear that these species are ubiquitous, and variations in their abundances tend to reflect the clumped distribution of individuals characteristic of most ecological systems, rather than consistent patterns in biological or physical parameters (e.g. pollution gradients). On the other hand, many rare species will be present in very few of the samples, hence their occurrence is so sporadic that analysis based on these species tends to be too noisy to provide any insight into interesting patterns and relationships, especially if standard parametric modelling is used (see below). The significance of all this is that indicator species are hard, if not impossible, to decide upon *a priori*. That is to say, one cannot easily select a good indicator species before one sees the data set to be analysed. And here is the point: selecting an indicator species *after* having examined the data set, that is, *a posteriori*, is statistically unacceptable. This is because the selection is based on the idea that this species is better than others according to some criterion chosen by the analyst, and this introduces bias in the rest of the analysis.

2.2.4 Standard parametric modelling

Statistical testing can be broadly classified into two main types: **parametric tests**, which assume that the data follow some particular distribution (e.g. normal, binomial, linear); and **non-parametric tests**, that make no such assumptions. Parametric tests are usually more robust and powerful than non-parametric ones, but are useless if the actual distribution of the data departs significantly from that assumed. To remedy this, the data may be modelled, the distribution determined, and then mathematically transformed to fit the distribution assumed by the test (usually the normal distribution). However, this procedure is not very practical, and given its poor performance with actual ecological data, it is discouraged by many workers in the field (e.g. Clarke and Warwick, 1994)

2.2 Distributional methods

Several distributional methods of analysis have been proposed, however, although more robust than univariate methods, they are usually less powerful than multivariate analytical methods as they still largely ignore the multivariate nature of the data. These methods will not be treated in detail, given that the focus of this review is mainly on multivariate methods, however, *K*-Dominance Curves and plots of Abundance-Biomass Comparison (ABC plots) will be briefly discussed because of their increasing use in studies of benthic assemblages, particularly those concerned with pollution.
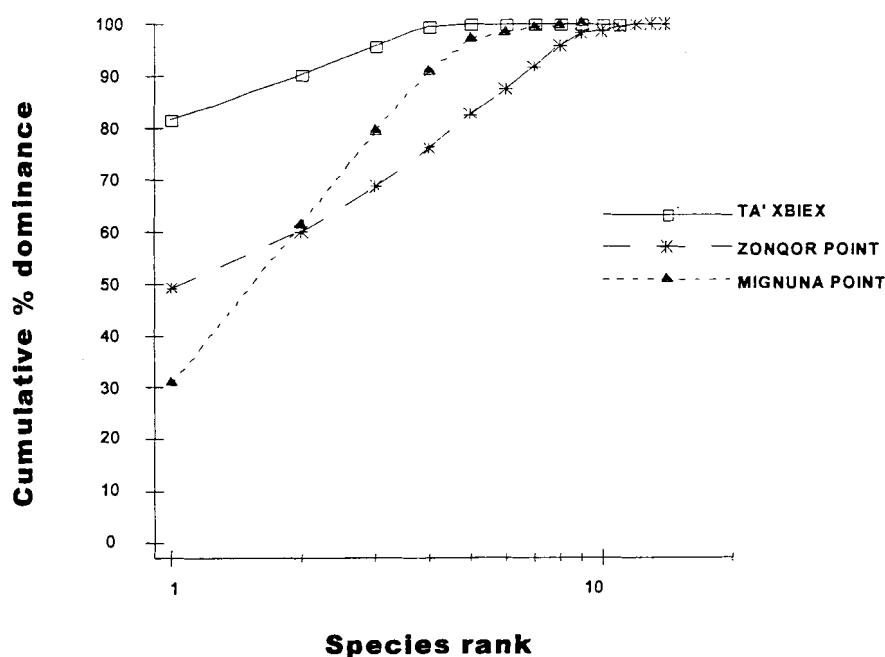

Figure 3.   *K*-dominance plot for the algal taxocene from a local benthic study (see Micallef, 1997 and Appendix B). Note that Ta' Xbiex, the most polluted site, had the lowest diversity.
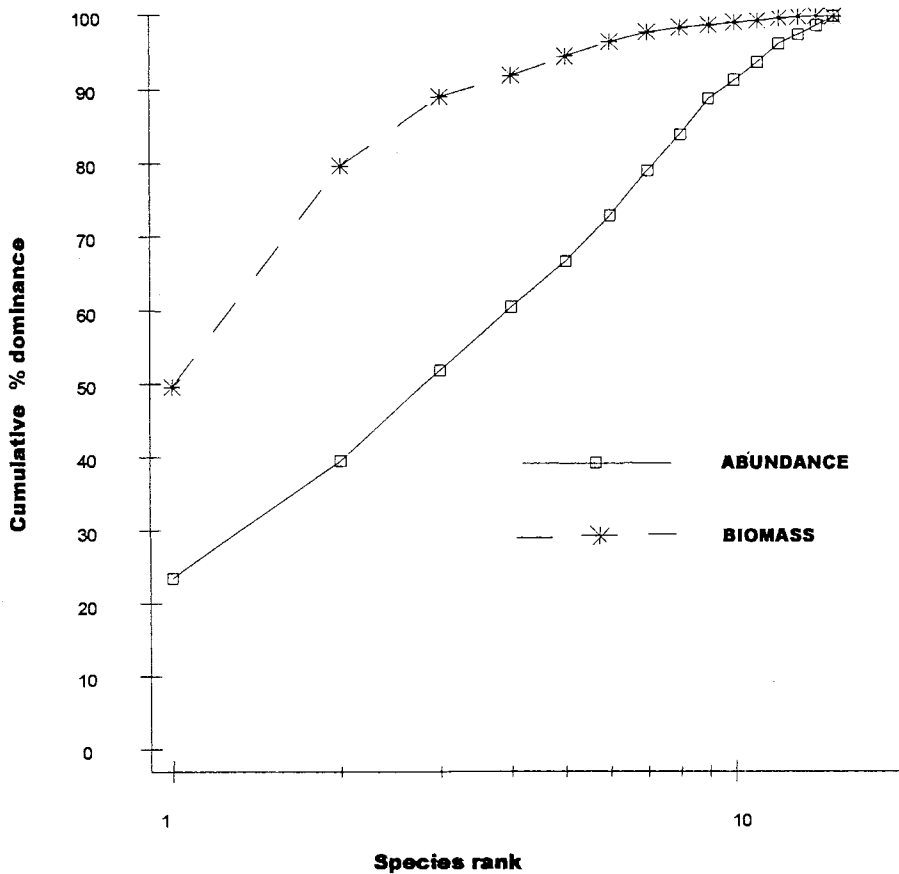
Figure 4.   Abundance-biomass (ABC) curves for the molluscan taxocene from a sample collected using a hand-net (60 strokes covering an area of ca.20m²) from a *Posidionia oceanica* meadow at a depth of 16m off the White Tower headland, Malta. (Hassan Howege, unpublished). This plot is typical of a non-polluted, non-disturbed system.

transform the y-axis for sets of samples wherein the curves approach a cumulative frequency of 100% for most of their length. Clarke (1990) proposes the use of the logistic transformation:

$$y_i' = log \left[(1 + y_i)/(101 - y_i) \right]$$

Some researchers have also proposed the use of partial dominance curves whereby earlier values cannot affect later positions on the curve (see Clarke, 1990 for details).

In **ABC plots**, the principle is extended such that two attributes are considered on the y-axis - cumulative percentage biomass and cumulative percentage abundance, and two curves result (Fig.4). Warwick (1986) hypothesises that in non-disturbed circumstances, the biomass curve rests over the abundance curve, but as pollution (and hence disturbance) increases, the relative positions are expected to shift so that the abundance curve resides above the biomass curve. Several recent studies (e.g. Reizopoulou *et al.*, 1996) have found this technique quite robust for use in marine ecology. A very clear account of the use of ABC plots is given in Clarke and Warwick (1994).

In *k*-dominance curves, each sample is represented by a plot, and comparison of sample-sites involves comparison of these plots. The species within the sample are ranked according to dominance (the highest in abundance or biomass first), and placed on the x-axis, which has a logarithmic scale. On the y-axis, the cumulative percentage abundance is plotted (Fig. 3). The purpose of such curves, as stated by Clarke (1990) is:

"to extract information on the dominance pattern within a sample, without reducing that information to a single summary statistic, such as a diversity index."

In a set of *k*-dominance curves one expects the curves with lowest diversity to reside on top of others with higher diversity. Thus in samples with low diversity, one usually finds a few species with very high abundances - these are hence the lowest in abundance rank and cause the first few y-values in the curve to be very high. More diverse samples would have less dominance and hence lower initial y-values, so that the plot is less elevated than for less diverse samples. The span of the curve in the x-dimension indicates the total number of species (species richness), that is, the second component of diversity.

A number of modifications may be introduced to render the plots clearer for the purposes of inspection. One may opt to compare dominance separately from number of species by re-scaling the x-axis from 1-100 (relative species rank), hence obtaining **Lorenz Curves**. Another option is to

Distributional methods can sometimes prove to be very useful, especially in the analysis of disturbed environments. For example, on the 10th April 1991, an oil spill (from the carrier 'Agip Abruzzo') occurred in the Ligurian Sea, and Danovaro *et al.* (1995) set out to investigate its effects using univariate, distributional and multivariate methods. Shannon-Wiener diversity, Hill's evenness and some other measures were used in the univariate analysis, and *k*-dominance curves were also plotted. Group average clustering, the ANOSIM test, and NMDS (both using Bray-Curtis similarities derived from 4th root transformed data - see below) constituted the multivariate techniques employed in this study. The data on nematodes, identified to genus level, gave very interpretable results in the multivariate analysis, but the *k*-dominance curves gave a clearer picture.

3. The multivariate nature of ecological data sets
3.1 Features of multivariate data sets
We have already stated that data sets in ecology are multivariate in nature, and as Clarke and Warwick (1994) emphasise, highly so.   This is because every sample is described by several species abundances, each of which is considered as a different variable, and, inversely, each species is described by its abundances in several samples.

"The need for multivariate analysis arises whenever more than one characteristic is measured on a number of individuals, and relationships among the characteristics make it necessary for them to be studied simultaneously" (Krzanowski 1972, as cited in Gauch 1982).

Therefore, the fact that each sample in a typical community study is described by the abundances of several species, several environmental factors, and several relationships and associations between these variables, clearly explains why ecological data sets are highly multivariate. Like all other multivariate data sets, they exhibit the following four characteristics.

1. Relationships

Several relationships are usually present in ecological data: those between samples, which are elucidated by Q-mode analysis; those between species, which are the objective of R-mode analysis; and combinations of both, investigated by special analytical techniques. Different samples may be taken from different areas or from the same area at different times. Relationships of species distributions with environmental factors are extremely important; if these are known, they reveal much about community structure, while on the other hand, knowledge of the latter can help detect changes in the former, effectively making community structure a bio-indicator.

2. Noise

Community data is usually very noisy, that is, many secondary patterns are present that obscure the more important and interesting underlying structure. Gauch (1982) gives one definition of noise in this context:

"Noise is variation in a species' abundances coordinated markedly less with variation in other species' abundances than the larger co-ordinations observed."

