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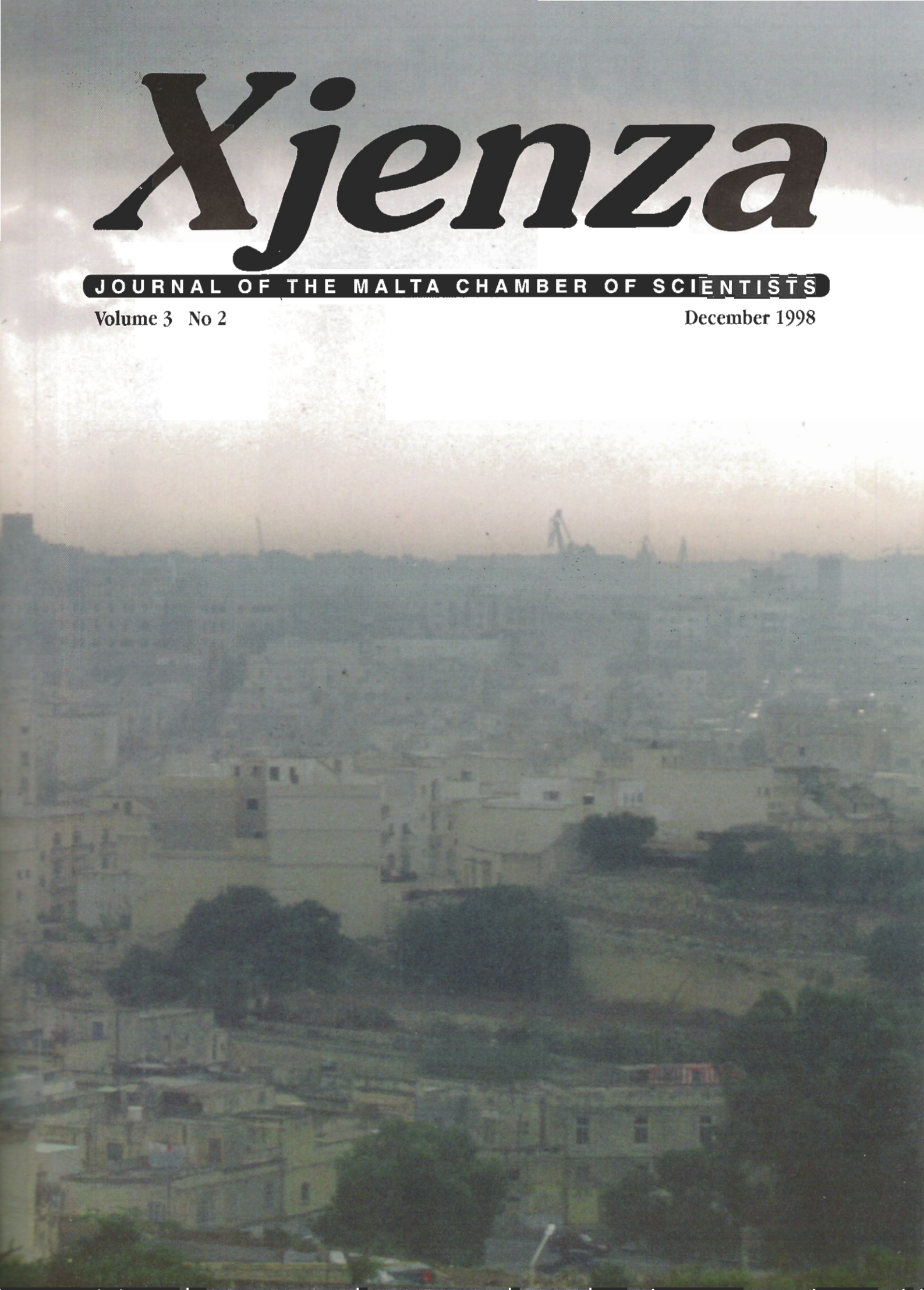

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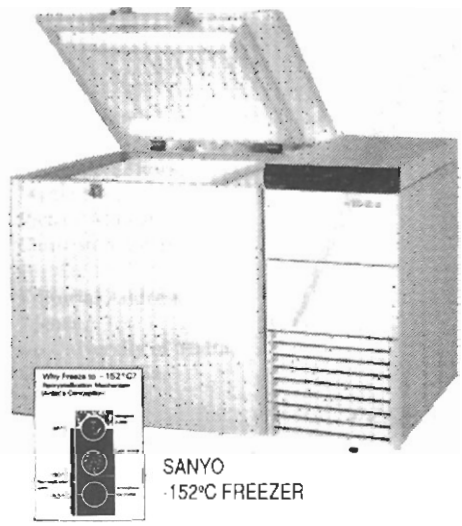

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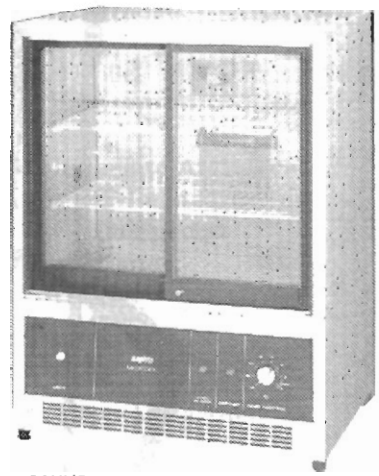

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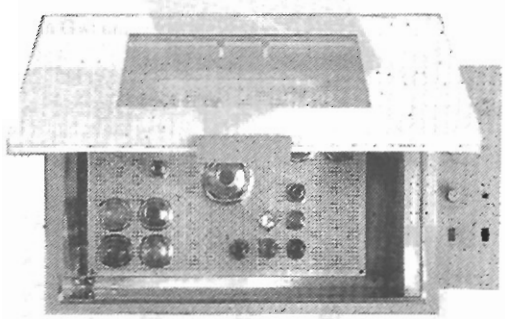

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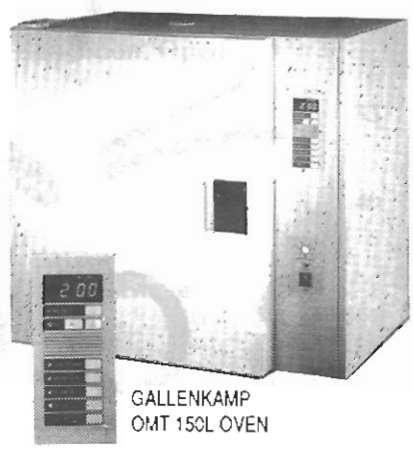

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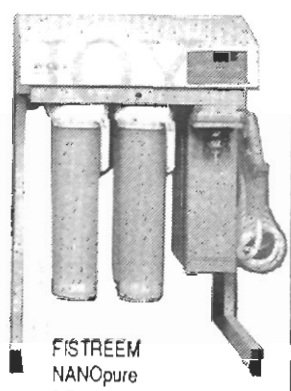

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The horizon over Marsa and Paola early in the morning, showing the brown haze of Nitrogen Oxides
(Photo: Dr. M.J. Ebejer)

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Editorial

Air Pollution: Whose Business Is It?

The subject of air pollution is warming up and there is a chance (soon, one hopes) that it may become one of the hot topics in the national media. And then, at last, politicians may begin to listen attentively and to act seriously where, for many years, they have been neglectful and passive.

In January 1997, during a postgraduate meeting for doctors, some of the environmental factors in the causation of lung disease were presented. The ensuing discussion served to confirm the impression that this area is replete with myth, misconception and a vacuum of knowledge even among many in the medical profession. In October 1998, Greenpeace submitted a report to Government outlining the health, social and economic problems of pollution from the burning of fossil fuels and suggested alternatives. In December 1998, several Local Councils followed the lead of Pieta Local Council and joined forces to organise a public seminar on air pollution with particular reference to that caused by the incinerator at St Luke‹s Hospital and the Marsa Power Station. There was much debate and discussion. The Minister for the Environment spoke well about what his office was actually doing about the problem and why it could not realistically be solved by the stroke of a pen, and why the whole issue of waste disposal was complex, and why it had to take into account the limitations of the Islands‹ demographic and geographic characteristics. The silence from the Ministers of Transport and Health was deafening! In January 1999, an article, by Dr Ray Ellul and Mr Michael Nolle, in the Sunday Times discussed some of the scientific issues involved in the study and monitoring of air pollution. A similar article, by the same authors, in this issue of Xjenza, outlines the scope of a forthcoming seminar on this topic.

The desire for economic growth is virtually synonymous with higher energy demands. Higher energy demands or production are the cause of air (and other) pollution. The contention is that air pollution is harmful. This is the crux of the matter. Is it harmful? When? To whom? How much? Is all ›smoke" - smoke? The scientific community understands much of what pollutes the atmosphere, it understands much of how it gets there and, how, at least in theory, it could be reduced or eliminated. The healthcare professions see the damage, and medical science is rapidly unravelling the mechanisms involved in the causation of this damage. However, debate continues on the extent of the risks of exposure and their time-related effects.

The Health Department has an efficient system of collating data on disease and mortality including that of the respiratory system, but in order to obtain meaningful indicators, the raw data, namely the diagnostic label put on the disease process, must be accurate and complete. In Malta, most likely this is not the case, particularly for those conditions caused by environmental factors. The medical profession has the obligation to educate itself on the subject, to obtain accurate data and to provide it to Government so that appropriate measures can be taken. These measures include the careful monitoring of relevant pollutants over a long enough time frame. The measures also include legislation (new if necessary) and enforcement of that same legislation. The persistence of turning a blind eye and a deaf ear simply will not do any more. For example, the emission of black smoke from diesel engines is totally out of control, yet legislation exists. Incentives to reduce pollution from vehicle exhaust, however, have been reversed by the recent Government Budget.

Few seem to have considered the effects of cumulative indirect exposure through the consumption of plant and animal food as well as through drinking water. The issues of health and pollution become more and more complex as attempts to understand them are made. But that is no excuse to ignore them. Clearly, there is a need for determining priorities and a need to set safe limits of exposure.

In the context of air pollution, it is important to note that whereas in the middle of this century it was black smoke, sulphur dioxide and other products of coal combustion that predominated in the pollution of the urban environment, today it is mainly vehicle exhaust (nitrogen oxides and particulates) which may be more relevant. Therefore, there is a need for more data (see Vella et al in this issue) and emphasis on different disease processes if realistic progress is to be made. Greater academic input from all scientific disciplines is required. For this to take place, Government, in turn, must appreciate that nothing can be achieved without adequate funding. Finally, if public opinion is to be moved it must first be informed with facts and not with misconceptions. Then, politicians too might make air pollution their business.

Martin J. Ebejer
Associate Editor

Communication

Atmospheric Pollution Background, Present and Future work

R Ellul and M Nolle
Department of Physics, University of Malta, Msida, Malta.

Introduction

The study of air pollution, its sources and its effects on human activity, as well as the transport and fate in the atmosphere of each component is today given considerable importance. Although two of the best known phenomena are global warming and ozone depletion, in fact these are only two facets of what is a very complex and incompletely known cycle.

An air pollutant may be any substance in the atmosphere which, at a high enough concentration, may be harmful to life or property. Such a pollutant may originate from natural or anthropogenic sources or both. Sources are many and varied. They include cars, smoke stacks and other industrial emissions as well as natural dust. Atmospheric conditions have a major effect upon the pollutants. Wind, temperature distribution, turbulence, depth of emission point, as well as the stability of the atmospheric conditions all play a part in the dispersal of pollutants.

Most commonly, pollutants harm life through their effects on the respiratory system. Vegetation may also be adversely affected. Buildings as well as metallic surfaces can easily be eroded by long term accumulation and concentration of various pollutants on their surfaces.

Description

Most of the air we breathe is composed of nitrogen and oxygen. About one percent is composed of naturally occurring constituents such as carbon dioxide and water vapour. A small part of this, however, is composed of pollutants including gases and particulate matter (suspended aerosols composed of solids and liquids).

Anthropogenic air pollution comes from both fixed and mobile sources. Mobile sources account for around 50% of such pollution. Natural sources include dust from farm fields and desert, smoke from forest fires and volcanic ash emitted into the troposphere and stratosphere. One can classify pollutants according to two broad categories: primary and secondary air pollutants.

Primary air pollutants enter the atmosphere directly from various sources and the five main ones are: carbon monoxide, hydrocarbons, particulate matter, sulphur dioxide and nitrogen oxides. Carbon monoxide which is a major pollutant in urban air is a product of incomplete combustion of fossil fuels. Apart from cigarette smoke the internal combustion engine is the principal source. This gas is extremely poisonous. The WHO specifies an upper limit of 10 ppb over an 8 hour average for human beings.Although stable it is relatively short lived because it is quickly oxidized to carbon dioxide by hydroxyl radicals.

Hydrocarbons (HCs) or volatile organic compounds (VOCs) are those composed of hydrogen and carbon. Methane is the most abundant and is an active greenhouse gas. Other volatile organics such as benzene and derivatives are highly carcinogenic. The major sources of all types of hydrocarbons are the natural decomposition of organic matter and the evaporation of gasoline from vehicles.

Particulate matter comprises solid particles or liquid droplets small enough to remain suspended in air. Such particles may be of a very complex chemical composition and include soot, smoke, dust, asbestos fibre and pesticide as well as metals (Hg, Fe, Cu, Pb). These particles are characterized by size. Particles larger than 10 microns settle out in less than a day while particles smaller than one micron remain suspended for weeks. High concentrations of particles containing sulphur and silica from volcanoes and deserts often reach the stratosphere and cause cooling of the earth's surface. Tropospheric particles cause human respiratory illness. Of particular interest are particles from diesel vehicles where these are strongly impregnated by highly carcinogenic aromatic compounds. This may be one of the important mechanisms giving rise to lung cancer in urban areas.

Most sulphur dioxide emissions come from the burning of fossil fuels containing sulphur. They cause acute respiratory problems and are an important source of acid rain. Most man-made emissions can be avoided by the use of low sulphur fuels and scrubbers.

Nitrogen oxides (NO_x) are formed mainly from nitrogen and oxygen during high temperature combustion of fuel in cars. This is another contributor to acid rain. The use of catalytic convertors in vehicles removes most of these emissions. It is thought that NO_x's contribute to heart and lung problems and may also be carcinogenic.

The second category of air pollutants comes from the chemical reactions between the above primary pollutants and other atmospheric constituents such as water vapour. These normally require the availability of sunlight. The result is photochemical smog. Smog is mainly composed of ozone (O_3), peroxyacetylnitrate (PAN) and other oxidants. Ozone formation is closely linked to

weather conditions namely, high temperatures, low winds, intense radiation and low precipitation. All these conditions are prevalent in the Mediterranean and favour smog production, which averages 40 ppb as opposed to continental Europe at 15 ppb. HC's are necessary for the buildup of ozone in the atmosphere. In the absence of HC's the reaction cycle would not be interrupted and ozone would not accumulate except from natural sources and stratospheric mixing.

Ozone is naturally present in the stratosphere and its absorption of UV acts as a natural shield against high UV levels reaching the earth's surface. It is hazardous as an oxidant in smog where it contributes to inflammation in the lungs and increases the incidence of acute episodes of asthma and reduces heart and circulatory functions. It is also poisonous to plant life. Factors that encourage the formation of smog include: numerous sources of primary pollutants, inversions that inhibit turbulent mixing of air, little cloud cover permitting high UV intensity, light winds unable to disperse pollutants and terrain that allows accumulation of pollutants.

Ozone and NO_x pollution in the troposphere is widespread and is not confined merely to urban areas. It is possible that emissions from soil contribute substantially to NO_x pollution. This tropospheric NO_x concentration influences the concentration of oxidants such as OH and O_3.

Local Experimental Work

Many questions remain unanswered concerning the reasons why ozone concentrations continue to be high in both urban and rural areas and work at the University of Malta is part of a European incentive aimed at tracking the dispersion of such pollutants across Europe.

We have at present installed three data gathering points: at Giordan lighthouse in Gozo, at the University building in Xewkija, Gozo, and at the main University campus at Msida. At all three locations ozone values are recorded every ten seconds and later averaged to half hourly values. Recently, recordings of carbon monoxide also began at Giordan lighthouse. Meteorological parameters such as wind speed and direction, temperature and humidity and daily weather maps are also recorded to enable interpretation of the results at a later stage. These data are being used in climate model predictions. The base stations set up so far are capable of being expanded to monitor far more pollutants than at present and may also be used to study the dispersion of local emissions, which will thus help to suggest ways of minimizing them. This work is coordinated closely with that of the Department of the Environment. One of the major questions which needs to be addressed is that involving particulate pollution from diesel vehicles. This programme is expected to start as soon as the necessary equipment arrives.

It is perhaps also worth stating that the data at Giordan lighthouse show unexpected night-time ozone 'occurrences' which can only be explained by stratospheric mixing due to the land effect. It is planned to study this further by raising a tethered meteorological balloon to a height of one kilometre with instruments suspended below to record the vertical profile over the boundary layer. Such work has not yet been performed in the Central Mediterranean.

Major Air Pollution Issues

Global Warming

Carbon dioxide is an important greenhouse gas. It is present naturally, but it is also a major product of fossil fuel burning.

Numerical climate model predictions indicate that increased warming by greenhouse gases may add about one degree Celsius to the global mean temperature by the year 2025 and three degrees within a hundred years. Although there is much uncertainty in the exact values quoted, warming appears inevitable with major long term consequences for life on Earth. Carbon dioxide is the most abundant greenhouse gas, but additional greenhouse gases are methane, nitrous oxide, CFC's (now being phased out) and ozone. All of these have been increasing substantially over the last few decades and the total contribution to global warming has been the subject of much detailed discussion. Some of the main questions which need to be resolved are: the effect on vegetation and plant growth rates, the rate of warming, the effect of this rate of warming on ocean levels, the effect of warming on cloud cover and aerosols, the change in rainfall patterns, the magnitude and distribution of temperature changes, the possible reduction of greenhouse gas emissions within the context of sustainable economic development, and the absolute accuracy of the numerical models, given that these are extremely complex.

Ozone Depletion

The depletion of ozone in the stratosphere as opposed to the troposphere is another global environmental concern related to pollution. Stratospheric ozone absorbs ultraviolet light and thus performs an essential function in reducing this to a level safe for life on earth. Removal of this stratospheric layer of ozone would have serious consequences for many forms of life on the planet. In the last decade major reductions of this ozone concentration have been observed and it is now fairly clear that this is due to man-made CFC's and NO_x's.

Conclusion

The subject of atmospheric pollution and its long and short term effects is an extremely complex one. This article has presented an oversimplified view in order to tempt more of the local scientific community to take an active interest in the subject and to participate in the forthcoming seminar on the subject on the 9th April 1999. The keynote speaker is Professor Paul Crutzen a Nobel Prize Laureate in atmospheric chemistry. The programmme is presented below. All those interested are encouraged to attend.

Atmospheric Pollution Seminar Program

Friday 9th. April 1999. L-Imgarr Hotel, Gozo

Morning Session Chairman: Dr R Ellul

9.00 AM	Opening,	Hon Dr Francis Zammit Dimech,	Minister for the Environment
		Hon Mrs Giovanna Debono,	Minister for Gozo
		Prof R Ellul Micallef,	Rector
		Dr Gerhard Kunz	Ambassador, FRG

9.20 AM	Prof. P Crutzen,	MPI Mainz,	'Ozone in Atmospheric Chemistry'
10.00 AM	Dr H Gusten,	IMK Karlsruhe,	'Urban Air Pollution'
10.30 AM	---------- Coffee break ---------- 10.45 AM		
10.45 AM	Mr M Nolle,	Univ. of Malta	'Background Measurements'
11.05 AM	Dr A Micallef,	Univ. of Malta	'Urban Hot-Spots Particle Modeling'
11.25 AM	Dr D Soderman	Eriee/FIS	'Modeling of Saharan Dust Transport'
11.45 AM	Dr A Vella,	Univ. of Malta	'Particulate Air Pollution and certain Health Problems in Malta'
12.05 PM	Mr D Bugeja	EPD	'Air Pollution Programmes'
12.25 PM	----------Lunch ---------- 2.00 PM		

Afternoon Session Chairman: Dr C Sammut

2.00 PM	Prof A Vella,	Univ. of Malta	'Review of Studies on Pollution in Urban Malta'
2.20 PM	Mr M Spiteri,	EMDP	'Non Spatial Environmental Planning'
2.40 PM	Dr L Korugic,	Univ. of Malta	'Environmental Effects of Plastics and Rubbers'
3.00 PM	Mr J Sacco	Univ. of Malta	'Relationship between Atmospheric and Blood Lead Concentrations'
3.20 PM	Ms M Attard,	Univ. of Malta	'Role of Transport in Urban Environments'
3.45 PM	---------- Coffee Break ---------- 4.00 PM		
4.00 PM	Dr A Micallef,	Univ. of Malta	'FUELFAC for Mapping Fuel Consumption on Roads'
4.30 PM	Prof E Mallia,	Univ. of Malta	'Alternative Electric Vehicles'

5.00 PM Discussion and Closing, Chairman: Dr R Ellul

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.

Keywords: multivariate analysis, diversity, similarity, ordination, classification, benthos, biotic communities, Maltese Islands.

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.

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 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.

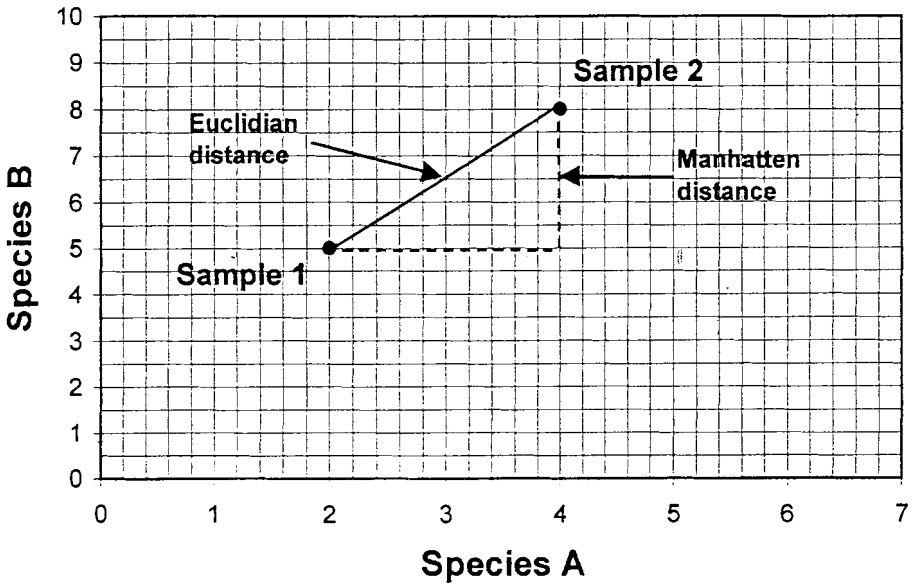

Figure 1. The multidimensionality of data sets in community ecology.

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.

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).

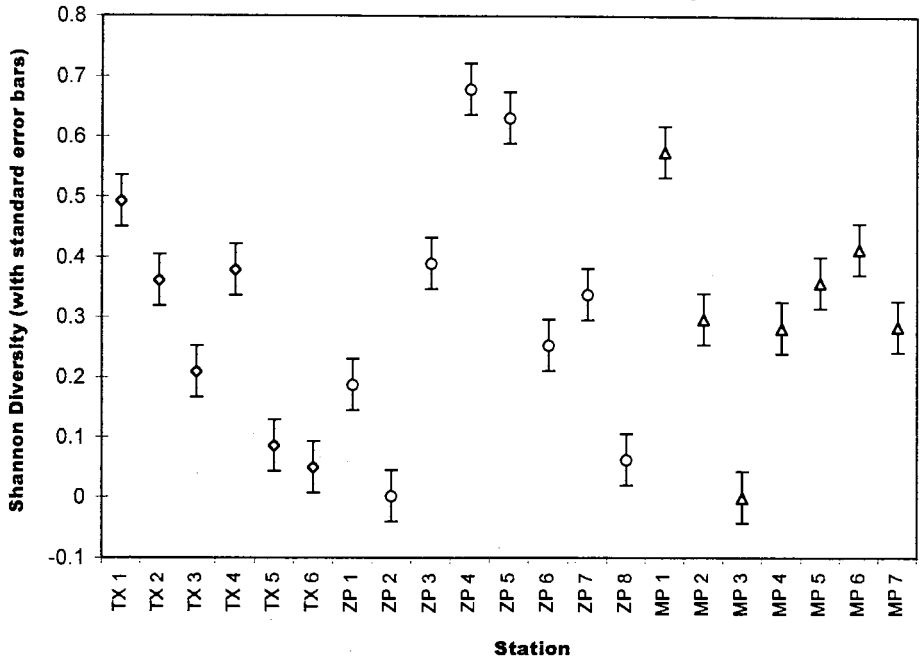

Figure 2. Univariate measures: Shannon-Wiener diversity with standard error bars for a local benthic data set (see Micallef, 1997 and Appendix B). TX (diamonds) = Ta' Xbiex site; ZP (circles) = Zonqor Point site; MP (triangles) = Mignuna Point site. For each site, the stations are arranged on the x-axis 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.

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

[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)

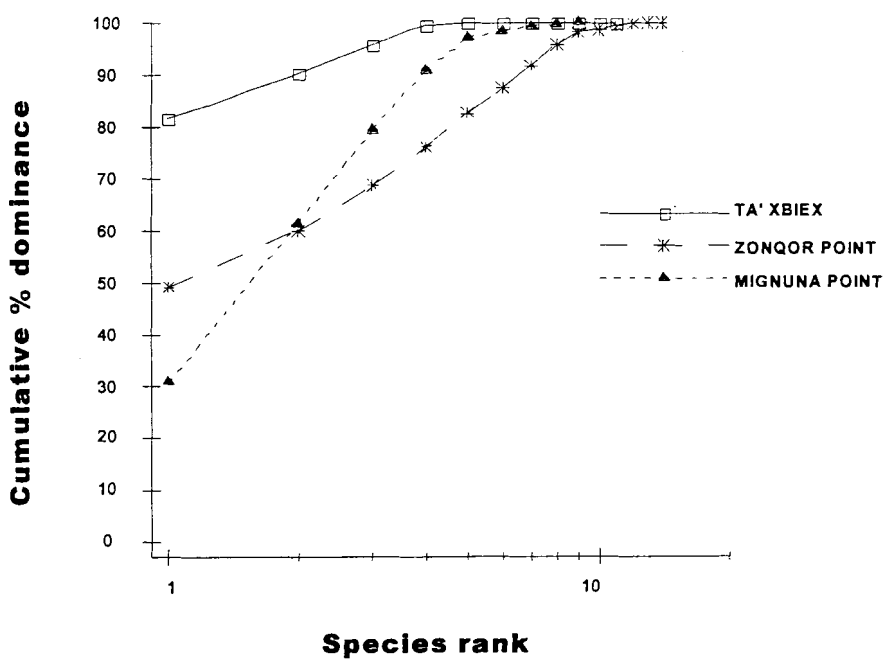

Figure 3. *K*-dominance plot for the algal taxocene from a local benthic study (see Micallef, 1997 and Appendix B). Note that Ta' Xbiex, the most polluted site, had the lowest diversity.