Such a definition renders the distinction between noise and significant relationships (or co-ordinations) rather subjective, but then, this depends very much on what one intends to study. Causes of noise include local disturbances, environmental heterogeneity at scales smaller than that of the sample area, and chance occurrence and establishment of species. The goal of analysis is to summarise the data in such a way as to eliminate noise and yet retain all the interesting data structure.

3. Redundancy

Redundancy may be considered as the opposite of noise. Normally, one is looking for recurrent patterns in the data, and the more clearly these are brought out, the more evident does noise (elements of the data that do not fit the pattern) become, and hence the easier it is to remove. The elucidation of patterns is enhanced by the presence of redundant data.

A definition of redundancy states that it *"involves co-ordinated species' responses and similar samples"* (Gauch, 1982). In other words, samples that are similar to the ones already present (such as replicates) do not provide any extra information, they are redundant. Species can also be redundant if their abundances reflect directly the abundances of other species (e.g. their predators), hence the term 'co-ordinated species' responses'.

From the above, it should be clear that redundancy in the data is desirable for statistical analysis as it enhances the patterns being sought and distinguishes between interesting relationships and noise, such that the noise can be excluded. However, after making use of the redundancy in the raw data for this purpose, the techniques themselves must remove it in the summarisation they produce. This is because redundancy left within the results of analysis increases the bulk of the data but adds nothing to what is already revealed. For a good discussion about redundancy and techniques for quantifying it in ecological communities, see Clarke and Warwick (1998).

4. Outliers

"An outlier is a sample of peculiar species composition that has low similarity to all other species" (Gauch, 1982)

This concept can be extended to species (species outliers) and to groups of samples/species. When a data matrix is composed of two or more blocks that differ considerably from each other (a situation known as *disjunction*), one block, usually the smaller one, can be considered as an outlier.

In the final result, however, *statistical outliers* are also present: a sample/species may seem different from the others simply because of gaps in sampling and loss of dimensionality during analysis (for example, the typical set of points not falling perfectly on the theoretical regression line in any scientific experiment). An ideal analytical method is expected to

produce few statistical outliers.

Techniques however must also be evaluated on their method of dealing with *community outliers*. In community data these are mainly due to disturbance and environmental heterogeneity. Some statistical techniques give considerable importance to outliers, treating them as representing very long gradients, and hence compressing the rest of the data into a very tiny space. This makes it very difficult, if not impossible, to notice more significant relationships. The solution here is to remove the outliers and re-analyse the data. The most obvious of outliers is an empty sample (all entries are zeros); before analysis using multivariate techniques, all such samples must be removed.

Considering the first point (relationships), it is preferable to work with a matrix (in Q-mode analysis) of the similarity of each sample with every other sample (e.g. multivariate analysis), than with a list in which each sample is reduced to a single index value (univariate analysis). Clearly, multivariate analytical methods are more suited than univarate techniques to explore multivariate data sets.

The second point determines which techniques succeed and which fail in giving a representation of the data acceptably close to reality. Statistical techniques assuming a random distribution of species between samples (Q-mode analysis) usually are not suitable, since in biological communities, the distribution of individuals is often clumped (see Burd *et al.*, 1990). The clumping differs from one community to another, so techniques that make no assumptions about the pattern of distribution of the data are preferable. Basic statistics do not offer sufficiently powerful methods of this kind, hence more specialised techniques have been 'borrowed' from a wide range of disciplines and introduced into the field of community ecology. While some of these techniques can be extremely powerful if well applied, others can give totally misleading and insignificant results. The point here is that one must select very carefully which technique to use. Employing an improper statistical analytical technique may render useless years spent collecting data, as the results obtained will probably be incorrect or at least very poor.

3.2 Treatment of data matrices prior to multivariate analysis: the implications of standardisation and transformation, and truncation of rare species.

One of the major problems with the use of univariate measures and the analysis of the indices they produce (see below) using parametric statistical techniques (e.g. ANOVA - analysis of variance[2]), is that such techniques assume normality while actual community data tend to have a skewed distribution. Appropriate *transformations* help to reduced the skewness and increase the symmetry

so that these techniques can be applied. Since the emphasis of this review is on multivariate methods, we shall limit ourselves to referring the reader to Clarke and Warwick (1994), Downing (1979) and Burd *et al.* (1990), who provide concise yet very clear introductions to the subject.

When applying non-parametric statistics, such as Classification and NMDS (Non-metric Multidimensional Scaling), transformation of data sets is useful for a very different reason - to weight the contributions of common and rare species. A typical data set in marine benthic community work would contain a few dominant (very abundant) species, a good number of moderately abundant species, and some very rare species. The first two categories are usually the most relevant, since the recorded abundance of the very rare species often does not reflect reality[3].

To avoid the useless bulk[4] and the noise that very rare species confer to data sets, many workers recommend their removal. This operation, which we shall hereafter refer to as '*truncation*' must, however, be carefully performed. Field *et al.* (1982) recommend that all species that never constitute more than *p*% of the total abundance (or biomass) of any sample be removed, where *p* is arbitrarily chosen such that a suitable number of species are left (typically 50 to 60 species). It is very important not to be 'overzealous' in truncation - for metric ordination techniques (e.g. Principal Components Analysis), which are very prone to noise caused by the presence of rare species, it is essential, but NMDS requires very little truncation, if any.

Removing the rare species leaves the very common and moderately common species. Often, the difference in abundance between these two groups of species is considerably large. Since many similarity measures place greatest emphasis on the most abundant taxa, the moderately common species, that may be as informative as the very abundant species (or more), are given secondary importance. Transformation seeks to address this issue, by decreasing the differences in abundance and hence increasing the importance of moderately abundant species in the calculation of similarities[5].

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[3] Very rare species cannot be acceptably sampled using the normal size of samples usually collected in most benthic work; their capture is therefore very dependent on chance. In other words, if one individual of species A is recorded in a 0.1m x 0.1m quadrat, this does not necessarily mean that A has an abundance of 100/m² - in fact, this is most probably incorrect. Similarity, a zero abundance in the quadrat sample does not mean that the species is absent in the area. Sometimes, species are also found in unusual places (for example, in the case of macrofauna within harbours where fishermen sort catches obtained from deep water and dump the unwanted material at their berth). Rare records (e.g. a single specimen in a whole data set), besides being obviously useless for analysis, add to the bulk of the data set and increase the noise.

[4] Additional data that is redundant, that is, it adds to the size of the data matrix (increasing analysis time immensely) but only serves to emphasise a pattern that is evident even from a very small part of the data set.

[5] Transformations must be used with care. In a recent study Olsgard et al. (1997) have shown that under certain circumstances, for example, when organisms are only identified to taxonomic levels higher than species, the results of the analysis are greatly influenced by the transformation used and the effects of transformation become stronger as taxonomic level increases; moreover, taxonomic resolution and transformation affect the results of analyses in different and unrelated ways.

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[2] See Underwood (1997) for a thorough account on the use of ANOVA in ecological experiments.

The most widely used transformations fall in the class of **power transformation** where, for an abundance y, the transformed abundance y^* is given by

$$y^* = y^\lambda$$

for example:

$\lambda = 0.5$ for square root transformation, and

$\lambda = 0.25$ for 4th root (double square root) transformation.

Logarithmic transformations can be considered as part of this family, since $(y - 1)/\lambda$ becomes equal to $log_e\, y$ as $\lambda \to 0$. Hence, since λ tends to zero in such transformations, they are more severe than double square root transformations. One cannot, however, use $log\, y$ as such, as when $y = 0$ this tends to negative infinity (and zero values abound in community ecology data sets), rendering the calculation of similarity indices impossible. Instead, $log\, (y + 1)$ is used since this gives zero when $y = 0$. Strictly speaking, this transformation does not fall within the power class of transformations. From a review of recent scientific literature on the subject (see below), it appears that some workers opt to use double square root transformation, while others use $log\, (y + 1)$. Clarke and Warwick (1994) claim that:

"there are rarely any practical differences between cluster and ordination results performed following $y^{0.25}$ or log (1 + y) transformations; they are effectively equivalent in focusing attention on patterns within the whole community, mixing contributions from both common and rare species."

The only problem with logarithmic transformation is the addition of a constant (1) to the abundance value. The results would tend to differ if data is standardised to abundance per square metre or abundance per $10m^2$, with the effect of the constant being less felt in the latter case. Double square root transformation is therefore better recommended, as it does not suffer from this problem.

The most extreme method of transformation is the reduction of abundances to presence-absence data (that is, all non-zero values are converted to 1). This shifts importance decidedly to the moderately abundant and rare species, since, for most assemblages, these constitute a larger portion of the data set than the dominant species.

The choice of **which transformation** to use is a biological, rather than a statistical question, and depends on the objective of the study. If the main interest is changes in the abundance of the most dominant species, a weak transformation (square root) is desirable. If the focus of attention are the moderately-abundant species, then a more severe transformation (4th root or log) should be applied. Alternatively, if the object of the study are the rare species, then presence-absence transformation may be the most suitable, possibly with very little or no truncation (although this protocol is severely prone to noise, as discussed above). The choice **whether to transform or not** is not merely a biological one, however. Some amount of transformation is statistically necessary in most cases. For instance, a biomass NMDS may be completely distorted by a

chance capture of a very large-bodied species. Similarly, in an abundance data set, a small-bodied species (e.g. barnacle spat) can attain very large abundance values and render the presence of all other species insignificant.

3.3 A brief comparison of measures of similarity/distance

There are two main classes of similarity (or distance) coefficients, as has been hinted above. One group considers only presence-absence (**binary coefficients**), while the second considers both presence-absence and relative abundance (**quantitative coefficients**).

An example of the first class is **Jaccard's Coefficient**. For two samples, j and k, this considers the number of species common to both (the higher this number, the larger the similarity) and balances this out with the number of species found only in j or in k (this reduces the similarity). A scaling factor is introduced in the denominator so that some independence of the actual number of species present is achieved and so that the coefficient takes a value between 0 and 1 (or 0 and 100%).The more popular binary measures are **Sørensen's Coefficient** (also known as the Dice or Czekanowski coefficient), **McConnaughey's Coefficient** and **Ochiai's Index**. The main problem with these measures is that they implicitly perform a presence-absence transformation, which is usually too severe and prone to errors due to the chance occurrence of rare species, as discussed above.

One of the biggest debates on the subject of similarity coefficients is whether to introduce joint absences or not. If all but two of the samples in a data set contain a certain species, would those two samples be somewhat more similar to each other than to the rest? In certain scientific disciplines, that consider other attributes rather than species, the answer may well be yes, but in ecology, where data sets abound with zero values, it makes no sense to consider joint absences. As Field *et al.* (1982) put it:

"Taking account of joint absences has the effect of saying that estuarine and abyssal samples are similar because they both lack outer-shelf species."

Table 2 lists the more popular coefficients which consider abundance. The idea of introducing abundances in a similarity measure was first proposed by Bray and Curtis in 1957, who modified the Sørensen coefficient to obtain the measure now known as the **Bray-Curtis Coefficient** (see Southwood, 1978). Many have criticised this measure, mainly because of the importance it gives to dominant species (it obviously does not transform the data as do binary measures), however, with appropriate transformation, many now recognise its robustness. In the same category as the Bray-Curtis distance, one finds the **Canberra Metric**, which is also very popular among ecologists. There are two objections to the use of this measure. The first is that the scaling term in the denominator is placed within the summation, the consequence of which is that rare species are given too much importance. The second objection is that when no individuals of a species are present in one sample but are present in the other sample, the index attains its maximum value (see Krebs, 1989).