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.

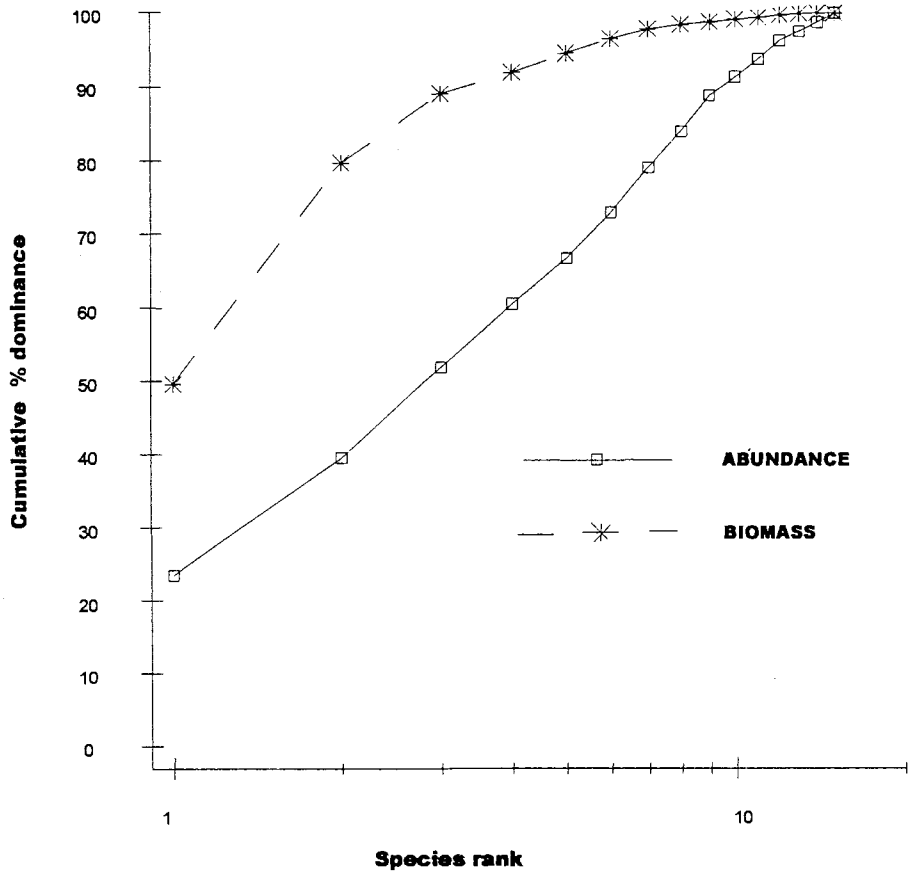

Figure 4. Abundance-biomass (ABC) curves for the molluscan taxocene from a sample collected using a hand-net (60 strokes covering an area of ca.20m^2) from a *Posidionia oceanica* meadow at a depth of 16m off the White Tower headland, Malta. (Hassan Howege, unpublished). This plot is typical of a non-polluted, non-disturbed system.

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 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:

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

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).

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].

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

[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/m^2 - 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.

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 $\lambda \rightarrow 0$. 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	$S_{bc} = \frac{\sum_i \lvert y_{ij} - y_{ik} \rvert}{\sum_i (y_{ij} + y_{ik})}$	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	$S_e = \sqrt{\sum_i (y_{ij} - y_{ik})^2}$	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	$S_{ch} = \sqrt{2 \left(1 - \left\{ \sum_i (y_{ij} y_{ik}) \Big/ \sqrt{\sum_i y_{ij}^2 \sum_i y_{ik}^2} \right\} \right)}$	3, 5
Kulczynski Distance	$S_k = 1 - \frac{1}{2} \left[\frac{\sum_i \min(y_{ij}, y_{ik})}{\sum_i y_{ij}} + \frac{\sum_i \min(y_{ij}, y_{ik})}{\sum_i y_{ik}} \right]$	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

[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.

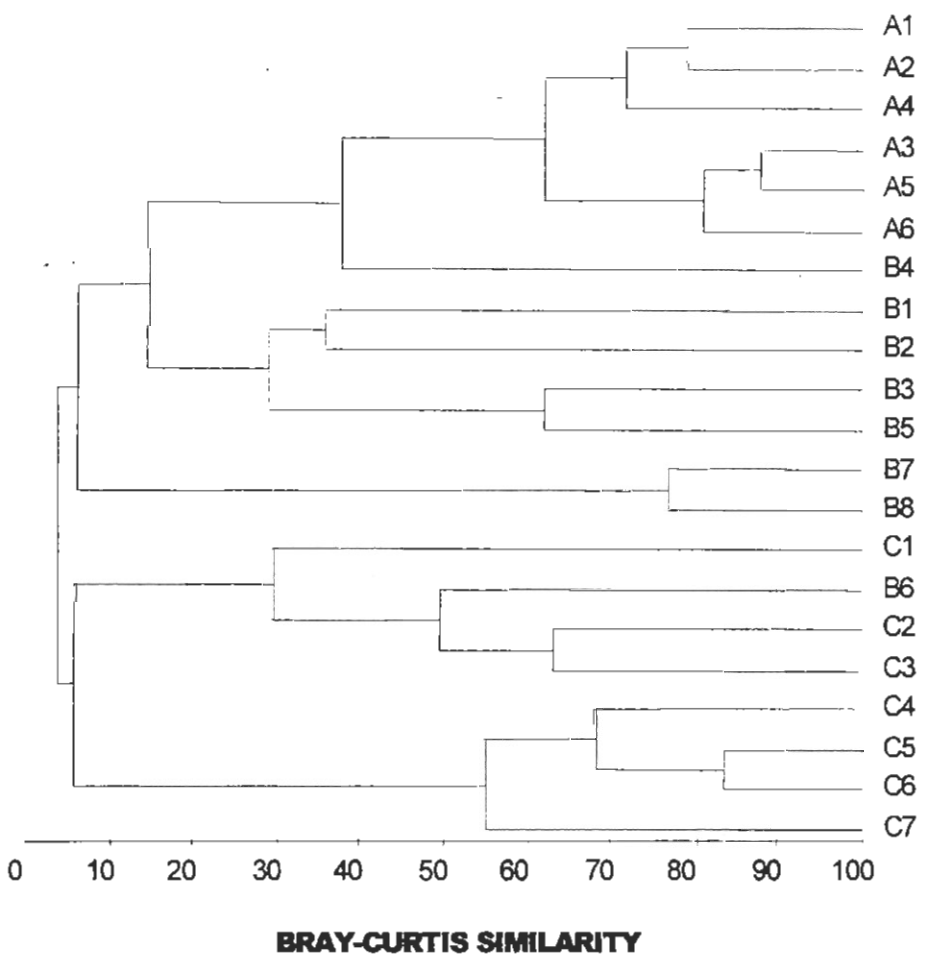

Figure 5. Dendrogram showing hierarchical classification of samples from a local benthic study (see Micallef, 1997 and Appendix B) based on the similarity of the algal taxocene aggregated to genus level. Note that samples labelled C (Mignuna Point) are separated from practically all the other samples 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%.

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).

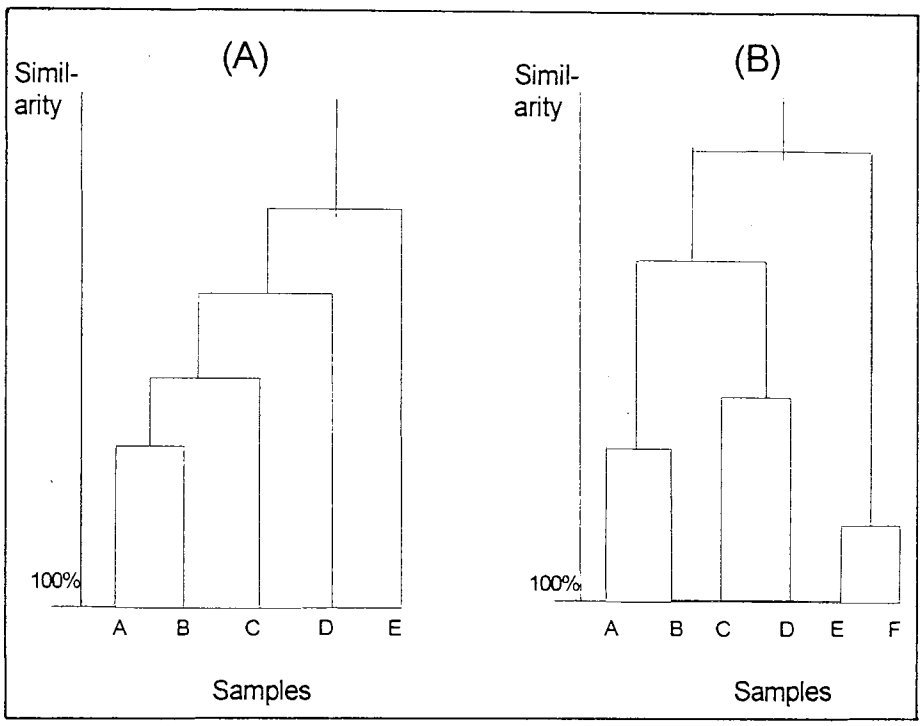

Figure 6. Typical results obtained using (A) single linkage, and (B) complete linkage, in polythetic hierarchical agglomerative classification of five samples.

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).

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

[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

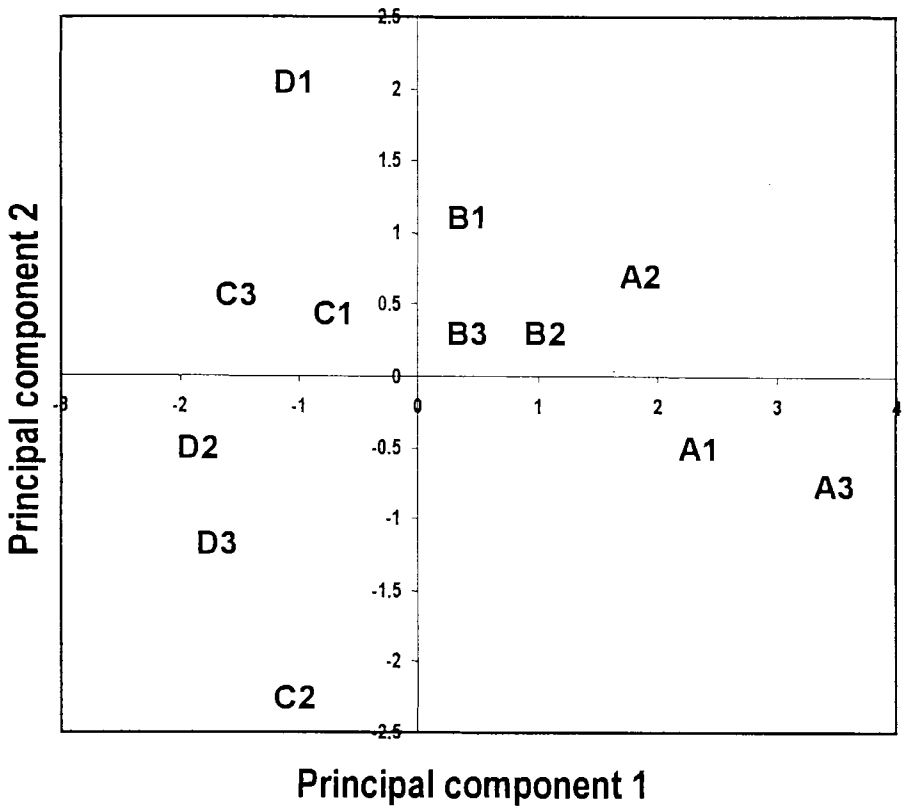

Figure 7 Two-dimensional PCA ordination of the abundance of decapod crustaceans (double square root transformed) in 12 suction samples collected from a local *Posidonia oceanica* meadow. Samples were collected from 6m (A), 11m (B), 16m (C) and 21m (D). The numeral indicates the collection period: 1 - 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.