Coefficient	Equation[a]	References[b]
Bray-Curtis Distance	$$S_{bc} = \frac{\sum_i \lvert y_{ij} - y_{ik} \rvert}{\sum_i \left(y_{ij} + y_{ik} \right)}$$	1, 2, 3, 4, 5
Canberra metric (Adkins form)	$$S_{cm} = \frac{1}{n} \sum_i \left(\frac{\lvert y_{ij} - y_{ik} \rvert}{y_{ij} + y_{ik}} \right)$$	1, 2, 4, 5
Euclidian Distance	$$S_e = \sqrt{\sum_i \left(y_{ij} - y_{ik} \right)^2}$$	1, 2, 3, 5
Manhattan (Absolute) Distance	$$S_m = \sum_i \lvert y_{ij} - y_{ik} \rvert$$	2, 3, 5
Relative Manhattan (Absolute) Distance	$$S_{rm} = \sum_i \left\lvert \left(\frac{y_{ij}}{\sum_i y_{ij}} \right) - \left(\frac{y_{ik}}{\sum_i y_{ik}} \right) \right\rvert$$	3, 5
Chord Distance	$$S_{ch} = \sqrt{2\left(1 - \left\{ \sum_i \left(y_{ij} y_{ik} \right) \middle/ \sqrt{\sum_i y_{ij}^2 \sum_i y_{ik}^2} \right\} \right)}$$	3, 5
Kulczynski Distance	$$S_k = 1 - \frac{1}{2} \left[\frac{\sum_i \min\left(y_{ij}, y_{ik} \right)}{\sum_i y_{ij}} + \frac{\sum_i \min\left(y_{ij}, y_{ik} \right)}{\sum_i y_{ik}} \right]$$	5

Table 2. The more popular quantitative coefficients (that is, those considering abundances) for comparing two samples j and k.

(a) *Symbols*: i = row (species) no.; n = number of species present in one or both of the samples (species richness of j and k when pooled together); $y_{\text{row column}}$ = abundance (or biomass) in the given row and column of the data matrix.

(b) *References*: 1 = Krebs (1989); 2 = Clarke and Warwick (1994); 3 = Ludwig and Reynolds (1988); 4 = Brower *et al.* (1990); 5 = Faith *et al.* (1987).

A second category of quantitative coefficients is the Absolute Euclidian family of measures, the simplest of which is the **Manhattan** (Absolute, City-Block) distance that provides the link with the previous category (Bray-Curtis and Canberra). Actually, the Bray-Curtis and the Canberra measures are forms of the Manhattan metric, with different standardisations (scaling terms in the denominator), as can be observed in Table 2. The question of standardisation comes back in **Euclidian Distance**, which also ranges from zero to infinity and so may cause some problems in metric ordinations and classification if it attains very large values[6] (see Clarke and Warwick, 1994). The formula recalls Pythagoras' theorem for the determination of the hypotenuse of a triangle. Geometrically, it is the 'as the crow flies' distance between two points, while the Manhattan distance (sum of the shorter sides of the triangle) is the absolute distance between two points, hence the alternative name, 'Absolute'[7] (see Fig.1).

Euclidian group distances can be relativised (standardised, scaled) using more formal mathematics than in the Canberra or Bray-Curtis renderings of the Manhattan metric. In this group, known as the Relative Euclidian family, we find the **Relative Euclidian** (range $0 \rightarrow 2$), **Relative Manhattan** (range $0 \rightarrow 2$) and the **Chord distance** (range $0 \rightarrow 2$) (see Ludwig and Reynolds, 1988). These last two measures have been found quite robust by Faith *et al.* (1987) using simulated data.

The Kulczynski measure, although not very popular, was also found to be among the most robust measures available by Faith *et al.* (1987), who recommend its use (together with the Bray-Curtis measure and Relative Manhattan). We have also found it to be slightly superior to the Bray-Curtis measure in the analysis of simulated

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[6] This is especially so in data sets with several zero entries, as is typical in community ecology.

[7] If one imagines a city with streets laid out in a regular grid (maybe Valletta, or as the name suggests, Manhattan in New York), it is the shortest distance one has to walk to go from one place to another along the streets, hence the alternative name 'City Block'.

	Sample 1	Sample 2	Sample 3	Sample 4
Sample 1	--	--	--	--
Sample 2	Similarity 1/2	--	--	--
Sample 3	Similarity 1/3	Similarity 2/3	--	--
Sample 4	Similarity 1/4	Similarity 2/4	Similarity 3/4	--

Table 3. Triangular matrix of similarities for a data set consisting of four samples

	Sample 1	Samples 2+4	Sample 3
Sample 1			
Samples 2+4	Similarity 1/(2+4)		
Sample 3	Similarity 1/3	Similarity (2+4)/3	

Table 4. Reduced triangular matrix for the data in Table 3.
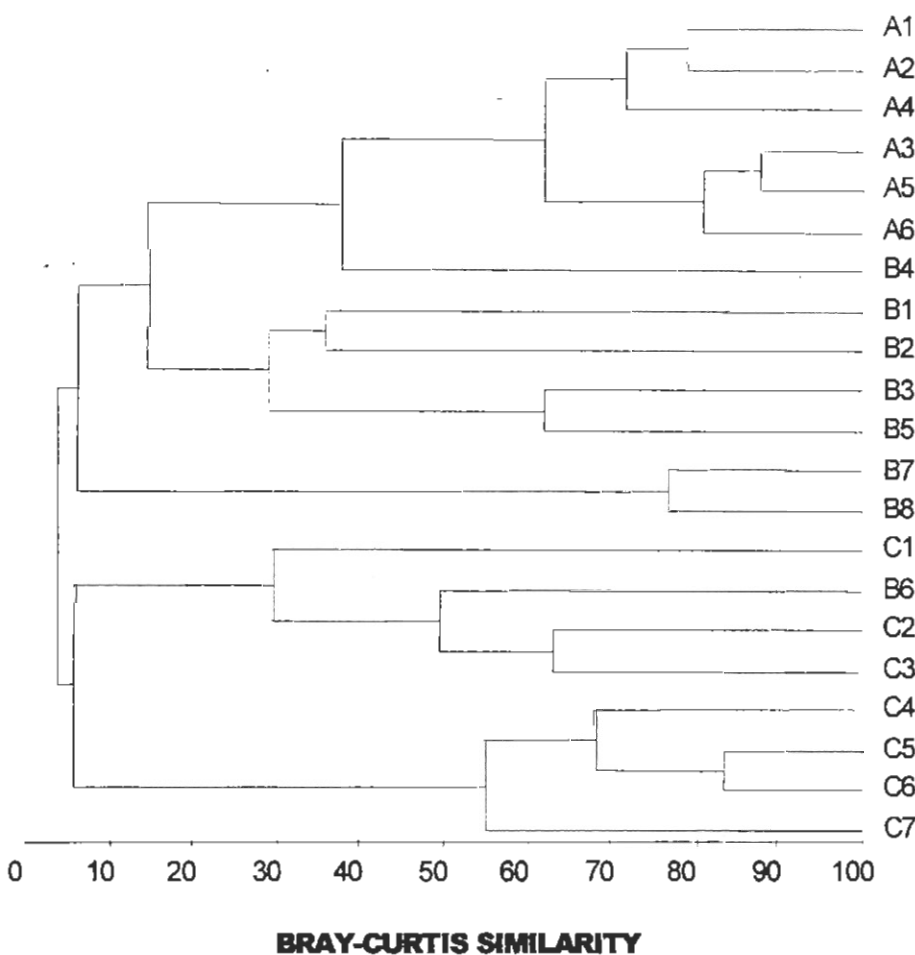
Figure 5. Dendrogram showing hierarchical classification of samples from a local benthic study (see Micallef, 1997 and Appendix B) based on the similarity of the algal taxocene aggregated to genus level. Note that samples labelled C (Mignuna Point) are separated from practically all the other samples at a similarity level of ca.6%, while those labelled A (Ta' Xbiex) and B (Zonqor Point) are separated at a similarity level of ca.15%.

BRAY-CURTIS SIMILARITY

data modelled on local marine benthic assemblages (Micallef, 1997).

4. Classification and ordination
4.1 Classification techniques
Classification, or cluster analysis, involves organisation of the units being analysed into groups, according to a similarity (or distance) measure calculated between the units. The result is typically presented as a *dendrogram*, a plot that appears rather like a family tree or an organisation chart (Fig. 5). Such a plot assumes hierarchical classification, however, reticulate clustering is also possible, whereby the units overlap like a net. Hierarchical methods are by far the most common, being easier to visualise and understand (Krebs, 1989).

Usually, in community ecology, one takes the similarities between each pair of samples or species to construct a *triangular similarity matrix* (Table 3).

As can be seen, the similarities along the top left to bottom right diagonal, for instance that between Sample 1 and Sample 1, are left out, being obviously 100%, and only the triangular section beneath this diagonal is filled, as the triangle on the other side is a mirror image (i.e. Similarity 2/1 equals Similarity 1/2).

Let us suppose that the highest similarity in Table 3 is that between samples 2 and 4 (Similarity 2/4). One can group samples 2 and 4 together, and produce a second matrix (Table 4)
Similarity 1/(2+4) can be derived by several ways. One may, for instance, take the average between Similarity 1/2 and Similarity 1/4, a technique known as **group average linkage**. Alternatively, one may take the higher of the values (**single linkage**) or the lower (**complete linkage**). The procedure we have followed is **agglomerative** in nature, as it proceeds with the units being brought together at ever lower

levels of similarity until a matrix containing only one value results. Another way to combine similarities is to assume that all units form part of a group, and then to break that group down into subgroups, a technique termed **divisive classification**. Theoretically, divisive methods are considered superior to agglomerative ones, because in the latter, anomalies at very low levels are fairly common, and since these are locked up in the structure as it forms, the bad combinations cascade. In other words, due to the limitations of the similarity measures, if two similarity values (such as Similarity 2/3 and Similarity 2/4 in Table 3) are very close, two different indices may not differentiate between the two (for example, Morisita's Index could consider Similarity 2/4 as higher than Similarity 2/3, but Jaccard's coefficient may provide values the other way round). Obviously, there is only one correct natural ranking (although none of the techniques available may be able to reproduce it faithfully for all values and in every analysis) and a discrepancy may cause the whole clustering pattern to be disrupted, since the actual similarities are forgotten as grouping proceeds. Divisive methods should also be less computationally demanding, as one is usually interested in the higher-level groups, and a small number of operations are needed to divide a group into a few major subgroups. However, although this argument makes much sense, divisive methods are nevertheless more computationally demanding since a good method must introduce enough sophistication to deal with the next point (monothetic/polythetic strategies - see below). Due to this problem, divisive methods have not, as yet, gained much ground in marine ecology, where very large data sets are commonplace. In plant ecology, however, divisive clustering using techniques such as TWINSPAN have been used extensively.

The easiest divisive strategy to conceive is **monothetic**.