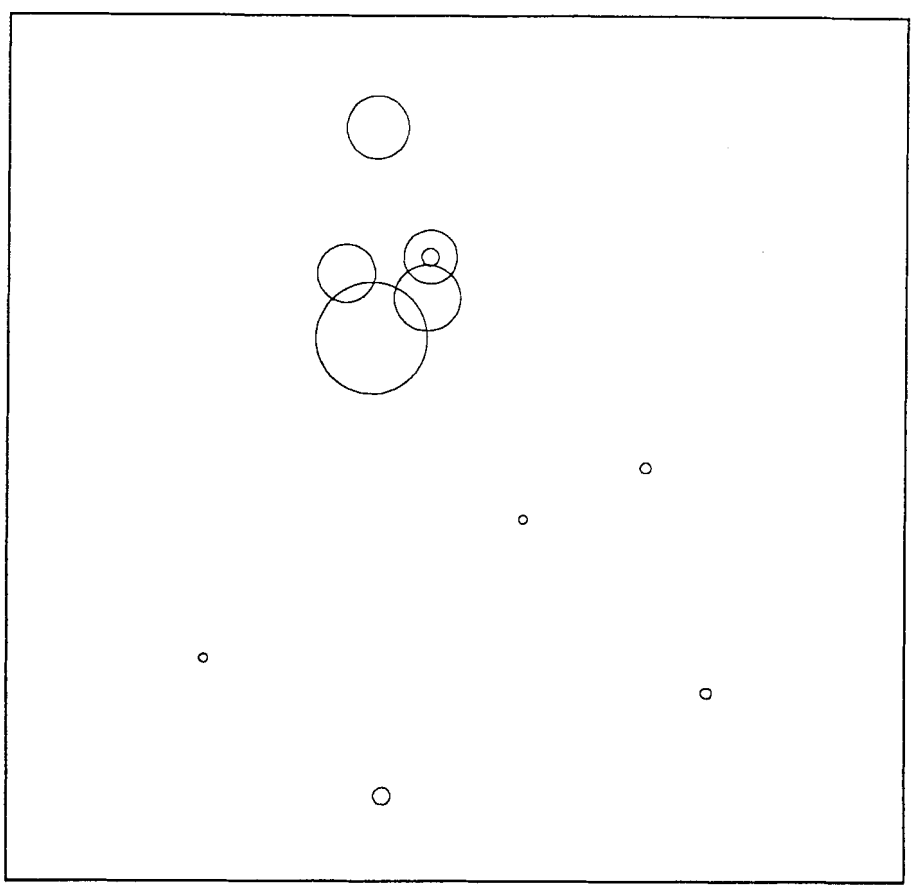

Figure 8 NMDS plot for the molluscan taxocene from a local *Cymodocea nodosa* meadow, based on abundance, after truncation at the 3% level, double square root transformation and using the Kulczynski similarity measure. Stress = 0.12. The radius of the circles is proportional to the *Cymodocea* shoot 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).

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

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):

[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.

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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.

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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

[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).

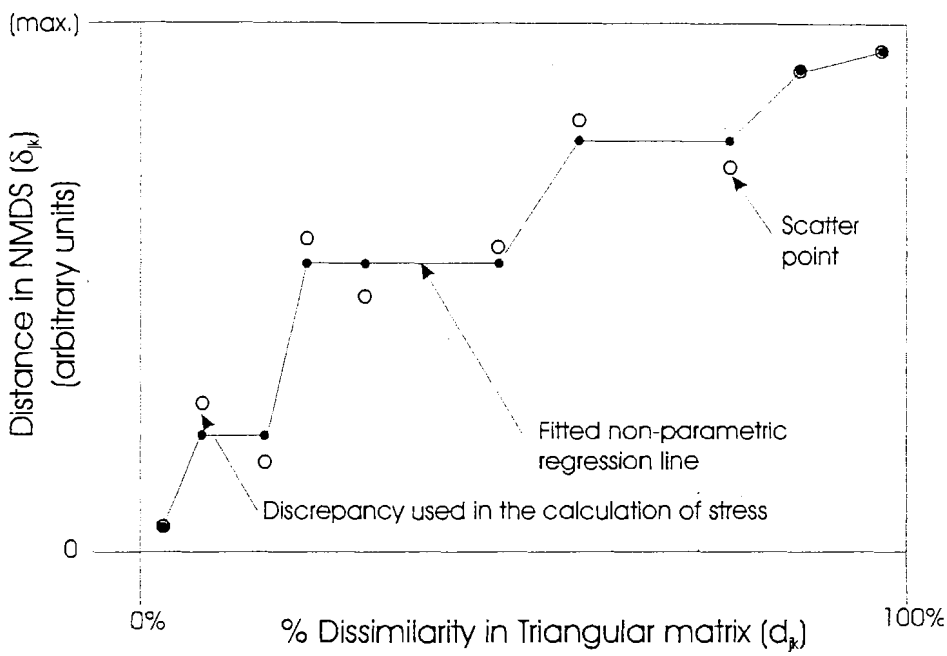

Figure 9. Shepard Diagram.

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.

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.

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

Research Article

Benzene and Toluene in Urban Air in Malta

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Summary: *This is the first published report on the presence of benzene and toluene in street air in Malta. The data refers to one hour average concentrations obtained from active samplers positioned at 1.5 m height in urban, suburban and rural sites and in a road tunnel. Two sampling time periods were chosen, one just before and the other after the morning rush hour traffic. Both benzene, a known carcinogen, and toluene are found to be present in higher concentrations in street air from urban sites than from suburban sites and are not detectable in rural areas unless these sites are visited by motor traffic as on weekends. In general, concentrations of these hydrocarbons increase after the passage of morning traffic. Benzene concentrations ranged from 7 to 84 μg m^{-3} and toluene from 26 to 306 μg m^{-3}. It is suggested that the air quality in Malta with respect to the presence of benzene and toluene is probably poor for sites where automotive traffic is prevalent but is generally good for rural areas. Air quality can be improved with the phase-out of leaded petrol which will allow the introduction of catalytic converters in petrol-powered vehicles.*

Keywords: benzene, toluene, air pollution, Malta.

Introduction

Although there is much public concern regarding the quality of air in Malta, very little data on air quality have been published and the work has dealt with pollution from inorganic species, principally sulphur dioxide and combustion-derived particles (Vella et al 1993; Vella *et al* 1996) and airborne lead (Sammut and Savona-Ventura, 1996).

This is the first published report on the presence of volatile organic compounds (VOCs) in urban air in Malta and concerns the aromatic hydrocarbons benzene and toluene. It is based on a pilot study performed by one of the authors (OG) as a dissertation project conducted during 1996-7 (Gaerty, 1997).

VOCs are released in vehicle exhaust gases either as unburned fuels or as combustion products and are also emitted by the evaporation of motor fuels and solvents. Benzene and toluene are constituents of leaded and unleaded petrol and toluene is also employed as a solvent ("thinner") in automotive and other paints. Benzene is of particular concern because it is a human carcinogen. No EC or WHO guideline value currently exists for benzene concentration in air although the UK government is considering adopting a value of 5 parts per billion (ppb) by volume (16.6 μg m^{-3}) as an air quality objective with a target value of 1 ppb. Chronic low-level exposure to benzene has been shown to cause bone marrow depression and leukaemia; benzene also depresses the immune system; exposure to levels of 10 parts per million (ppm) or less for one year has been found to cause a 560-fold increase in cancer risk (US EPA, 1994). Long term exposure to toluene at ppm levels causes kidney, liver and respiratory tract damage but there is insufficient evidence to classify toluene as a human carcinogen.

In addition to the health risks posed by these hydrocarbons when present as primary air pollutants, they also contribute towards the formation of secondary air pollution including the photochemical generation of ozone, a process which is favoured by abundant sunlight.

Materials and Methods

The analytical method involved passing a known volume of air through a charcoal sorbent tube, eluting the analyte with carbon disulphide and analysing the eluate by capillary column gas chromatography using flame ionization detection.

Air was sampled using a battery operated SKC Universal Sampling Pump operated at a constant flow rate of 200 mL min-1: the pump was equipped with a low-flow adaptor (from SKC) which enabled accurate calibration in the low flow rate (< 500 mL min^{-1}) region. The flow rate was determined using a soap bubble calibrator supplied by the pump manufacturer.

Air sampling sites were chosen to represent urban, suburban and rural environments as well as a road tunnel. The locations are shown in Table 1.

Sampling was performed at approximately 1.5 m height and the sample tube was kept in a vertical position to minimise channelling (Lodge, 1988). Once the pump was switched off, the sorbent tube was removed from the sampling train, its open ends were sealed using plastic caps, placed in stoppered glass tubes and refrigerated pending analysis. The air temperature was recorded and wind speed was obtained from the Meteorological Office.

Commercially available charcoal sorbent tubes (SKC, ST226-01) containing two sections of 20/40 mesh charcoal (100 mg front section and 50 mg backup) were employed. Carbon disulphide was used as solvent to elute the sorbed hydrocarbons. The purity of several commercially-available brands of carbon disulphide with respect to benzene was found to be inadequate for this work and the solvent required purification prior to use. This consisted of heating the solvent (BDH, Analar Grade, 500 mL) with a mixture of concentrated sulphuric acid (BDH, GPR Grade; 10 mL) and concentrated nitric

Table 1. Benzene and Toluene concentrations in urban air in Malta.

Location	Location type & site number	Date & Time	Wind speed (knots) & direction	[Benzene] $\mu g\ m^{-3}$	[Toluene] $\mu g\ m^{-3}$
St Anne St., Floriana	urban, 1	25/3/97 5:50-6:50	10-15; WNW	72	132
"	"	25/3/97 7:45-8:45	15-20; WNW	63	79
Independence Ave., Mosta	urban, 2	9/4/97 5:45-6:45	15-20 E	24	29
"	"	9/4/97 7:52-8:52	18-25 E	29	63
Bisazza St., Sliema	urban, 3	10/4/97 6:00-6:50	14-20 NE	74	41
"	"	10/4/97 7:45-9:00	15-22 ENE	44	107
Birkirkara Rd., San Gwann	urban, 4	11/4/97 6:00-6:50	5-10 NE	80	170
"	"	11/4/97 7:45-8:35	10-15 NE	84	306
Naxxar Rd., Lija	urban, 5	14/4/97 5:58-6:58	4 SW	20	28
"	"	14/4/97 7:45-8:45	4-10 NE	43	79
Burmarrad Rd., Burmarrad	sub-urban 6	16/4/97 6:05-6:55	8-13 WNW	nd(*)	nd
"	"	16/4/97 7:45-8:45	8-15 NW	18	29
Saqqajja Hill, Rabat	urban, 7	17/4/97 6:23-7:03	5-10 SSW	7	26
"	"	17/4/97 7:30-8:30	5-10 S	58	122
Tan-Nadur, l/o Rabat	rural 8	18/4/97 5:30-6:40	20-30 NE	nd	nd
Howard Gardens Rabat	sub-urban 9	24/4/97 9:10-10:00	9-15 NW	31	38
"	"	24/4/97 17:10-18:00	8-12 N	nd	nd
National park, Ta' Qali	rural 10	27/4/97 9:10-10:00	10-15 WSW	13	nd
"	"	27/4/97 17:10-18:00	12-18 SW	12	nd
"	"	28/4/97 9:10-10:00	10-15 SW	nd	nd
"	"	28/4/97 17:10-18:00	10-15 WSW	nd	nd
Qammieh, Mellieha	rural 11	1/5/97 9:00-10:00	7-10 NE	nd	nd
"	"	1/5/97 16:30-17:30	3-8 SW	nd	nd
Regional Rd., St. Venera	Tunnel 12	30/4/97 5:50-6:20	20-30 WNW	507	nd
"	"	30/4/97 7:45-8:15	20-30 WNW	197	405

(*) nd = not detected

acid (BDH, GPR grade; 5 drops) under reflux for 3 hours with magnetic stirring. The solvent was then separated, washed and dried over sodium sulphate and re-distilled. The purity of the solvent was checked by blowing down under nitrogen a 1 mL portion to 50 (L and analysing the product by gas chromatography to check for the absence of a benzene peak.

Desorption of the hydrocarbons from charcoal was achieved using 0.50 mL purified carbon disulphide which had been chilled to about -5°C prior to use and the solution was spiked with internal standard (10 ppm isopropyl-benzene); even the charged sorbent was chilled before extraction with cold solvent to avoid loss of analyte. The desorption efficiency for benzene and toluene was determined by spiking the sorbent with standard amounts of analytes and using isopropylbenzene as internal standard. For benzene, the desorption efficiency was 87% while that for toluene was 82%.

A Perkin Elmer Model 8600 gas chromatograph fitted with a BP1 fused silica bonded phase capillary column (SGE) was employed; a Finnigan Matt ion-trap detector was used for mass spectrometric confirmation of chromatographic peaks. Analysis was performed within 48 hours of collection, each section of sorbent tube being analysed separately and in triplicate. Gas chromatographic conditions were as follows: 47°C for 0.3 minutes then to 55°C at 10°C min^{-1}, hold for 3 minutes, then to 300°C at 30°C min^{-1}; injector temperature 180°C; detector temperature 185°C, carrier gas (He) head pressure 10 psig; injection volume 3 µL, splitter ratio: 40. The instrumental detection limit for benzene and toluene in carbon disulphide solution was 1ppm.

Results and Discussion

The results are shown in Table 1. The values represent one-hour averages and the hydrocarbon concentrations in air clearly reflect the traffic density associated with the study area. Thus, for urban sites, benzene and toluene concentrations were generally higher than for sub-urban sites and are much higher than for rural sites, at which sites, concentrations are frequently below detection limits. In general,

both benzene and toluene were found to be more abundant in air during the sampling time corresponding to the morning traffic rush hour than earlier in the morning. Thus the post-7:30 am samples typically yielded higher concentrations of both hydrocarbons, but especially of toluene.