We have been assuming during this discussion that clustering is based on similarity measures. Taking two hypothetical samples, 1 and 2, a rudimentary similarity measure can be constructed by considering the number of species found in both, out of the total number of species. This is a presence-absence measure, as it does not take relative abundances into account. We can, of course, consider abundances, to achieve measures such as the Bray-Curtis similarity. On the other hand, we may not work with similarity measures at all, but take the abundance of a single species, or, in general, any particular variable, and use it to compare the units (samples). We have thus constructed a univariate version of cluster analysis, better known as a monothetic classification. In divisive strategies, the easiest way to divide a group is to find a single attribute found in some of the members but not in others, and separate the units on this basis. This is monothetic divisive clustering, and it suffers from the severe limitations we met in other univariate techniques. Dividing groups by taking several criteria into account at once is also possible, but requires sophisticated algorithms demanding much computational power, and that are more difficult to understand (Williams, 1971), hence turning the analysis into a black-box. Because of this, many ecologists have steered clear of divisive techniques, and for the purposes of the present review, it would not make much sense to delve further into this abandoned area of statistical ecology. The clustering method of choice for the analysis of benthic assemblages is polythetic hierarchical agglomerative (PHA) classification.

A final point about linkage needs to be made. Single and complete linkage are theoretically attractive since they are non-metric. If, instead of the original triangular similarity matrix, one alters the matrix so that the actual values are replaced by similarity rank, an identical dendrogram would be obtained. This is an advantage over group-average clustering. However, single linkage has a tendency to form chains of linked samples, with each successive stage in the agglomerative process simply adding another sample to an ever growing group (Fig. 6A), while complete linkage has the opposite effect - it produces many small clusters at an early stage (Fig. 6B).

In practice, most workers prefer group-average linkage (see literature review below) as it has been found, from experience, to achieve an acceptable balance between these two extremes. Classification of simulated data sets modelled on local marine benthic assemblages has confirmed this to be true also for local situations (Micallef, 1997).
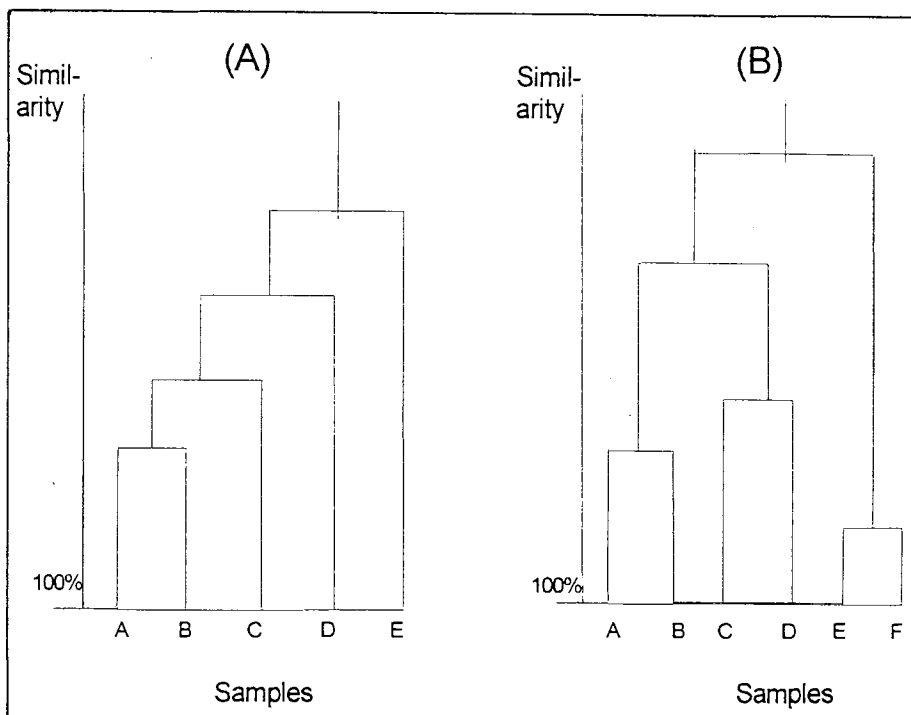

Figure 6. Typical results obtained using (A) single linkage, and (B) complete linkage, in polythetic hierarchical agglomerative classification of five samples.

Several researchers have found that group-average hierarchical

agglomerative clustering is efficient in the detection of changes in the structure of benthic assemblages along a pollution gradient, particularly when combined with other methods as recommended by Clarke and Warwick (1994). For example, Simboura *et al.* (1995) applied ABC curves and this clustering strategy after transforming the original data matrix using $\log_{10}$, and comparing the samples using the Bray-Curtis similarity measure. Univariate measures (Shannon-Wiener's diversity, Pielou's evenness) were also used. The techniques confirmed one another and provided a clear picture of the situation. Reizopoulou *et al.* (1996) compared macrozoobenthic assemblages in three Mediterranean lagoons (Tsopeli in the Ionian Sea, Vivari in the Aegean Sea, and Goro in the northern Adriatic) having different levels of disturbance. Comparisons of diversity (Shannon-Weiner's index), ABC plots and group-average cluster analysis (of $\log_{10}$ transformed data, using the Bray-Curtis similarity measure) were performed. The latter two methods separated the three lagoons, and the dredged site at Goro was clearly distinguished from the other sites that were not as disturbed.

4.2 Ordination techniques

Ordination seeks to depict a multidimensional data set as a low-dimensional plot in which similar entities are placed close together and dissimilar ones far apart. The variety of techniques available is immense, and here we shall only consider the major ordination strategies of relevance to community ecology.

One group of ordination techniques, that has become known as **geometric projection methods** (Kenkel and Orlóci, 1986), was very popular among ecologists in the 1960s and 70s, possibly since these methods were developed by plant ecologists rather than by researchers in other disciplines. The original, and most popular, technique was proposed by J. R. Bray and J. T. Curtis in a 1957 paper published in *Ecological Monographs* entitled "An ordination of the upland forest communities of southern Wisconsin". This technique is formally known as **Polar Ordination,** however, it is often termed Bray-Curtis or Wisconsin Ordination, for obvious reasons. Although the technique is now out of favour with ecologists, this seminal paper has had a great influence:

• It introduced the Bray-Curtis similarity measure (see Krebs, 1989), now very popular among ecologists due to its proven robustness (Faith *et al.*, 1987; Clarke and Warwick, 1994). This was also the first similarity measure to consider relative abundance.

• It gave rise to a whole range of ordination methods that had the effect of promoting ordination techniques in ecology, where they were scarcely known.

• It also stimulated research in the distribution of species along environmental gradients (see Whittaker, 1975), that eventually gave rise to the development of direct gradient analysis (see below).

Polar Ordination involves selecting two samples to serve as poles at two extremes of an environmental gradient

(typically the samples which the distance measure considers most dissimilar), and placing these samples at two ends of a line, at a distance given by the dissimilarity measure multiplied by a factor *x* (to convert the measure to actual distance on the plot paper or screen). The distance measure between each of the other samples and the two poles is calculated, multiplied by *x*, and geometrically speaking, placed at the intersection (on one side of the line) between two circles centred at the poles and with a radius equal to distance from the respective pole multiplied by *x*. Obviously, this is in practice achieved algebraically with a computer program.

The major flaw in Polar Ordination is the selection of the poles. The greatest distance is usually that between two samples of which at least one is an outlier (see Gauch, 1982). Choice of the poles is therefore subjective, and must be done *a priori* (as has been discussed above), hence, very evident gradients are needed (Kenkel and Orlóci, 1986).

A second group of ordination methods are termed **metric ordination methods,** indicating that the actual *value* of the distance measure between any two samples is used in the analysis and is somewhat conserved even in the final plot (Minchin, 1987). On the other hand, in the third group, **non-metric techniques,** only the *rank order* of the distances between the samples is used.

The oldest and best-known metric method is **Principal Components Analysis** (PCA) and involves the reduction of dimensionality by maximisation of variance along a few main axes. In other words - taking the two-dimensional case - it works by extrapolating points on a two-dimensional graph, normally onto the line of best fit. Doing this in the multidimensional case produces the principal component (axis 1) such that when all the points are extrapolated onto this line, the greatest distance (least clumping, maximum variance) between the points is achieved (Fig. 7). The second axis must be perpendicular to the first, and, given this criterion, must achieve maximum variance. In the three-dimensional case, the third axis is defined by virtue of the first two and the criterion that it must be perpendicular to both, but in *n* dimensions, there are *n-1* axes that can be produced independently (*n-1* degrees of freedom). Using a statistic, one obtains the variance (information) explained by each component, and for an acceptable two-dimensional plot (axis 1 vs. axis 2) a considerable amount of the variance (typically >70%) in the data must be given by these two components.

The concept of 'line of best fit' is derived from the application of sum of squares and cross-products (SSCP) techniques, which assume linearity of the data. Formally, this type of analysis is termed eigenanalysis. PCA works directly on the original data matrix, having an in-built distance measure (the Euclidian distance). One PCA variant, PCoA **(Principal Co-ordinates Analysis)** works on triangular distance matrices (hence allowing much more flexibility[8]) - this is also termed metric

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[8] In other words, it does not use Euclidian distances (and hence does not suffer the problems relative to this class of measures discussed in the last part of section 3.3)

multidimensional scaling. PCA, and PCoA are conceptually simple and computationally straightforward, however, they have the following limitations:

• The amount of distortion introduced at each reduction in dimensionality builds up and cannot be removed down the chain - hence, their distance-preserving properties are poor.

• They give too much weight to the actual value of the distance coefficient - hence, they are extremely sensitive to outliers that in practice compress most of the relevant sample (or species) points to a tiny area at the centre of the plot, making interpretation impossible unless the outliers are removed *a posteriori*.

• They assume independence (orthogonality) of factors (components), which must therefore be perpendicular to one another. This adds to the rigidity and care must be exercised in interpretation as mathematically independent factors need not represent independent patterns in nature (James and McCulloch, 1990).

• They assume linearity, that is, that distribution of the proportions is approximately normal. To some extent, this requirement may be satisfied by proper transformation of the original data set (e.g. logarithmic transformation).

• PCA is very rigid in defining similarity (does not apply for PCoA).

Another PCA variant is known as **Factor Analysis**. This does not require orthogonality (independence) of factors, and seeks the maximal correlation among the variables, rather than maximum variance.

Correspondence Analysis (CA, also known as Reciprocal Averaging) is the basis of another family of metric techniques, based on an older and quite subjective method known as **weighted averages ordination** (WA). In the latter, species (for example) are given weights (quite subjectively), and the matrix is transformed to multiply each cell by the respective species weight. A sample score in the ordination results from the averaging of the transformed entries for each species within that sample. Hence, one obtains sample scores from species scores (the weights). It is of course also possible to do the reverse analysis, that is, to obtain species scores starting from sample scores.

Correspondence analysis starts by arbitrary assignment of species scores (weights) and thence calculates sample scores (or vice versa). The second iteration works out species scores from the sample scores calculated in the first iteration, and so on, until the scores stabilise. The scores thus converge to a unique solution after a number of iterations, which solution is not influenced by the initial (arbitrary) choice of scores (hence removing the subjectivity in WA). Although at first sight this seems to have very little to do with PCA, it is nonetheless another form of eigenanalysis (see James and McCulloch, 1990). The final scores are actually the first axis, comparable to PCA's principal component. The second axis of CA (and further components, if required), is obtained in a similar iteration procedure, but the linear effects of the first axis are factored out (see Palmer, 1993).

The method suffers from what is known as the *arch effect* (curvilinear distortion, horseshoe effect; Clarke and Warwick, 1994; Minchin, 1987; Palmer, 1993; James and McCulloch, 1990; Gauch 1982). To add yet another metaphor to an already ample list, an ordination suffering from this effect appears as a 'rainbow' of sample (or species) points, with the points well separated along one
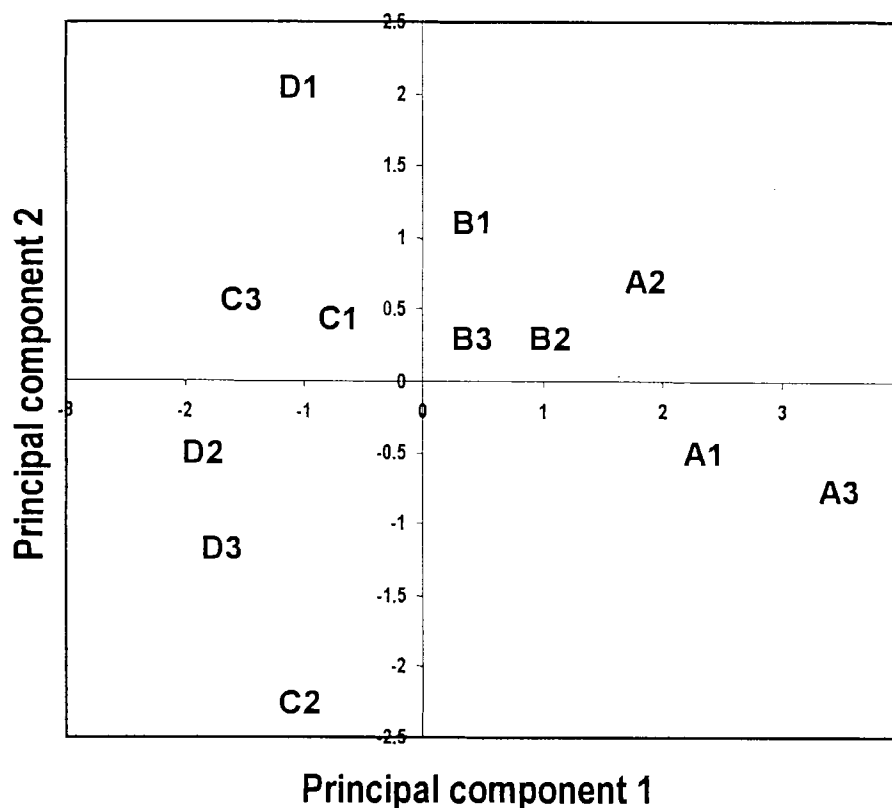

Principal component 1

Figure 7   Two-dimensional PCA ordination of the abundance of decapod crustaceans (double square root transformed) in 12 suction samples collected from a local *Posidonia oceanica* meadow. Samples were collected from 6m (A), 11m (B), 16m (C) and 21m (D). The numeral indicates the collection period: 1 - August 1993, 2 - December 1993, 3 - April 1994. PC1 (x-axis) and PC2 (y-axis) together account for 78% of the total sample variability. Note segregation of the shallow water samples (6m and 11m) from the deeper water samples (16m and 21m). (Data re-analysed from Borg and Schembri, 1998).

axis and very poorly distanced along the perpendicular axis. PCA and PCoA also suffer considerably from this phenomenon. Hence, a variant of CA has been proposed, known as **Detrended Correspondence Analysis** (DCA or DECORANA), that essentially splits the CA ordination space into segments, and stretches or shrinks the scale in each segment accordingly to remove the arch effect. Although this appears to be too manipulative to some, it is done with considerable objectivity. However, some arbitrary decisions are in-built in the algorithm and hidden from the user - thus it "[erects] *a communication barrier between the data analyst and ecologist*" (Clarke and Warwick, 1994) as the algorithm is conceptually complex.

The third class of ordination methods that we shall discuss is that of **non-metric methods**. The best known technique is **NMDS** (Non-Metric Multidimensional Scaling) which, as has been stated above, uses rank order of the values in the triangular similarity (or distance) matrix. The sample points are typically spread out randomly in a three-dimensional space, and their positions optimised (to reflect the distance rank order, either locally or globally - see Minchin, 1987) by a mathematical algorithm until a minimum value of stress is reached (Fig. 8). The data are similarly reduced from

3- to 2-dimensions (see Clarke and Warwick, 1994). The procedure has a tendency to find local stress minima and get locked inside these 'small holes' without finding the 'big crater', using the analogy of a ball thrown on rough ground. To minimise the possibility of not finding the minimum stress configuration, the procedure must be repeated several times (with different starting positions) from which the final configuration with the minimum stress value is adopted. (More details on the NMDS algorithm are in Appendix A.)

Another form of ordination known as **Gaussian Ordination** (Gauch and Chase, 1974; Gauch *et al.*, 1974) seeks to maximise the least-squares fit of the abundance of each species in a sample to a Gaussian curve, hence obtaining sample points (from a first-guess arrangement) by *"iterative fitting that changes the ordination values to produce optimal Gaussian fit for all the species together"* (Gauch *et al.*, 1974). After several evaluations of the technique by a number of workers, Gauch himself dismissed it due to its computational complexity (Gauch 1982) while Minchin (1987) found the method to be highly sensitive to quantitative noise. The technique is now out of favour with most ecologists and is hardly mentioned in the literature.

A mathematical treatment of most of the above techniques can be found in Afifi and Azen (1979) and in Everitt and Dunn (1991), amongst others.

5. Other statistical methods
Hereunder we discuss some other statistical techniques pertinent to the analysis of community ecology data.

1. **Direct Gradient Analysis** embraces a set of techniques that display the distribution of organisms along gradients of important environmental factors. One way this is done is to fit a statistical distribution (typically the normal or log-normal curve) to each species within samples taken along an environmental gradient. This form of direct gradient analysis can be used to construct models on which simulations can be based. A very interesting technique, **(Detrended) Canonical Correspondence Analysis**, is a Canonical Analysis variant involving the fusion of species-sample data with environmental data to produce a separate ordination method (see ter Braak, 1986; Palmer, 1993; ter Braak and Verdonschot, 1995; Thiolouse *et al.*, 1995).
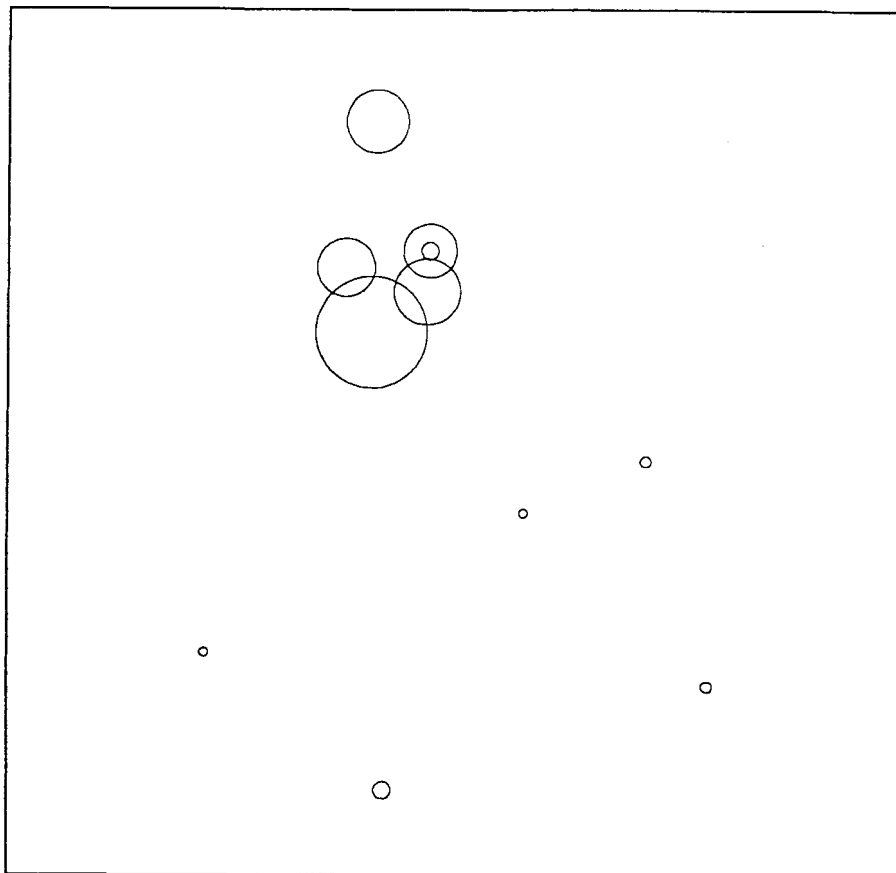
2. Ordinations can be compared

Figure 8   NMDS plot for the molluscan taxocene from a local *Cymodocea nodosa* meadow, based on abundance, after truncation at the 3% level, double square root transformation and using the Kulczynski similarity measure. Stress = 0.12. The radius of the circles is proportional to the *Cymodocea* shoot density. Note the clear separation into two groups: an upper one consisting of samples collected from 4m where shoot density was high, and a lower group collected from 8m where shoot density was low. (Data re-analysed from Howege and Schembri, 1997).

using a technique called **Procrustes Analysis** (see Schönemann and Caroll, 1970). Manually, this involves standardising the axis ranges (from 0 to 100) of two ordination plots, constructing the plots on transparencies, and rotating the two until the best fit of one plot on the other is achieved. The root mean square average of the distances between the observed and expected sample points gives a very robust statistic for evaluation of the ordination method. Procrustes analysis is the mathematical rendering of this plot-fitting algorithm.

3. One type of non-parametric multivariate test for differences between groups of samples is **ANOSIM** (analysis of similarity, by analogy with ANOVA, analysis of variance), first described in Clarke and Green (1988). ANOSIM is used to test the hypothesis that a number of samples in the data matrix constitute a group[9]. Starting from the triangular similarity matrix converted to rank order similarities, one computes a test statistic comparing the within-site differences (in the groupings being tested) to the between-site differences, then recomputes the statistic under permutations of the sample labels to create a permutation distribution from which the significance level of the first value can be estimated. The method can be extended to more complex two-way grouping designs (see Clarke and Warwick, 1994).

4. In most studies on marine assemblages, the ultimate aim is usually to determine what is causing the community structure to be the way it is. Abiotic variables for each sample, such as water temperature and depth, suspended matter, sediment granulometry, organic content of the sediment, and the concentration of chemical pollutants, are often determined in typical studies. The analysis of these parameters is less demanding on techniques than that of biotic variables -- metric ordinations such as PCA, and distance measures such as Euclidian distance, which are not robust for species-samples matrices, give acceptable results in such cases, where zero values are rare.