The benzene air concentration in urban sites found in the early morning measurements, which are presumably indicative of "background" levels, appear to be rather high in places. The values ranged from lower or very close to the UK environmental quality guideline concentration (e.g. sites 2, 5 ,6 and 7) to values as high as four times this guideline (sites 1, 3, 4). Just after the morning rush hour, at most of the sites, benzene concentrations increase and in places become four to five times higher than the guideline value.

Studies in Austria in 1995 showed that ambient levels of benzene in urban areas ranged from 5 to 28 μg m^{-3} while for rural sites the values ranged from 2 to 5 μg m^{-3} (Hanus-Ilnar and Hrabcik, 1995). These values, however, are not readily comparable to the Malta data because they refer to 14-day means obtained from passive samplers. Monitoring studies carried out in 1994 in North America (Montreal, Toronto and California) revealed a mean benzene concentration in air equal to about 4 μg m^{-3} (Wallace, 1996) although again this value represents a 24-hour average rather than a 1-hour average.

In air pollution studies, concentrations of toluene usually correlate with those of benzene and this was found to be generally true for Malta air. Toluene is much more abundant in petrol than benzene as the gas chromatograms in Figure 1 for locally imported leaded and unleaded petrols demonstrate. However, the ambient air concentrations of these two hydrocarbons will be expected to be only partly determined by the abundance ratio in fuels; other factors which are expected to play a role include (a) the relative rate of oxidation of the two compounds in air and (b) the fate of these hydrocarbons during combustion in internal combustion engines. Vehicle exhaust gas contains benzene both as an unburned fuel but also as the dealkylation product of substituted aromatic hydrocarbons. In exhaust gas from unleaded petrol-powered internal combustion engines, the mass abundance ratio of toluene/benzene is approximately 2 (Butler, 1979). The mean abundance ratio in Malta air is 1.9 which is similar to 1.8 reported for Swedish air (Gustavsson, 1996). This strongly suggests that the presence of these hydrocarbons in air is indeed mainly derived from pollution by petrol-powered engines.

It is interesting to note that at the National Park in Ta' Qali, benzene was detected in air on 27/4/97, which was a Sunday, but not on the next day. Benzene in air at this site is unlikely to have originated from traffic travelling on roads upwind from the study site: this is because, on both sampling days, wind direction and speed were similar and if the benzene detected on Sunday represented transported pollution from these roads, such pollution would probably have been present on Monday as well since this is a busier day for traffic. Rather, benzene air pollution at this site more likely reflects the influx of motor traffic into the picnic and leisure area of the park during the weekend break.

For site 1 in Floriana, the increase in abundance of both benzene and toluene in air after the morning rush hour did not occur but a reduction in values was actually measured. This result is possibly an analytical artifact, although it may also be related to the presence of a

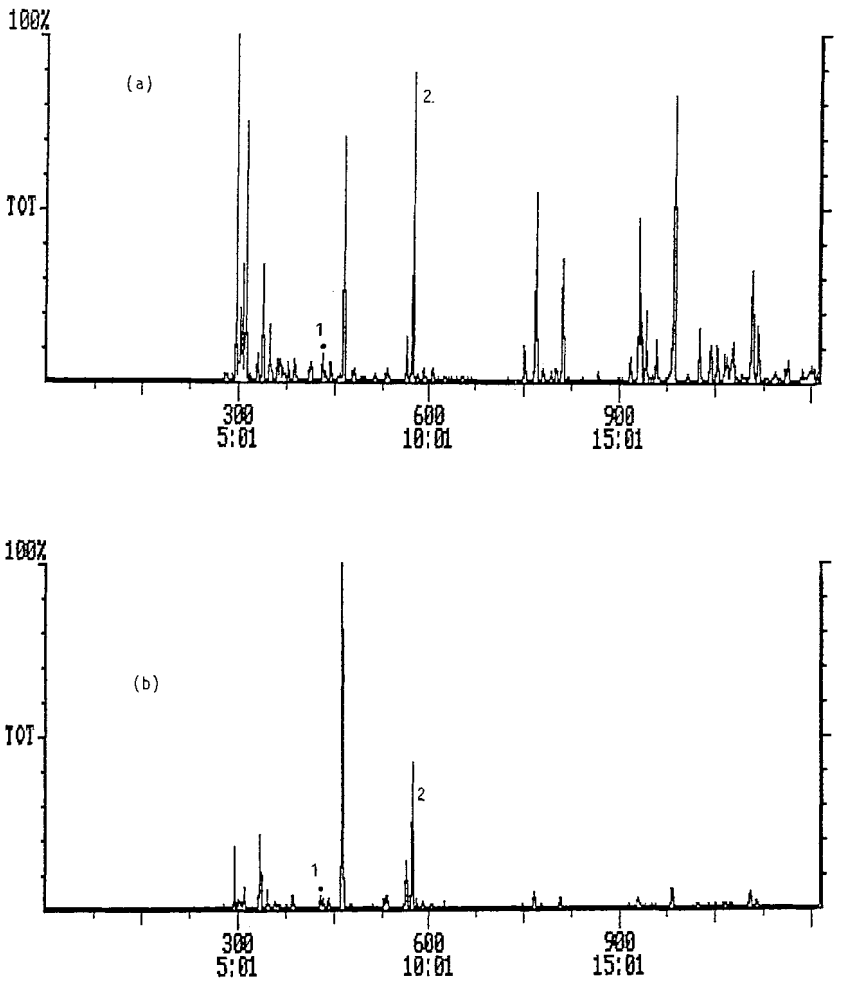

Figure 1. Gas chromatograms for leaded (a) and unleaded (b) petrol as imported in Malta. Peak 1 = benzene; peak 2 = toluene. Chromatographic conditions: analytical column: BP1, 50 m, narrow bore ; temperature programme: isothermal at 100°C for 5 minutes, ramp at 10°C min-1 to 275°C.

roadside service station sited about 300 m from the sampling site which may have received a fuel consignment during the early morning. Such deliveries are expected to introduce into street air a considerable quantity of petrol vapour from the displacement of head space air from underground reservoir tanks. The effect of roadside service stations on air quality is currently being investigated in our laboratory.

Finally, the situation obtaining inside the road tunnel in site 12 deserves comment. The very high concentration of benzene (507 μg m^{-3}) measured in the tunnel prior to the arrival of heavy morning traffic is noteworthy; the absence of toluene at this time is possibly the result of efficient degradation processes occurring by dark reactions (e.g. with nitrate radicals). The reduced benzene concentration measured during the period 7:45 to 8:15 after the passage of morning rush traffic could possibly result from ventilation effects caused by traffic traversing the tunnel. It is interesting to note that the toluene/benzene mass concentration ratio found at this time (= 2.1) is very close to the value in fresh car exhaust gas.

According to the Material Safety Data Sheet (Penn State Univ.) for benzene, the occupational threshold limit value is 3 mg m^{-3} and this is rather close to the concentration values measured in the tunnel. Clearly, applying the precautionary principle, it would appear sensible to consider protective measures for workers involved with repairs inside the tunnel.

Conclusions

The results of this pilot study suggest that the quality of Malta air with respect to benzene and toluene is probably poor for those areas where automotive traffic is very prevalent but appears to be generally good for rural sites. These are preliminary conclusions and require confirmation by additional work on a larger sample size. Also, for purposes of comparison with data from other countries, longer time period sampling using passive techniques is required. However, it is felt that the data as it stands does confirm the view that these hydrocarbons in Malta air do originate principally from petrol-powered vehicular exhaust. It is therefore clear that the air quality situation will continue to worsen if the current rate of increase of vehicular traffic on Malta's roads persists in future. In view of the toxicity of the hydrocarbons, and especially that of benzene, this situation is expected to exert a negative impact on the health of the population. Measures that reduce hydrocarbon emissions from vehicular exhaust can address this problem but such measures, which require the introduction of catalytic converters for petrol engines, cannot be implemented until leaded petrol is phased out. Several countries in Northern Europe have phased out or are close to a phase out of leaded petrol and the use of catalytic converters is supported by EU requirements (COWI, 1998). It is hoped that similar measures be introduced to Malta as soon as is practically possible.

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Research Article

Personal Development Profiles and Models of Learning in Teachers' Continuing Professional Development in Primary Science, A Case Study from the Centre for Science Education, Open University, UK.

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Summary. *Guidelines issued by the Department for Education and Employment in the UK have maintained that programmes of Continuing Professional Development in Science (CPD) which qualify for special funding from Government must have as a priority the development of a personal knowledge of science by the participating teachers. This paper is concerned with finding measures of the progress which could define teachers' progress as they strive to develop and apply an enhanced personal knowledge of science to practice. It also aims to identify some of the key elements which contribute to successful CPD in primary science.*

Working with more than 400 teachers over a period of three years, the following measures were set in place and then examined, to look for evidence of progress in the acquisition of science knowledge and a resultant change in the practice of teaching science within the primary curriculum:

- responses to tutor-marked assignments (TMA's)
- end-of-course evaluations
- entries in reflective diaries.

Responses to assignments
The type, level and range of skills being displayed by teachers in three episodes of assessment during an Open University CPD Course in Primary Science were used to construct a framework for illustrating the potential for teachers to achieve a personal development profile in science and science teaching (see Figure 1).(See Tresman et.al.1997)
Participants progress through the course could then be tracked by plotting their acquisition of core skills (as demonstrated in their response to the assignments) with time.

Core skills included:
research experience/teacher as researcher
organisation
communicating
writing
record keeping
scientific knowledge in key areas supporting Key Stage 1 and 2 of the UK National Curriculum, e.g. Life, Diversity and Evolution, Materials, Energy & Forces, Electricity, Planet Earth, Ecosystems & Environment.
practical skills

The complexity of the assessment tasks and level of skills being tested increased with time, culminating in a substantial end-of-course project. (See Figure 1. (Tresman et.al. 1997 and 1998.))

A quantitative measure of performance on each of these assessment events was then obtained for a pilot sample. Such early results would appear to indicate that teachers with varied levels of science qualifications, are able to achieve similar levels of attainment in a course of professional development in Primary Science. The results also enable the formulation of a model of learning during Programmes of CPD that comprises a personal learning journey through five phases as detailed in Figure 2. Encountering these five phases enables teachers to access the post training experiences indicated in the sixth and seventh column to the right of Figure 2.

Reflective Diaries
What do teachers say about the relevance, as they perceive it, of an enhanced personal understanding of science to teaching it in primary classrooms?
Information was collected about the perceptions of more than 100 teachers engaged in primary science CPD. The data was obtained using a technique in which teachers wrote reflective diaries on their experiences on six INSET courses with four separate providers.
We were interested in what common threads might emerge from groups working with different tutors on different programmes in different parts of the country. In all, several thousand diary entries were produced, reflecting on their experiences. These illustrated the major issues addressed by teachers and tutors engaged in Courses of Continuing Professional Development lasting between 5 and 20 days. (Tresman and Fox 1996 for a full account of research and extracts from reflective diaries).
The key issues were as follows:

- Science is difficult, and the most difficult concept is force and the various concepts related to and derived from force - including floating and sinking, weight and density. This difficulty was expressed both in terms of personal understanding and introducing it into primary classrooms.
- The application of force can be readily experienced and teachers were enthusiastic about making, calibrating and using a forcemeter.
- Teachers placed great value on practical work which they could engage in to progress their own understanding. This seemed to be a process by which teachers moved from early positions of anxiety, misconceptions and difficulty with concepts to more enlightened and confident

Table 1. The two student subgroups with TMA and Student Project Scores.

				Assignment Marks / 100				
ID Code	**Region**		**Returned Questionnaire**	**TMA01**	**TMA02**	**Project**	**Grand Total**	**Highest Science Qualification**
□ 3 O-levels		**Passmark**		**40**	**40**	**40**		
3	2		Yes	82	62	67	211	3 O-levels
4	2		Yes	78	60	55	193	1 O-level
7	2		Yes	67	72	87.5	226.5	1 O-level
14	5		Yes	88	88	83	259	3 O-levels
15	6		Yes	59	66	46	171	1 GCSE
16	6		Yes	68	77	69	214	2 O-levels
17	6		Yes	57	85	85.5	227.5	None
21	6		Yes	46	55	65.5	166.5	None
22	6		Yes	67	82	71	220	1 O-level
23	6		Yes	49	56	62.5	167.5	1 CSE
26	10		Yes	45	47	58	150	1 O-level
28	10		Yes	76	63	73.5	212.5	1 O-level
Mean				**65.2**	**67.8**	**68.6**	**201.5**	
□ 1 A-level								
20	6		Yes	57	55	53	165	2 A-levels
33	2		Yes	79	59	57	195	1 A-level
38	2		Yes	93	95	83.5	271.5	1 A-level
40	2		Yes	46	60	53.5	159.5	BSc
41	2		Yes	84	93	77.5	254.5	MSc
Total			**18**					
Mean				**73.2**	**74.2**	**64.9**	**201.4**	
Total Mean				**67.8**	**69.9**	**67.5**	**201.5**	

positions.