Linking environmental variables to ordinations can be performed at several levels as described below, using NMDS as the ordination technique of choice.

a. Single abiotic variable:

If this is a discrete quantity (for example, mean sediment particle size or sea-grass shoot density), one can give the different values of this parameter different numbers or symbols and plot these on the NMDS instead of the sample names (as has been done in Fig.8).

b. Two or more abiotic variables (comparison of plots):

For two abiotic variables, a scatter diagram may be plotted. Otherwise an NMDS or PCA of the abiotic variables can be performed. The resulting scatter diagram or ordination plot may be compared to the biotic ordination using Procrustes analysis. The problem with

this method is that the results depend on the dimensionality of the final plot.

c. Two or more abiotic variables (comparison of triangular matrices):

One may rank the similarities in the triangular similarity matrix for the biota, and in that for any two abiotic variables, and then compare the ranks of the biotic and abiotic triangular matrices using a weighted Spearman (or harmonic) rank correlation, a standard non-parametric statistical procedure. A statistic is obtained that takes values between -1 (ranks in complete opposition) and +1 (ranks identical). The significance of this cannot, however, be evaluated from statistical tables, since the similarities that are used for constructing the ranks are not independent. However, one can create a permutation distribution similar to that for ANOSIM (for example, the PRIMER suite of programs [Clarke and Warwick, 1994] includes one called RELATE) that can build such a distribution to test significance of the statistic.

The potential of this method extends much farther. Let us take, for example, the case where we have three abiotic variables. A triangular similarity matrix of abiotic variables 1 and 2 (subset 1,2) is constructed (using Euclidian distance[10]), ranked, and compared to the ranked biotic triangular matrix to obtain a value of $\rho(\rho_{1,2})$. This is repeated using abiotic variables 1 and 3, and again for variables 2 and 3, to construct the abiotic triangular similarity matrix[11] and determine ρ for each case ($\rho_{1,3}$ and $\rho_{2,3}$). The combination of environmental variables giving the highest value of ρ should therefore indicate the variables that are influencing the community structure most.

Conclusion

The availability of a set of powerful statistical techniques for data analysis, we hope, will serve as an incentive for more studies on the benthic assemblages of the Maltese Islands. These techniques may also be applied to the re-analysis of existing data-sets, which may not have yielded very useful results when analysed using more traditional methods. The potential benefits of re-analyses of old data-sets have been amply demonstrated by, for example, Papathanassiou and Zenetos (1993) who re-analysed the data of Zenetos and Papathanassiou (1989). This was a study of the recovery of the benthic assemblages after the introduction of a tannery-effluent treatment plant in the Gulf of Geras (Aegean Sea), which reduced chemical pollution considerably. The re-analysis employed univariate measures (Shannon-Weiner's diversity index, Pielou's evenness), k-dominance curves, average-linkage hierarchical agglomerative classification, non-metric MDS and ANOSIM tests (the latter three techniques being performed on triangular similarity matrices derived from $\log_{10}$ transformed data, using the Bray-Curtis similarity measure). The authors conclude their 1993 report thus (Papathanassiou and Zenetos, 1993):

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[9] The group must be selected *a priori*: one should not apply this test on clusters resulting from multivariate analysis (see Clarke and Warwick, 1994)

[10] As suggested by Clarke and Ainsworth (1993).

[11] A PRIMER procedure called BIO-ENV calculates ρ for all possible combinations of variables. More details are given in Clarke and Ainsworth (1993) and in Clarke and Warwick (1994).

"The multivariate techniques and graphical descriptors (k-dominance curves) have detected the effects of recovery from pollution. Univariate methods, however, can only be applied with success in obvious cases of marked community disturbance and pollution impact..."

Locally, Micallef (1997) re-analysed the data in Borg (1991), Mallia (1991) and Mallia (1993), again with fruitful results.

However, given the wide array of methods available, and the numerous formulations in which they can be applied, one has to find out which protocol works best in a particular situation. Techniques that work well, say in the Atlantic and North Sea, (for example the PRIMER protocol) may not be so efficient in a different ecological setting, such as the oligotrophic central Mediterranean. Zenetos *et al.* (1991) made use of Margalef's index[12] of diversity to compare benthic communities in the Cyclades Plateau (Aegean Sea) together with NMDS and group-average clustering on $\log_{10}$ transformed data applying the Bray-Curtis similarity measure. They compared ecological and palaeoecological data sets, and concluded that care must be applied when drawing conclusions from limited data sets using these methods, since the expected separation into depth groups was observed when the analysis was performed on the total living fauna (329 taxonomic units) and the dead Mollusca (211 taxonomic units), but it was not so marked when only the living Mollusca (41 taxonomic units) were used.

Recent work by the authors has sought to establish a protocol of techniques best suited for local data sets (Micallef, 1997). Using standard parametric modelling, information was extracted from typical local data sets and used to create simulated data sets with the characteristics of local benthic communities. These were sampled using a known pattern, that NMDS and Cluster Analysis were expected to reproduce. Using a variety of simulated communities, transformations, truncation methods and similarity indices, we noted which techniques gave ordinations and classifications that best reflected the original sampling pattern[13] and hence established an optimum protocol of procedures.

It was found that the removal of species that have abundances of less than 3%[14] in all samples, such that a data set with not less than 30 species is obtained, helps analysis as it removes rare species (a considerable source of noise). Transformation of abundances to 4th root was found to be necessary to decrease the differences in the effects of rare and dominant species on similarity measures. The Kulczynski similarity measure was found to be very robust, followed closely by the Bray-Curtis similarity measure. Other results derived from this study will be discussed in a future publication.

Acknowledgements

References

Afifi A. A. and Azen S. P. (1979) *Statistical analysis: a computer oriented approach.* (2nd edition). Academic Press, New York. iii + 207 pp.

Aldenderfer M. S. and Blashfield R. K. (1984) *Cluster analysis.* Sage Publications, California. 87pp.

Anderson E.W., Rolé A. and Schembri P.J. (1992) Coastal zone surveys of the Maltese Islands: onshore and offshore. In: Suárez de Vivero J.L. [ed.] *The ocean change: management patterns and the environment.* pp.139-152; Sevilla, Spain: Departamento de Geografia Humana, Universidad de Sevilla.

Aradas A. and Benoit L. (1870) Conchigliologia vivente marina della Sicilia e delle isole che la circondano. *Atti dell' Accademia Gioenia di Scienze Naturali in Catania,* **3**, 1-342.

Axiak V. [ed.] (1993) *Elective project abstracts B.Sc. 1990-1992.* Department of Biology, University of Malta, Msida, Malta. v + 36pp.

Axiak V. [ed.] (1994) *Biology abstracts B.Sc. 1993-1994, M.Sc. 1993.* Department of Biology, University of Malta, Msida, Malta. v + 29pp.

Axiak V. [ed.] (1995) *Biology abstracts B.Sc. 1994-1995, M.Sc. 1994-1995.* Department of Biology, University of Malta, Msida, Malta. iv + 22pp.

Bannister J.V. (1970) *Some adaptational differences in the limpets* Patella caerulea *and* Patella lusitanica *in relation to zonation.* M.Sc. dissertation, Department of Biology, Royal University of Malta, Msida, Malta. ix + 105pp.

Bannister J.V. [ed.] (1974) *Contributions in marine science.* Royal University of Malta, Msida, Malta. vii + 172pp.

Benoit L. and Gulia G. (1872) Fauna maltese: indice dei molluschi terrestri ed acquatici (parte prima). *Il Barth* (Malta), **10**, 198-200.

Biggs H.E.J. and Wilkinson C (1966) Marine Mollusca from Malta. *Journal of Conchology,* **26**, 52-65.

Borg J.A. (1991) *Vertical zonation of shallow water benthic macrofauna in an inlet on the northern coast of Malta.* Unpublished B.Sc. Dissertation. Department of Biology, University of Malta, Msida, Malta. v + 210pp.

Borg J.A. and Schembri P.J. (1993) Changes in marine benthic community types in a Maltese bay following beach rehabilitation works. Conference proceedings *Clean Seas 93.* Mediterranean Conference Centre, Valletta, Malta 9-11 November 1993.

Borg J.A. and Schembri P.J. (1995a) Preliminary data on bathymetric and temporal changes in the morphology of a Maltese *Posidonia oceanica* (L.) Delile meadow.

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[12] A univariate measure

[13] Two-dimensional plots were compared by Procrustes Analysis.

[14] Of the total abundance of all species within a sample.

Rapport du Congrès de la Commission Internationale pour l'Exploration Scientifique de la Mer Méditerranée, **34**, 20.

Borg J.A. and Schembri P.J. (1995b) Epibenthic macrofaunal assemblages and bottom heterogeneity in the shallow infralittoral of the Maltese Islands. *Rapport du Congrès de la Commission Internationale pour l'Exploration Scientifique de la Mer Méditerranée*, **34**, 20.

Borg J.A. and Schembri P.J. (1995c) The state of *Posidonia oceanica* (L.) Delile meadows in the Maltese Islands (Central Mediterranean). *Rapport du Congrès de la Commission Internationale pour l'Exploration Scientifique de la Mer Méditerranée*, **34**, 123.

Borg J.A. and Schembri P.J. (1998) Bathymetric distribution of decapods associated with a *Posidonia oceanica* (L.) Delile meadow in Malta (Central Mediterranean). *Proceedings of the Fourth International Crustacean Congress, Amsterdam 20-24 July 1998* (in press).

Borg J.A., Mallia A., Pirotta K., Schembri P.J. and Vassallo A. (1997a) A preliminary report on the marine macrobenthos and the demersal fish fauna of the island of Filfla (Maltese Islands, Central Mediterranean). *The Central Mediterranean Naturalist* (Malta), **2**(4), 136-151.

Borg J.A., Micallef S.A., Pirotta K., and Schembri P.J. (1997b). Baseline marine benthic surveys in the Maltese Islands (Central Mediterranean). In: Özhan, E. [ed.] *Proceedings of the third international conference on the Mediterranean coastal environment, MEDCOAST 97, November 11-14, 1997 Qawra, Malta* Vol.1 pp. 1-8; MEDCOAST Secretariat, Middle East Technical University, Ankara, Turkey.

Brower J.E., Zar J.H. and von Ende C.N. (1990) *Field and laboratory methods for general ecology.* (3rd edition). Wm.C. Brown, Dubuque, Iowa. xi + 237pp.

Burd B.J., Nemec A. and Brinkhurst R.O. (1990) The development and application of analytical methods in benthic marine infaunal studies. *Advances in Marine Biology*, **26**, 169-247.

Caruana A.A. (1867) *Enumeratio ordinata molluscorum Gaulo-Melitensium of the late Mr Giuseppe Mamo.* British Press, Malta. 78pp.

Caruana Gatto A. and Despott G. (1919a) Materiali per una malacofauna marina delle isole Maltesi. *Archivium Melitense* (Malta), **3**, 431-446.

Caruana Gatto A. and Despott G. (1919b) Materiali per una malacofauna marina delle isole Maltesi. *Archivium Melitense* (Malta), **4**, 38-46.

Cassie R.M. (1962) Frequency distribution models in the ecology of plankton and other organisms. *Journal of Animal Ecology*, **31**: 65-92.

Clarke K.R. (1990) Comparisons of dominance curves. *Journal of Experimental Marine Biology and Ecology*, **138**, 143-157.

Clarke K.R. and Ainsworth M. (1993) A method of linking multivariate community structure to environmental variables. *Marine Ecology Progress Series*, **92**, 205-219.

Clarke K.R. and Green R.H. (1988) Statistical design and analysis for a 'biological effects' study. *Marine Ecology Progress Series*, **46**, 213-226.

Clarke K.R. and Warwick R.M. (1994) *Change in marine communities: an approach to statistical analysis and interpretation.* Natural Environment Research Council, Plymouth, U.K. 144pp.

Clarke R.B. and Warwick R.M. (1998) Quantifying structural redundancy in ecological communities. *Oecologia* **113**, 278-289.

Colinvaux P. (1986) *Ecology.* John Wiley and Sons, New York. ix + 725 pp.

Colinvaux P. (1993) *Ecology 2.* John Wiley and Sons, New York. xvi + 688 pp.

Crossett R.N. and Larkum A.W.D. (1966) The ecology of benthic marine algae on submarine cliff faces in Malta. In: Lythgoe N. and Woods J.D. [eds] *Symposium of the Underwater Association for Malta 1965.* pp. 57-61. Underwater Association for Malta, London, UK.

Crossett R.N., Drew E.A. and Larkum A.W.D.(1965) Chromatic adaptation in benthic marine algae. *Nature*, **207**, 547-548.

Dandria D. [ed.] (1996) *Biology abstracts B.Sc. 1995-1996, M.Sc. 1995-1996.* Department of Biology, University of Malta, Msida, Malta. iv + 28pp.

Dandria D. [ed.] (1997) *Biology abstracts B.Sc. 1996-1997, M.Sc. 1996-1997.* Department of Biology, University of Malta, Msida, Malta. v + 39pp.

Danovaro R., Fabiano M. and Vincx M. (1995) Meiofauna response to the *Agip Abruzzo* oil spill in subtidal sediments of the Ligurian sea. *Marine Pollution Bulletin* **30**(2), 133-145.

Despott G. (1919) The Mollusca of Marsascirocco Harbour, Malta. *Proceeding of the Malacological Society*, London, **13**, 168-183.

Downing J.A. (1979) Aggregation, transformation and the design of benthos sampling programs. *Journal of the Fisheries Research Board of Canada*, **36**: 1454-1463.

Drew E.A. (1969) Photosynthesis and growth of attached marine algae down to 130 metres in the Mediterranean. *Proceedings of the International Seaweed Symposium*, **6**, 151-159. [Santiago de Compostela, Spain, September 1968]

Everitt B.S. and Dunn G. (1991) *Applied multivariate data analysis.* Edward Arnold, London. v + 135 pp.

Faith D.P., Minchin P.R. and Bebin L. (1987) Compositional dissimilarity as a robust measure of ecological distance. *Vegetatio*, **69**, 57-68.

Fenech M.F. (1980) *Aspects of the biology of the vermetid mollusc* Serpulorbis arenaria. Unpublished M.Sc. dissertation, Department of Biology, University of Malta, Msida, Malta. iii + 144pp.

Field J.G., Clarke K.R. and Warwick R.M. (1982) A practical strategy for analysing multispecies distribution patterns. *Marine Ecology Progress Series*, **8**, 37-52.

Gamito S. and Raffaelli D. (1992) The sensitivity of several ordination methods to sample replication in benthic surveys. *Journal of Experimental Marine Biology and Ecology*, **164**, 221-232.

Gauch H.G. (1982) *Multivariate analysis in community ecology.* Cambridge University Press, Cambridge, U.K. x + 294 pp.

Gauch H.G. and Chase G.B. (1974) Fitting the Gaussian curve to ecological data. *Ecology*, **55**, 1377-1381.

Gauch H. G., Chase G.B. and Whittaker R.H. (1974) Ordination of vegetation samples by Gaussian species distributions. *Ecology*, **55**, 1382-1390.

Grieg-Smith P. (1980) The development of numerical classification and ordination. *Vegetatio*, **42**: 1-9.

Gulia G. (1873) Fauna maltese. Indice dei crostacei. *Il Barth*, Malta **15-16**, 414-315.

James F.C. and McCulloch C.E. (1990) Multivariate analysis in ecology and systematics: panacea or Pandora's box? *Annual Review of Ecology and Systematics*, **21**, 129-166.

Howege H.M. and Schembri P.J. (1997) Seasonal variation in abundance and biomass of the molluscan assemblages associated with the sea-grass *Cymodocea nodosa* (Ucria) Asch. in the Maltese Islands. *Deuxièmes Journées Maghrébines des Sciences de la Mer sur le theme "Ressources Halieutiques et Environnement Marin". Agadir, Morocco 20-22 Décembre 1997.* (In press).

Kenkel N.C. and Orlóci L. (1986) Applying metric and nonmetric multidimensional scaling to ecological studies: some new results. *Ecology*, **67**, 919-928.

Krebs G.J. (1989) *Ecological methodology.* Harper and Row, New York. xii + 654 pp.

Kruskal J.B. and Wish M. (1978) *Multidimensional scaling.* Sage Publications, California. 93pp.

Larkum A.W.D., Drew E.A. and Crossett R.N. (1967) The vertical distribution of attached marine algae in Malta. *Journal of Ecology,* **55,** 361-371.

Lincoln R., Boxshall G. and Clark P. (1998) *A dictionary of ecology, evolution and systematics* (2nd edition). Cambridge University Press, Cambridge. ix + 361pp.

Ludwig J. A. and J. F. Reynolds. 1988. *Statistical ecology: a primer on method and computing.* Wiley-Interscience, New York. xviii + 337pp.

Lythgoe N. and Woods J.D. [eds] (1965) *Symposium of the Underwater Association for Malta 1965.* Underwater Association for Malta, London, UK. 71pp.

McAndrew R. (1850) Notes on the distribution and range in depth of Mollusca and other marine animals observed on the coasts of Spain, Portugal, Barbary, Malta and Southern Italy in 1849. *Reports of the British Association for the Advancement of Science,* **1850,** 264-304.

McNaughton S.J. and Wolf L.L. (1970) Dominance and the niche in ecological systems. *Science,* **167,** 131-139.

Mallia A. (1991) *Zonation patterns on a rocky shore under the influence of a sewage outfall.* Unpublished B.Sc. Dissertation. Department of Biology, University of Malta, Msida, Malta. iv + 276 pp.

Mallia A. (1993) *Effect of exposure on rocky shore zonation patterns in the Maltese Islands.* Unpublished M.Sc. Dissertation, Department of Biology, University of Malta, Msida, Malta. vii + 243pp.

Mallia A. and Schembri P.J. (1993) Rocky shore community structure as an indicator of low level organic loading on Maltese coasts (Central Mediterranean). Conference proceedings *Clean Seas 93.* Mediterranean Conference Centre, Valletta, Malta 9-11 November 1993.

Mallia A. and Schembri P.J. (1995a) Strategies for the conservation of the coastal and shallow water ecosystems of the Maltese Islands. In: Özhan E. [ed.] *Proceedings of the second international conference on the Mediterranean coastal environment, MEDCOAST 95, October 24-27, 1995 Tarragona, Spain.* Vol.1 pp 187-201; MEDCOAST Secretariat, Middle East Technical University, Ankara, Turkey.

Mallia A. and Schembri P.J. (1995b) Detecting low-level sewage pollution using rocky shore communities as bio-indicators. *Rapport du Congrès de la Commission Internationale pour l'Exploration Scientifique de la Mer Méditerranée,* **34,** 140.

Medlycott W. (1870) Notes on the geology, botany, and natural history of Malta. In: Seddall H. *Malta: past and present, being a history of Malta from the days of the Phoenicians to the present time.* pp. 335-355. Chapman and Hall, London.

Micallef R.M. (1997) *Application of multivariate and other statistical methods to the analysis of marine communities from the Maltese Islands.* Unpublished B.Sc. Dissertation, Department of Biology, University of Malta, Msida, Malta. vi + 250 pp.

Minchin P.R. (1987) An evaluation of the relative robustness of techniques for ecological ordination. *Vegetatio,* **69,** 87-107.

Nicolaidou A., Zenetos A., Pancucci M-A. and Simboura N. (1993) Comparing ecological effects of two different types of pollution using multivariate techniques. *P.S.Z.N. I: Marine Ecology,* **14**(2), 113-128.

Olsgard F., Sommerfield P.J., and Carr M.R. (1997) Relationships between taxonomic resolution and data transformations in analyses of a macrobenthic community along an established pollution gradient. *Marine Ecology Progress Series* **149,** 173-181.

Palmer M.W. (1993) Putting things in even better order: the advantages of canonical correspondence analysis.

Ecology, **74,** 2215-2230.

Papathanassiou E. and Zenetos A. (1993) A case of recovery in benthic communities following a reduction in chemical pollution in a Mediterranean ecosystem. *Marine Environmental Research,* **36,** 131-152.

Pirotta K. and Schembri P.J. (1997a) Characterization of the major marine biotopes of the hard substrata around the Maltese Islands. In: (Özhan, E. [ed.] *Proceedings of the third international conference on the Mediterranean coastal environment, MEDCOAST 97, November 11-14, 1997 Qawra, Malta.* Vol.1 pp. 9-24; MEDCOAST Secretariat, Middle East Technical University, Ankara, Turkey.

Pirotta, K. & Schembri, P.J. (1997b) Characterization of the major marine biotopes of the soft substrata around the Maltese Islands. In: (Özhan, E. [ed.] *Proceedings of the third international conference on the Mediterranean coastal environment, MEDCOAST 97, November 11-14, 1997 Qawra, Malta.* Vol.1 pp. 25-37; MEDCOAST Secretariat, Middle East Technical University, Ankara, Turkey.

Reizopoulou S., Tessalou-Legaki M. and Nicolaidou A. (1996) Assessment of disturbance in Mediterranean lagoons: an evaluation of methods. *Marine Biology,* **125,** 189-197.

Richards G.W. (1983) Molluscan zonation on rocky shores in Malta. *Journal of Conchology,* **31,** 207-224.

Rohlf F. J. (1972) An empirical comparison of three ordination techniques in numerical taxonomy. *Systematic Zoology,* **21,** 271-280.

Schembri P.J. and Jaccarini V. (1978) Some aspects of the ecology of the echiuran worm *Bonellia viridis* and associated infauna. *Marine Biology,* **47,** 55-61.

Schembri P.J. (1995) Threatened habitats as a criterion for selecting coastal protected areas in the Maltese Islands. *Rapport du Congrès de la Commission Internationale pour l'Exploration Scientifique de la Mer Méditerranée,* **34,** 128.

Schönemann P. H. and Carroll R. M. (1970) Fitting one matrix to another under choice of a central dilation and a rigid motion. *Psychometrica,* **35,** 245-255.

Simboura, N., Zenetos A., Panayotidis P. and Makra A. (1995) Changes in benthic community structure along an environmental pollution gradient. *Marine Pollution Bulletin,* **30,** 470-474.

Sneath P.H. and Sokal R.R. (1973) *Numerical taxonomy.* W. H. Freeman, San Francisco. xv + 573 pp.

Sommier S. and Caruana Gatto A. (1915) *Flora melitensis nova.* Firenze, Italy. viii + 502pp.

Southwood T.R.E. (1978) *Ecological methods - with particular reference to the study of insect populations.* (2nd edition). Chapman and Hall, London. xxiv + 524pp.

ter Braak C.J.F. (1986) Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. *Ecology,* **67,** 1167-1179.

ter Braak C.J.F. and Verdonschot P.F.M. (1995) Canonical correspondence analysis and related multivariate methods in aquatic ecology. *Aquatic Sciences,* **57,** 225-289.

Thiolouse J., Chessel D. and Champley S. (1995) Multivariate analysis of spatial patterns: a unified approach to local and global structures. *Environment and Ecological Statistics,* **2,** 1-14.