- Electricity was the form of energy most often indicated as being difficult, but teachers typically reflected favourably on opportunities to experience practical sessions with appropriate equipment backed by 'content' session on related theory.
- More comments were made about the relevance of CPD courses which concentrate on developing enhanced personal knowledge in science, than any other issue, i.e. in order to sustain motivation, courses had to be seen to be relevant to science needs of primary teachers.
- There was some recognition about the more indirect relevance of teachers reflecting on the valuable insights they were gaining from their own experiences as learners.
- A majority of comments focused on the way teachers would take experience of the course into the classroom.
- The notion of relevance was perceived by many to include responsibilities of co-ordinator or subject leader roles.
- The issue of levels - adult ... children's was central to the issue of relevance. Some found it very difficult to focus on the longer term aims for their practice of an enhanced personal knowledge of science. Their compulsion to find classroom relevance detracted from the opportunities for learning at their level. Role of tutor or course materials was crucial in successfully mediating and negotiating these longer-term aims.
- Groups were always composed of teachers with varied science backgrounds, and many teachers found that previous experience had not provided them with the understanding they had previously assumed.
- Successful courses designed into them activities targeted to making teachers question existing concepts and resolve partial understandings/ misconceptions.
- Sufficient emphasis was needed on the time-demanding processes of science. Many important processes can be practised without traditional practical activities requiring equipment and materials e.g. hypotheses framed, planning carried out for experiments, analysis and interpretation of data, conclusions drawn.
- Teachers expressed feelings of increased confidence to handle science in the classroom as they become more knowledgeable about science. By the end of their programmes of professional development, most participants recognise that they have participated in a significant route to improving the quality of their classroom practice. They recognise that science is difficult but are prepared to meet the challenge.

Some additional perceptions were revealed in a study of end of course evaluations. These included, (1) the high

Figure 1. Personal Development Profiles for Primary Teachers participating in the OU Course 'Primary Teachers Learning Science' : An Illustration.

	Core Skills						
TARGETS SET BY STUDENTS IN THEIR END OF COURSE PROJECTS	Assessing the consequences for teaching of new knowledge. Sustained changes in science teaching; impact on practice.						Baseline assessment of competence = pass continuous assessment (TMAs 01 & 02) and examined component (The Project)
Tested in TMA03	(New targets (Evaluating achievements (Reflecting on learning. ((Teacher as researcher (disseminating new knowledge. (Supporting colleagues (Managing science in the school.					<u>Pass</u>	
Tested in TMA02	(Planning research work. (Reflective learning for teachers (writing skills. Teaching (investigative science. (Monitoring learning outcomes (for children. Record keeping.			<u>Pass</u>	<u>Pass</u>		
Tested in TMA01	(Organisation and (communication of ideas in (science. Scientific knowledge. (Children's ideas. Learning (outcomes from activities Various starting points in basic skills in science						
		Orientation	Familiarisation	Assignment 1 Immersion Phase	Assignment 2 Reflective Phase	The Project Bridging to practice	Post course agenda and new professional targets Application Phase

Figure 2. A model for learning during programmes of continuing professional development

orientation phase ∅	familiarisation phase ∅	immersion phase ∅	reflective phase ∅	bridging to practice ∅	POST-TRAINING EXPERIENCES application to teaching, longer term impact on practice ∅	Greater Understanding of Children's learning
•reflecting on professional situation •verbalising, assessing, auditing current knowledge and perceptions of purpose of teaching science in primary curriculum •expectations, needs and targets for course consequences of present knowledge for key tasks you are involved in •fixing time lines and organising workload •reassurance •motivation - role of tutor/course materials long term aims	•breaking down barriers •confidence building •showing science to be accessible •raising awareness of contribution personal knowledge of science can make to practice •familiarity of possible outcomes of learning for teaching •guidance on the processes of learning, reflection, active learning, group interactions	•learning key concepts in science skills and processes of science •working with course materials •mixed media resources •designing and participating in tutorials •completing assignments •correcting misconceptions •increased confidence in confronting new and difficult ideas in science	•analysing processes of own learning •trying alternative learning strategies at personal level •opportunities to access and record progress in personal learning •requirement to focus explicitly on using own knowledge to change teaching •collecting evidence to show how developing scientific knowledge is enhancing professional role(s) •focusing on personal achievements	•process new information insights and experiences into classroom strategies •test new ideas, record (for assignments) •critical evaluation of new practice, through stages and sequential tasks within framework for course assessment •considering consequences for teaching of personal achievements •creation of new 'building blocks' for primary practice •effective questioning and intervention to diagnose children's partial understanding and misunderstandings	•long term-post course, but where framework is established within an assessed course •reflecting on changed professional situation and appropriate future targets •support and dissemination techniques gained through course to enable work with colleagues	•management of opportunities for learning science in the school •confidence in eliciting children's ideas and analysing them in order to plan activities •judging influence of teaching on the development of children's scientific ideas •teacher as researcher - sharing new knowledge in the science education community

Table 2. An Example of a Ten-day Government funded Course.

TERM 1		**BBC/OU RESOURCES**
June	Pre-course twilight - introduction to proposed course, staff etc, attended by course participants and Headteachers 2 day introduction to course	
Late September	Ways of studying the workbooks. OU assessment for the Certificate. Introduction to the learning file and assignments Audit of science skills of course participants Diversity and Evolution tutorials and workshop Investigative work in science, reporting on and recording children's achievements and experiences	Workbook 1: Life: diversity and evolution Assessment booklet Teaching Today [1] Broadcast and Notes
October	1 day, tutorials and workshop on materials	Workbook 2: Materials: Physical and Chemical changes Teaching Today [2] Broadcast and notes
Late October	1 day in school researching tasks with children and preparing for course assignments 1 and 2 (with supply cover)	
November	1 day, tutorials and workshop on Forces and Energy	Workbook 3: Forces and Energy Teaching Today [3] Broadcast and notes.
ASSIGNMENT 1 DUE NOVEMBER		
Early December	1 Day workshop and tutorials Continuing Forces and Energy Differentiation in primary science Working with colleagues, school based INSET - strategies for and evaluation of (production of A$ resource sheets by course participants to be shared amongst all members of the group)	Workbook 3: Forces and Energy Workbook 2, Section 4
TERM 2		

January	1 day workshop and tutorials on Circuits and Magnets Planning for science, providing equal opportunities for science (5 minutes presentation by course participants)	Workbook 4: Electricity: Making connections Teaching Today [4] Broadcast and notes

ASSIGNMENT 2 DUE MARCH

February	1 day workshop and tutorials The Planet Earth Resourcing primary science	Workbook 5: the Planet Earth Teaching Today [5] Broadcast notes
Late February	1 day in school (with supply cover) researching tasks for assignment 3	
March	1 day workshop and tutorial on Ecosystems Assessment in science	Workbook 6: Ecosystems Teaching Today [6] Broadcast and notes
Twilight late March/April	Exhibition mounted by course participants in local professional development centre for Heads, Colleagues, Pupils, future course participants.	

ASSIGNMENT 3 DUE AUGUST

AWARD BOARD MEETS NOVEMBER TO OFFICIALLY AWARD CERTIFICATES

level of value placed by teachers on tutorials which explained specific scientific concepts in a non-threatening environment. (2) Tutorials should be held in the company of experienced tutors of adult learners (in these cases combinations of Local Education Authority science advisory staff and Open University science tutors). (3) Acknowledgement of the large amount of time needed for study and work for assignments. (4) The appreciation of high degree of organisation needed by teachers to synthesise their study, to reflect on the use of the science in school and to try out ideas for assignments in the classroom. (These sentiments were influential in validating the course as a post graduate certificate within the Open University's MA in Education.)

On the basis of research outlined above,the following are identified as critical aspects of training programmes in primary science. Programmes which include these factors should enable teachers to achieve success in learning science and applying it to their practice in school and classroom.

CRITICAL FACTORS

1. Explicit links should be made, between selected content at the level of the adult learner and the potential for this to impact on primary science practice. This link must be included in the course resources and tutorial provision.
2. Techniques for reflective learning should be introduced at an early stage.
3. Explicit statements on core skill and subject specific outcomes should occur at key points in the programme of training.
4. There should be opportunities for teachers to record evidence of their progress in learning/applying science.
5. Opportunities should be provided for teachers to demonstrate the processes involved in their development of new science knowledge (the personal learning strand).
6. Opportunities should be provided for interaction with concepts/activities in workbooks/course materials and tutorials - especially core skill activities, which can feed into course assessment.
7. The role of the tutors is central to establishing the quality of the learning experience through providing appropriate tuition and feedback. This requires adequate levels of support for tutors with quality assurance checks being built into the system.
8. CPD courses need to include needs analysis and audits of knowledge, and providers should use this data to establish differentiated provision for teachers with varied backgrounds.

9. Support of head teachers and senior/other colleagues is crucial in the reconciliation of personal needs of participating teachers and priorities of school development in science.
10. A sequence of assessment tasks focused on practice which involve critical evaluation of teaching and a coherent, progressive guided set of tasks across a period of months facilitates maximum impact on practice.
11. The provision of a framework for assessment which allows participating teachers to function as researchers within the context of a project at the end of the course. (Tresman et.al. 1997.) (Gibson, 1996.)
12. The design and implementation of an appropriate and varied tutorial programme e.g. Table 2 preferably through establishing partnerships between teachers, schools and Higher Education Institutions. (Gilroy and Day, 1993.)

On reflection: A model of learning

A number of critical factors have been assimilated into a model of learning shown in Figure 2. This model is relevant to the design of programmes of primary science professional development, established to meet UK Government guidelines (1990 onwards), but may contain appropriate guidance for all providers of Continuing Professional Development in primary science wherever they are working in partnership with practising teachers.

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Abstracts

BIOMONITORING OF ORGANIC POLLUTANTS IN LOCAL COASTAL WATERS USING EROD ACTIVITY IN FISH

Paula Bonnici
Supervisor: Victor Axiak

Mixed Function Oxidases are a group of detoxification enzymes which can be induced in fish livers by the presence of organic contaminants in the seawater or in sediments. Quantification of this group of enzymes in fish livers is nowadays being used in conjunction with chemical monitoring to assess the extent of organic pollution present in a particular area.

The main aim of this biomonitoring exercise was to determine the induced levels of 7-ethoxyresorufin O-diethylase (EROD) activity in the fish *Serranus cabrilla* collected from three sites along the Wied Ghammieq sewage pollution gradient, namely from Xghajra, Zonqor Point and Delimara areas and from Wied iz-Zurrieq area, a sewage-free reference site. The existence of the sewage pollution gradient from Xghajra to Zonqor Point to Delimara has been demonstrated by means of microbiological tests. Sample collection from every site was performed every season for a period of one year to investigate seasonal variation in specific and total hepatic EROD activity levels. The values of the biological parameters Liver Somatic Index and Composite Condition Factor were also determined. Seasonal variations in these parameters as well as correlations between them, and with the hepatic EROD activity values measured were investigated.

Mean specific hepatic EROD activity levels from *Serranus cabrilla* was found to be 2.06 ± 0.13 picomoles of resorufin formed per minute per mg protein. These values are lower than the mean specific hepatic EROD activity levels quoted by other authors for *Serranus cabrilla* species caught in other Mediterranean countries. However, they are comparable to specific hepatic EROD activity levels in other members of the *Serranus* species, like for example in *Serranus scriba* which were caught from local coastal areas and from French and Corsican coasts, as well as to levels in *Serranus hepatus* caught from the Atlantic coast of France. The maximum level detected was 14.98 whilst the minimum detected level was 0.27 picomoles of resorufin formed per minute per mg protein.

The occurrence of a sewage pollution gradient was reflected in specific hepatic EROD activity values during spring and winter; in the total hepatic EROD activity values during spring as well as in Liver Somatic Index values in summer and winter. Moreover, mean annual Liver Somatic Index values of Xghajra samples were found to be relatively higher than values of samples collected from the other sites. On the other hand, no significant variation in the Composite Condition Factor values was recorded amongst samples collected from the three sites situated along the sewage pollution gradient.

Monoxygenase activity in *Serranus cabrilla* was found lowest during the reproductive season. Conversely, maximum Liver Somatic Index values in samples collected from the exposed sites were found to coincide with the reproductive season of the fish or occurred slightly before. As for composite condition factor, Xghajra and Zonqor Point samples were found to exhibit maximum values prior to or during the reproductive season.

The suitability of Wied iz-Zurrieq as a reference site was questioned from the biomonitoring results obtained. In every season, specific hepatic EROD activity values in fish collected from the Wied iz-Zurrieq area were higher than the specific hepatic EROD activity values measured in fish collected from the Delimara area. Furthermore, during summer and autumn, these were also higher than specific hepatic EROD activity values measured in fish collected from the Xghajra area.

In the present work, the effects of bromoform exposure via an intraperitoneal injection, on hepatic EROD activity levels were studied in the seabream *Sparus aurata* to investigate further the effects of increasing bromoform concentrations in the marine environment through discharge of chlorinated waters on local fish populations, as well as to investigate the effect of bromoform on biomonitoring in general. Bromoform is the major chlorine disinfection by-product present in seawater. The major point sources of bromoform are situated in the Delimara and in the Wied Ghammieq area. No data as regards bromoform concentration in coastal seawater is yet available in Malta. However, it is expected that bromoform concentration in the exposed sites which were investigated in the biomonitoring exercise is higher than in the other coastal areas. In the exposure experiment, a dose of 50(g/kg bromoform was administered. A negative control group using ethanol and a positive control group using (-naphthoflavone were also used to determine any significant induction.