Tokeshi M. (1990) Niche approportionment or random assortment: species abundance patterns revisited. *Journal of Animal Ecology,* **59,** 1129-1146.

Underwood A.J. (1997) *Experiments in ecology: their logical design and interpretation using analysis of variance.* Cambridge University Press, Cambridge. 504 pp.

Warwick R.M. (1986) A new method for detecting pollution effects on marine macrobenthic communities. *Marine Biology,* **92,** 557-562.

Warwick R.M. and Clarke K.R. (1991) A comparison of some

methods for analysing change in benthic community structure. *Journal of the Marine Biological Association of the United Kingdom*, **71**, 225-244.

Warwick R. M. and Clarke K. R. (1993) Comparing the severity of disturbance: a meta-analysis of marine macrobenthic community data. *Marine Ecology Progress Series*, **92**, 221-231.

Whittaker R.H. (1965) Dominance and diversity in land plant communities. *Science*, **147**, 250-260.

Whittaker R.H. (1975) *Communities and ecosystems*. (2nd edition). Macmillan, New York. 385pp.

Wilkinson C., Bevan J. and Balaam C. (1967) Distributional studies of marine molluscs in Malta. *Underwater Association Report* **1966-67**, 89-99.

Williams W.T. (1971) Principles of clustering. *Annual Review of Ecology and Systematics*, **2**, 303-326.

Williams W.T. and Lambert J.M. (1959) Multivariate methods in plant ecology. I. Association-analysis in plant communities. *Journal of Ecology*, **47**, 83-101.

Zammit V.A. (1972) *Aspects of the ecology and physiology of selected rock pool biota*. Unpublished M.Sc. dissertation, Department of Biology, Royal University of Malta, Msida, Malta. 187pp.

Zenetos A. and Papathanassiou E. (1989) Community parameters and multivariate analysis as a means of assessing the effects of tannery effluents on macrobenthos. *Marine Pollution Bulletin*, **20** (4), 176-181.

Zenetos A., Papathanassiou E. and Van Aarsten J.J. (1991) Analysis of benthic communities in the Cyclades Plateau (Aegean sea) using ecological and palaeoecological data sets. *P.S.Z.N. I. Marine Ecology*, **12**(7), 123-127.

Appendix A: NMDS
- a brief description of the underlying algorithm

From recent work in which the various ordination methods discussed above have been compared, NMDS has emerged as the most robust of the techniques available. To cite but one example of such research, we shall discuss the work of Nicolaidou *et al.* (1993). These authors challenged a number of techniques to distinguish between polluted and clean sites, and among the polluted sites, between pollution due to organic wastes and that due to coarse metalliferous residues. Univariate measures (Shannon-Weiner Diversity) separated polluted from clean sites by indicating a decrease in diversity for the polluted stations, but did not distinguish between the two types of pollution, except where the solid wastes were dumped on finer sediments. K-dominance curves were found to be sensitive only in an organically enriched area. Classification and PCA were not helpful either, although an elucidation of the differences between the extreme cases was provided by Correspondence Analysis. Non-metric MDS, however, proved to be markedly superior to the other techniques, distinguishing clearly clean from polluted stations, fine from coarse sediments, as well as organic and metalliferous pollution.

NMDS was also found to be very robust in the analysis of data sets from the Maltese Islands (Micallef, 1997). It is therefore fitting to discuss this technique in more detail, especially since it is becoming the ordination method of choice among marine ecologists worldwide. There are several implementations of the NMDS algorithm, and here we shall discuss the implementation found in the PRIMER[15] package (Clarke and Warwick, 1994). A detailed outline of the procedure is given by

Kruskal and Wish (1978) and summarised in Clarke and Warwick (1994). The following main steps are involved:

1. The number of dimensions, m, for the final ordination is specified.

2. A starting configuration with the n sample points is constructed. This is usually done by a randomisation procedure, but the initial configuration may also be entered from another ordination technique, e.g. PCA.

3. The inter-point distances from this plot are regressed on the corresponding dissimilarities in the triangular distance matrix. One can represent this step by plotting a scatter graph of distance in the NMDS plot (d_{jk}, y-axis) against distance value in the triangular distance matrix (δ_{jk}, x-axis). If a similarity coefficient has been used, the resulting triangular similarity matrix is converted into a distance matrix by the program. A non-parametric regression line[16] is fitted onto the plot, which is known as a Shepard Diagram (Fig. 9).

4. The goodness of fit of the non-parametric regression is measured by calculating the deviations of the points from the line of best fit. The amount of deviation is termed stress. Stress is defined[17] as:

$$Stress = \frac{\sum_j \sum_k \left(d_{jk} - \hat{d}_{jk}\right)^2}{\sum_j \sum_k d_{jk}^2}$$

where: $\hat{d}_{jk}$ = dissimilarity δ_{jk} scaled to the units of d_{jk} (so that they can be subtracted).
Stress tends to zero as this value becomes equal to d_{jk} for all values.

5. The current configuration is slightly modified in such a way that stress is reduced. Actually, this is done by a complex mathematical technique that seeks to "*perturb the configuration in a direction of decreasing stress*" (Clarke and Warwick, 1994). The non-parametric regression has one assumption - that as y increases x

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[15] Plymouth Routines In Multivariate Ecology Research - a suite of programs developed and distributed by the Plymouth Marine Laboratory, Plymouth, U.K. See Clarke and Warwick (1994), a text that serves as a manual for the suite.

[16] In standard linear regression, a line, commonly called the 'line of best fit', is fitted to a set of points. This assumes that the y-axis parameter varies linearly with the x-axis parameter ($y \propto x$). If $y \propto x^2$, a quadratic regression ('curve of best fit') would fit much better than a linear one, and so on with other polynomials. These regressions are called parametric, as one assumes there is a straightforward relationship between x and y - using these models, one obtains metric MDS. In NMDS, the best fitting 'line' is much more flexible: it takes the shape of the scatter plot, typically appearing as a 'staircase' rising from the origin. This is non-parametric regression and gives NMDS its flexibility.

[17] This is the original equation. In some versions, the denominator ('scaling term') is modified, but the advantages of these modifications seems to be counterbalanced by the increased risk of convergence to local minima (see Clarke and Warwick, 1994).

[18] This is known as the monotonicity assumption, as opposed to the linearity assumption in metric techniques.

increases[18]. The actual values of x and y are not as important as the fact that they increase together (the larger the dissimilarity index, the larger the distance on the plot). NMDS thus uses only the rank order of the similarities, and not the actual values. It follows that to reduce stress, y-values in the Shepard diagram (i.e. distances in the MDS) must increase correspondingly as the x-values increase (i.e. as rank of dissimilarities increases). Figuratively, one seeks a fit of 'steepest ascent' (or descent). Techniques that achieve this form part of a branch of mathematics called 'numerical optimisation'.

6. Steps (3) to (5) are repeated until further perturbation leads to no decrease in stress (convergence).

The main problem with this algorithm, besides its comparatively huge demands on computer power with respect to metric methods, is the risk of finding local minima. Imagine that there are two configurations with low stress, one of which has the least stress (global minimum), and that the two configurations differ considerably. If the algorithm happens to find the second configuration (local minimum), it is almost impossible to escape from this as the global minimum is different enough that it cannot be found with the slight perturbation of step 5. To minimise this risk, steps 1 to 6 should be repeated several times and the configuration with least stress adopted. This done, one can furthermore estimate the adequacy of an NMDS using several criteria, especially the stress value:

|	
Stress < 0.05	Excellent representation. Virtually no risk whatsoever of local minima.
Stress = 0.05 - 0.1	Good ordination. Very little risk of misinterpretation[19].
Stress = 0.1 - 0.2	Quite a useful ordination. If stress values are close to 0.2, however, the general appearance, rather than the details, is most reliable.
Stress = 0.2 - 0.3	Not very useful. If stress values are close to 0.3, the resulting ordination is not acceptable.
Stress > 0.3	Not acceptable. Points placed randomly in an NMDS plot (starting configuration) often attain stress values of 0.35 to 0.45.
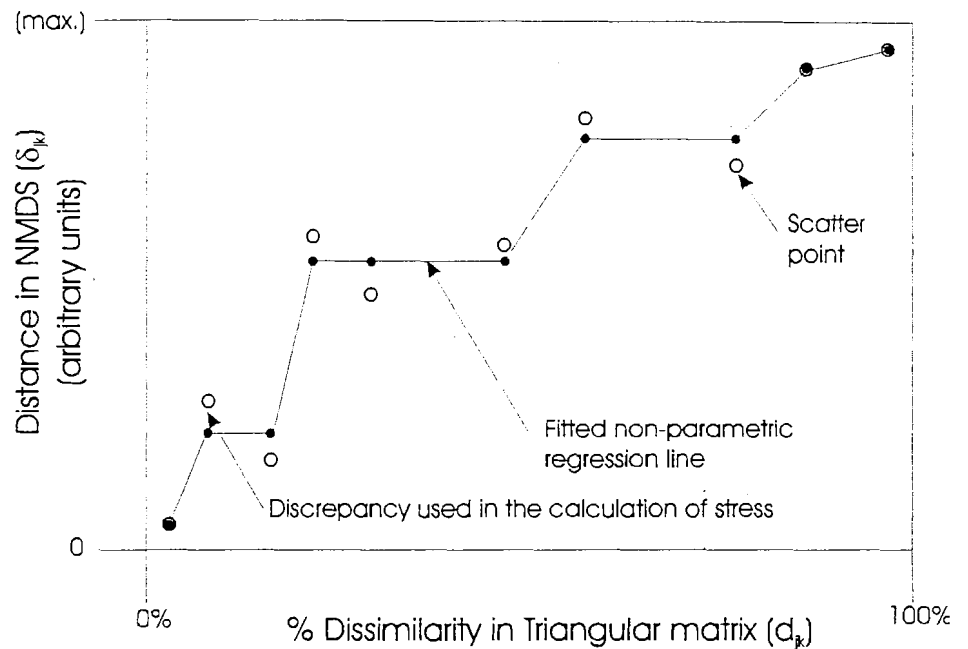
Figure 9. Shepard Diagram.

Other methods of evaluating an NMDS ordination include inspection of the final Shepard diagram and superimposing groups from cluster analysis on the NMDS plot.

Appendix B: Local benthic study

Some of the examples we have used to illustrate this review were taken from a local study on the benthic assemblages from three areas round the Maltese coast, subject to different degrees of anthropogenic impacts. This work was undertaken by a group from the Department of Biology of the University of Malta and was partly funded by the European Commission through its MedSPA Programme (Project leader: Prof. V. Axiak).

The three sites sampled, Ta' Xbiex (within Marsamxett Harbour), Zonqor Point (Marsascala Bay), and Mignuna Point (St.Thomas Bay) had similar exposures, geology and topography, but differed in the degree of pollution, mainly that associated with boating activities. At each site, SCUBA divers laid transects perpendicular to the shore. Samples were collected from the rocky bottom at stations between which the water depth increased by 0.5m, until sediment was encountered; in effect this amounted to 8-9 samples per transect. At each station, all biota within a 35cm x 35cm quadrat were picked out or scarped off. In the laboratory, the biota were sorted into taxa and identified as far as possible. Animals were counted, while for algae, dry weights were determined. The results of this study will be presented in a future publication.

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[19] An ordination that converges to a local minimum is considered to misinterpret the data.