The Composite Condition Factor values obtained for the bromoform-exposed group were lower than the values for the other groups, confirming that bromoform stresses fish. However, no liver enlargement was observed. Specific EROD activity levels in the bromoform-exposed group were found to be lower than those of the negative control group, possibly indicating that bromoform is an inhibitor of Mixed Function Oxidases.

Mixed Function Oxidase inhibitors, if present in the marine environment, might mask the occurrence of Mixed Function Oxidase inducers, resulting in relatively lower hepatic EROD activity in the more contaminated sites. Consequently, the presence of bromoform in the exposed sites, especially in the Delimara area could explain why lower specific hepatic EROD activity values in fish collected from Delimara were obtained when compared to the specific hepatic EROD activity values obtained in fish collected from the Wied iz-Zurrieq area.

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PARASITIC HYMENOPTERA ASSOCIATED WITH SCALE INSECTS ON CITRUS TREES IN THE MALTESE ISLANDS

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Biological pest control entails the use of natural enemies (parasites or predators) to control other insect pest species damaging cultivated crops. These natural enemies are commonly known as beneficial insects and play an important role in nature by keeping in check other arthropod populations. Some well-known species are nowadays mass-produced on a commercial scale and sold to farmers as an alternative to chemical pesticides.

Since several pest species have become cosmopolitan through introduction with their host plants, there is a tendency for mass-produced beneficial insects to follow a similar route by artificial introduction. However, as with any introduced exotic species, there is always the potential risk that such species may adversely affect other native insect populations, the extent of which is difficult to quantify. For this reason and the fact that native beneficial insects tend to perform better, being more adapted to the local environmental conditions, it is more favorable to use indigenous natural enemies rather than exotic ones.

Since some beneficial insects are highly host specific, the correct species identification of both pest and its parasite is a key essential. The purpose of this work was therefore aimed to give a taxonomic account of the locally occurring beneficial insects of the order Hymenoptera parasitizing a group of pest species that are found on cultivated citrus trees. The latter pests belong to the order Homoptera, and are commonly known as scale insects.

Scale insects are sap-feeders and when present often occur in high numbers, covering mostly leaves and young twigs. These pests severely impair the plant's health through depletion of food and nutrients. As with most Homoptera, scale insects secrete considerable amounts of honeydew which permits the growth of a number of fungal species. The latter are often found growing on leaf surfaces wherever scale insects occur and they physically restrict the amount of sunlight that reaches the photosynthetic tissue.

Scale insect samples were collected from all over the islands of Malta and Gozo between January 1996 and December 1997. For identification purposes, several techniques were then used to rear the immature stages of the parasites that were feeding on the scale insects . Eleven species of scale insects were identified including *Aonidiella aurantii, Hemiberlesia rapax, Lepidosaphes beckii, L. gloverii, Parlatoria pergandii* and *P. ziziphi* (Diaspididae); *Ceroplastes rusci, Ceroplastes floridensis, Coccus hesperidum* and *Saissetia oleae* (Coccidae) and *Planococcus citri* (Pseudococcidae).

A total of twenty five hymenopteran parasites were found on these scale insects in the Maltese Islands. These include both parasites and hyperparasites from five families of the superfamily Chalcidoidea (Hymenoptera). For each species, information is provided on general distribution, diagnostic features, material examined and hosts species. Of these, twenty species are recorded for the first time. The new records include *Aphytis hispanicus, A. lepidosaphes, A. melinus, Coccophagus nr. rusti, C. scutellaris, Encarsia citrina, E. herndoni and Marietta exitiosa* (Aphelinidae); *Anagyrus pseudococci, Anicetus italicus, Cerapterocerus mirabilis, Comperiella bifasciata, Encyrtus lecaniorum, Gyranusoidea advena, Leptomastidea abnormis and Metaphycus nr. stanleyi (Encyrtidae); Aprostocetus nr. toddaliae* (Eulophidae); *Dicopus citri* (Mymaridae); *Pachyneuron concolor* (Pteromalidae) and *Chartocerus kurdjumovi* (Signiphoridae).

Coccophagus lycimnia, Metaphycus flavus and *Microterys nietneri* were previously recorded by Borg, P. (1919) and again by Borg, J. (1922) who also recorded *Scutellista caerulea* for the first time. *Signiphora flavopalliata* was later recorded by Borg, J. (1932).

Original drawings of important morphological features of all recorded parasitic species are included. Dichotomous keys for the identification of females are also provided.

Most species of Aphelinidae and Encyrtidae are endoparasitic. Eggs are laid free within the host or attached to the host integument and the hatched larva starts to feed on the internal tissues of the host. The pupa is then formed within the integument of the host after the entire body contents of the latter have been consumed and meconia (faecal material) have been voided into the resulting cavity.

Species of *Aphytis* are ectoparasites. The eggs are laid beneath the scale of the host, on or beneath the body of the scale insect. Protected by the covering host scale, the *Aphytis* larva feeds externally by sucking the body fluids from the scale insect (Plate 3). The fully grown larva then excretes characteristic meconial pellets and pupates underneath the empty scale.

Five of the beneficial species were recorded as hyperparasites. These include *Cerapterocerus mirabilis, Chartocerus kurdjumovi, Marietta exitiosa, Pachyneuron concolor* and *Signiphora flavopalliata.* Insect hyperparasitism involves the development of a secondary parasite (or hyperparasite) at the expense of a primary one. Hence, an insect hyperparasite attacks another insect that is itself parasitic on an insect host.

A number of factors determine the ability of parasitic insects to control pest populations, and consequently their potential use in biological control. These include host specificity, mode of parasitism, sex ratio of progeny, ontogeny and ease with which they can be mass-reared. However, from the results obtained, two

species have shown signs of being important controlling factors of *Coccus hesperidum* and *Aonidiella aurantii*, namely *Metaphycus flavus* and *Comperiella bifasciata* respectively. All three species of *Aphytis* were also very effective in controlling their respective host populations. Furthermore, parasite activity was highest during the period October to November, followed by the period March to May.

COLONISATION, SUCCESSION AND SEASONAL DYNAMICS ON A MALTESE ROCKY SHORE

Ruth Mallia
Supervisor Patrick J. Schembri

A two-year experiment was conducted to investigate the processes of colonisation and succession on cleared patches at Qalet Marku, a rocky shore on the North-eastern coast of Malta. Replicate patches were cleared mechanically in the supralittoral, mediolittoral (upper and lower subzones) and upper infralittoral zones, in November-January. Uncleared control patches in each zone, and two transects on the same shore which were sampled over a period of one year served to determine the natural patterns of seasonal changes in the shore assemblages.

The time taken for initial colonisation of the cleared patches and also for return to an assemblage with a composition approximating that of the uncleared surroundings was, in order of increasing rapidity: supralittoral < mediolittoral < infralittoral. A general successional sequence was observed as follows:

In the supralittoral, Cyanophyta colonised first. Seasonal changes in abundance and thickness were evident during the successional sequence. Following a first stage with low microalgal abundance, percentage cover increased during the winter season (nine months after clearing). The experimental patches were almost indistinguishable from the surrounding areas after two and a half years. *Littorina neritoides* was never found in large numbers in the experimental patches, unlike the controls, possibly because the clearing method left the rock surface in the patches smoother than the surrounding areas. However, seasonal variations in the density of *L. neritoides* in the cleared patches followed the same pattern as in the control patches.

In the mediolittoral, the general sequence was similar to that described for temperate and Mediterranean shores. Initial colonisation by encrusting corallines (and possibly microalgae) was very rapid (about 40 - 80% cover within two weeks), as was colonisation by limpets due to lateral migration and by larval settlement. Other rhodophytes and Cyanophyta colonised the patches at low cover. The early-appearing coralline algae continued to dominate the patches (4 - 17 weeks from clearing), while the cover of other rhodophytes (lithothamnoids, *Laurencia papillosa, Polysiphonia* species) increased, and chitons also colonised the patches. The cover of rhodophytes and *Rivularia* species increased after 17 weeks from clearing until the end of the first year (Plate 2), and chlorophytes and some phaeophytes appeared at low abundance. In the first six months of the second year from clearing, the density of limpets, chitons and juvenile vermetids increased, and juvenile barnacles appeared in some patches. In the last six months of the second year, juvenile vermetids decreased, the cover of lithothamnoids, *Laurencia papillosa* and *Cladophora* species increased, and that of the *Cyanophyta* declined. After two and a half years, the patches were similar to the surrounding assemblage, and resembled it in species composition, but not in certain other aspects (e.g. higher densities of limpets in the experimental patches).

In the infralittoral, the patches were indistinguishable from the surroundings after one year, although in some of the patches understorey and encrusting cover was more sparse than in the controls. Microalgal films dominated the cleared patches in the first four weeks after clearing, and *Cladophora* species colonised some patches. From 4 to 20 - 30 weeks after clearing, the microalgal films declined and *Cystoseira* species colonised the patches and reached up to 40% cover by 20 to 30 weeks from clearing. Jania species, lithothamnoids and *Cladophora* species also increased in cover, and other phaeophytes, rhodophytes and chlorophytes were present at low cover. In the third stage, lasting until 45 weeks from clearing, the cover of *Cystoseira* species increased. By about 14 months from clearing, the *Cystoseira* species had covered the patches completely. Some chlorophytes and rhodophytes were present as understorey species. The assemblages in the infralittoral patches were more variable among themselves than in the mediolittoral and supralittoral, but all were eventually dominated by *Cystoseira* species (in summer). In both the experimental and control patches, the number of species tended to be lower than in the mediolittoral patches.

In the mediolittoral zone the Rhodophyta were by far the most common and abundant group in the control patches, and in the three later successional stages (from the beginning of the second year) in the cleared patches. In the infralittoral the dominant algae were the phaeophytes *Cystoseira* and *Padina pavonica*. Chlorophyta, particularly species of *Cladophora*, were quite common as understorey species, and *Cladophora* species were also the most common chlorophytes in the mediolittoral patches. One feature of both the seasonal and successional changes was that along the shore, certain ephemeral or annual genera (*Polysiphonia, Herposiphonia, Ceramium, Chondria*) were found in particular seasons in all the patches of a zone, but different species of each genus were found in the different patches.

The sequences observed in the mediolittoral and infralittoral fit the accepted general sequence usually observed on rocky shores around the world, and were similar qualitatively to results from studies in the Mediterranean and other seas. The most obvious difference was the lack of ephemeral chlorophytes during the early stages of succession. The rate of succession in both the mediolittoral and infralittoral cleared patches was at the low end of the reported range from temperate and tropical shores. The different time

scales for colonisation in the three zones, the rate of change and the patterns of colonisation within the patches all indicate that colonisation is effected primarily by water-borne propagules, and is not so dependent on vegetative growth from the surrounding algae, even in such small patches (430 - 700 cm2), which have a high perimeter / area ratio. However, for the sedentary animal species - limpets and chitons in the mediolittoral zone and anemones in the infralittoral zone - lateral migration was an important means of colonisation. Statistical analyses of the data (hierarchical cluster analysis and ordination by non-metric MDS) resulted in similar groups. The main factors differentiating between groups were: the density of *L. neritoides* for the supralittoral patches; and location, seasonal changes and time from clearing for the mediolittoral and infralittoral patches.

The seasonal changes in the transects followed the annual pattern of a mild, wet winter (October to mid-May) and a hot dry summer (May to September), but occurred one to two months later than the climatological changes. In winter, *Cystoseira* species and ephemeral or annual algae (mostly *Polysiphonia*, *Jania* species and Phaeophyta) increased in cover, and there was a downward migration of *L. neritoides*, especially in the less exposed transect. In May-June the assemblages were characterised by increasing lithothamnoid cover and settlement of vermetids and limpets. During the summer, *L. papillosa, Rivularia* species and chlorophytes increased, and there was a downward migration of the limpets (species of *Patella*) and *Lepidochitona* corrugata. Most of the algal species had stable limits of distribution, with only three of the abundant species/taxa showing a seasonal change in their upper limit of distribution: lithothamnoids (the upper limit moved upshore in May-June, then downshore in summer), *L. papillosa* (the upper limit moved downshore in winter) and *Cystoseira* species (the upper limit moved upshore in winter).

An important factor was aerial exposure, particularly during January-March, caused by a combination of several days of good weather and a lower than usual sea-level. This was observed in successive years, and affected the organisms of the mediolittoral and upper infralittoral zones, with bleaching and death of some algae (predominantly Rhodophyta such as *lithothamnoids, L. papillosa* and *Ceramium*). Cluster analysis of the transect results showed that the zones remained stable throughout the year, although the changing distribution of the abundant species affected the width of the zones. The seasonal changes in the composition of the assemblage were also confirmed.

BIOMONITORING OF HEAVY METAL POLLUTION IN MALTA

Lisa Pace
Supervisor: V. Axiak

Heavy metals have been classified amongst the most potentially deleterious contaminants for biota and human consumers of sea food (Martin and Richardson, 1991). The need to gain further information on the heavy metal pollution status of coastal waters has thus been recognised on both a national and regional scale. In this regard, Malta is directing its efforts towards monitoring heavy metal pollution within the framework of a biomonitoring programme for the Mediterranean Region.

This investigation aimed to assess the feasibility of detecting heavy metal pollution using a biomonitoring approach. This entails measuring the biochemical response of an indicator species to contaminant exposure and thus provides an early warning signal of environmental metal contamination. The biological response or stress index which is being proposed is the induced synthesis of the metal-binding protein metallothionein in the presence of heavy metal contamination.

The suitability for heavy metal biomonitoring of two locally abundant mollusc species, the limpet *Patella rustica* and the neogastropod snail *Hexaplex trunculus* was assessed. Both species had been used in previous studies for monitoring of metals and tributyltin respectively. They were sampled from six locations along the north east coast of Malta. These included Qawra Point (reference area); Wied Ghammieq, Xghajra and Marsascala (constituting an environmental pollution gradient); Bahar ic-Caghaq (Maghtab Landfill area) and Manoel Island, two suspected 'hot spots' of metal pollution.

The molluscs were found to accumulate significantly high levels of cadmium, copper and zinc in Xghajra, Marsascala and Bahar ic-Caghaq when compared to the reference site. The high copper and zinc levels measured in the limpet in the present study were comparable to values in similar biota from foreign industrialised polluted areas. The results obtained seem to suggest that the north-east coast of Malta, along which the sampling points were considered, is subject to chronic low-level metal pollution. The site at Bahar ic-Caghaq was chosen with the specific intent of determining whether the Maghtab Landfill, situated a few metres away from the sampling point, constitutes a source of heavy metal leachate. The high heavy metal loads of both biota and sediment seem to support this possibility.

On the basis of chemical data (i.e. total metal content in tissues) and biological data (the role of metallothioneins in metal sequestration), *Patella rustica* was selected as the more suitable sentinel species for monitoring purposes and a valuable substitute to *Mytilus galloprovincialis*, the official MEDPOL indicator species.

Metallothioneins (MTs) constituted suitable biomarkers of metal pollution in *P. rustica* since measurements of protein levels, performed using gel-filtration chromatography, were generally correlated with heavy metal concentrations in the cytosol and limpet soft parts and thus provided a clear indication of the pollution status of the sites investigated. The correlation was weaker for zinc since this metal was bound mainly to high molecular weight proteins. Moreover, zinc was preferentially distributed in the non-cytosolic compartment of the cell.

The kinetics of cadmium accumulation and sequestration were studied in *Patella rustica* via in vitro exposure of the limpet to 500µgCd/L for three weeks. The induction of metallothioneins provided a quantifiable biological response to sub-lethal levels of cadmium.

The application of a relatively simple spectrophotometric assay (described by Viarengo et al., 1997) for the semi-quantitation of metallothioneins was evaluated. This technique can be considered as a compromise between sensitivity and cost and sees a practical application for routine monitoring purposes. It yielded the same results in terms of the identification of 'hot spots' of metal contamination as those obtained from gel-filtration chromatography.

Significant differences in protein levels amongst the sites investigated were observed in both species. Limpets and snails collected from Xghajra, Marsascala, Bahar ic-Caghaq and Manoel Island exhibited significantly higher MT levels than those in the reference site (as shown for Patella rustica in Figure 2 below). Data of MT concentrations were correlated with total tissue metal levels and with cytosolic cadmium and zinc in *P. rustica*. MT levels in Xghajra limpets were tentatively linked not only to metal contamination but also to pollution by organic compounds suggesting that MTs could be used as an index of general environmental stress.

Seasonal fluctuations in protein levels in the limpet were obtained with the highest levels measured in spring and the lowest in autumn. These variations were related to the reproductive and growth cycles of the limpet. Spring was suggested as the season which lends itself best to biomonitoring since the variations in protein levels due to the reproductive cycle can be excluded. The largest quantitative differences in MT levels between the sampling points and reference site were most evident in this season.

A set of recommendations and guidelines are proposed to assist in the planning and application of a local biomonitoring programme using *Patella rustica* as the sentinel species.

Since metal accumulation in limpets is size dependent, organisms of the same shell size should be collected. A shell length of about 15mm is suggested since at this length most individuals are male - this enhances the collection of a more homogeneous sample and reduces the variability in metal levels due to sex.

Spring is suggested as the season which lends itself best to biomonitoring. During this period P.rustica is sexually inactive. In this season, the limpet undergoes somatic tissue growth and assimilates heavy metals from the environment. The same sampling time should be kept annually to allow for a correct interpretation of the data.

The spectrophotometric method described by Viarengo et al., (1997) is recommended for the routine determination of total metallothionein levels in *P. rustica*. An adequate amount of replicate determinations from each site should be made. This data should be supplemented with chemical monitoring in the way of heavy metal determinations of the cytosolic fraction and limpet soft parts in order to determine the exact nature of the insulting metals.

The biological data obtained from biomonitoring can be used to generate an index of metal pollution if the mean rise in MT protein levels in seemingly polluted areas is compared with levels in a reference area. More extensive data from the sites investigated and from other sampling points is required to obtain a complete picture of the metal pollution status on a national scale.

Standardisation of the methods used to analyse the data is important. It would be of great benefit to set threshold limits to the levels of MT induction which could be used to indicate biological stress and environmental quality in general. The present work may be seen as a contribution to our knowledge which is essential in the setting-up of such threshold levels.

EVALUATING THE USE OF THE SEA WEED PADINA PAVONICA AS A DIET FOR THE MASS PRODUCTION OF THE ROTIFER BRACHIONUS PLICATILIS

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The success of marine fish larval rearing may be attributed to the use of the rotifer, *Brachionus plicatilis* as the first larval food. Marine fish larvae only measure around 3mm at hatching, with mouth openings of less than 0.1 mm, and a very primitive digestive system. This is the case with the gilthead sea bream, Sparus aurata, a commercially cultured species in Mediterranean countries.

The characteristics which make rotifers perfect prey for such marine larvae are the following: (1) they are relatively small in size (100-300 SYMBOL 109 \f "Symbol"m); (2) they are planktonic in nature; (3) they are cultured in high densities and reproduce rapidly; (4) they provide the digestive enzymes which are lacking in the early stages of larval development; (5) they can be enriched with the nutrients essential for larval growth and survival.

Padina pavonica is a brown alga belonging to the class Phaeophyta. It is found all the year round in the photic zone of colder and temperate areas, such as the Atlantic ocean and the Mediterranean sea. It grows on rocks or on other algae, principally on rocky coasts and underwater cliffs below the tidal zone down to depths of 30-40m. It is multicellular and relatively well-developed, showing a great variety of structure and form. The seaweed attaches to its growth substrate by means of rhizoids. The thallus, of width ranging from 4-10cm, often has concentric dotted striping. The fans are more or less conical, thin, serrated at the edges, and encrusted with lime. Its cells contain, in addition to chorophyll, a brown pigment known as fucoxanthin which gives the thallus a brownish appearance.

P. pavonica is also of commercial importance, and is at present being cultured in Maltese waters and other parts of the Mediterranean by the locally based company Institute of Bentique Algae (IBA) Ltd. The Padina cake used in this study is actually a waste product of a commercial process which is carried out in Malta. This process extracts a substance from the seaweed which has been shown to have an effect on calcium-fixation in human osteoblasts kept in vitro; when the extract is removed from the seaweed, a powdery cake is left, which apart from its nutritional value, is thought to have some of the active extract, originally found in the seaweed, left in it. The Padina cake could thus make an ideal food for organisms with exoskeletons such as rotifers.

The objectives of this study are: (1) to evaluate the suitability of using Padina cake as a rotifer diet, partially or totally replacing other commercial diets normally used in marine hatcheries; (2) to further evaluate the most suitable diets, determined in (1) above, on a commercial scale; (3) to determine the proximate nutritional value and the fatty acid profile of the rotifers cultured in the various experimental trials, as a preliminary step to evaluate the possibility of eliminating further rotifer enrichment before feeding to the sea bream larvae; (4) to perform a cost-benefit analysis for the most suitable diets determined through the commercial-scale trials.

The study was carried out using L-strain rotifers. The laboratory trials, carried out in 90L cylindroconical fibreglass tanks, varied depending on the diet used to feed the rotifers. Hence, the trials were differentiated on the grounds of using the Padina cake (PC), Culture SelcoSYMBOL 226 \f "Symbol" (CS), and microalgae (ALG) combined with Baker's yeast (BY), in different proportions. From all trials performed at laboratory scale, those of most commercial interest were selected and repeated in full-scale 900L cylindroconical tanks. Each trial required two experimental tanks to obtain duplicate results. The rotifers were fed 4 times per day using the standard feeding regimes for the various formulated diets.

Data collection was carried out from inoculation all the way until the death phase of the rotifers was reached. Each day the rotifer populations, their fertility, the percentage increases in total rotifer population from Day 1 of culture, and various growth parameters were determined. Quality of the culture medium was also monitored daily. The total proximate nutritional analyses and fatty acid profiles of the diets used in the trials and the rotifers cultured on these diets were carried out. The operating costs for Live Food culture required for the production of 1 million Sea Bream fingerlings was performed to evaluate the economic advantages, if any, of using Padina pavonica cake as a substitution diet. The results were analysed statistically using the Paired t-test and Wilcoxon test.

The best substitution ratios to use for both the CS and Alg+BY trials in the 90L chemostats, was found to be 50:50. The total amount of rotifers cultured using the 50:50 ratio in 1 tank for the CS trial is 1.48 times greater than when using the commercial Culture Selco diet alone, and 1.43 times greater for the Alg+BY trial. At the 900L chemostat level, the best substitution ratio was 60% CS and 40% PC. Rotifer growth was 1.45 times better using this diet then when using CS.

The 50:50 diets do not have a significant effect on the fertility of the rotifers, but maintain a growing population for longer periods than both the CS and Alg+BY diets because the ageing process of the rotifers is prolonged, and a clean culture medium is maintained. The same applies for the 60:40 trial at the 900L chemostat level. Although the growth parameters for these diet mixtures are relatively lower than for the rotifers fed the standard diets, rotifer growth performance is better in the long term. This is because rotifers can be maintained for longer periods in the late exponential phase of growth.

Rotifers fed the 50:50 diet, for both the CS and Alg+BY trials, show an increase in percentage crude protein and crude lipid content. Fatty acid profiles also indicated that rotifers feeding on the 50:50 diets showed adequate levels of EPA and DHA. PC therefore seems to induce lipid and fatty acid metabolism in rotifers.

As a result of using the 60:40 diet as a replacement for the standard Culture SelcoSYMBOL 226 \f "Symbol" on a commercial scale, Live Food Culture operating costs may be reduced by at least 25%. Hence, the use of PC as a rotifer diet is also of financial interest to the aquaculturist.

THE EFFECT OF pH AND PRESERVATIVES ON GROWTH AND THERMAL INACTIVATION OF PAECILOMYCES VARIOTTI BAINIER ISOLATED FROM A SPOILED NON-CARBONATED BEVERAGE

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Paecilomyces variotti Bainier (IMI 371343 R) was isolated and identified from a spoiled non-carbonated beverage and shown to be the causative agent of spoilage in this particular case. Growth and heat resistance studies carried out on this organism strongly suggest probable shortcomings in the pasteurization process which had been applied to this beverage and possibly also in its formulation.

P. variotti grows well in the beverage when compared with its growth in a defined sucrose (2.5% w/v) citrate liquid medium. In Malt Extract Agar optimum growth was obtained at pH values 3.5, 4.5 and 5.5, with much better tolerance being shown to acidic rather than alkaline conditions. This classifies this organism as an important spoilage organism in the case of acidic foods and beverages such as the non-carbonated beverage investigated in this study.

It was established that the spoiled beverage containing 127 ppm benzoic acid was inadequately preserved against P. variotti. Sorbic acid was shown to be a much

better preservative against *P. variotti* than benzoic acid. In fact, at pH 3.5, P. variotti was shown to be resistant to benzoic acid concentrations of up to 400 ppm, while only 100 ppm of sorbic acid was enough to completely inhibit macroscopic growth of this organism. The use of a combination of sorbic acid and benzoic acid was recommended with respect to the beverage under investigation.

Thermal inactivation studies on *P. variotti conidia* and chlamydospores revealed that the pasteurization regime which had been applied to the spoiled beverage was grossly ineffective against chlamydospores. Spoilage manifested itself as a submerged mycelial mass. It is only under this condition that chlamydospores are produced. These clamydospores were shown to be much more heat resistant than the aerial conidia. These facts ruled out airborne contamination and pointed towards defective raw material or plant sanitation.

Heat resistance of conidia of *P. variotti* at 60°C, was found to increase significantly with increasing pH in the range of 2.7 to 3.9 and with decreasing concentration of preservatives benzoic acid and sorbic acid at pH 3.3. At equimolar concentrations of the undissociated form of these preservatives, benzoic acid was found to be significantly more inhibitory than sorbic acid with respect to heat resistance. Also, at 300 ppm sorbic acid, the presence of other ingredients (tea extract, caramel, aspartame, sodium saccharine and flavours), significantly increased the heat resistance of conidia suggesting that these ingredients provide a protective effect against heat.

Observed and calculated values of the logarithmic inactivation factors for *P. variotti*, at three different pasteurization regimes, were found to be comparable. Both the observed and calculated values indicate that while all three treatments provided sufficient kill (>12D) with respect to conidia, only the most severe treatment was marginally effective (>4D) against the chlamydospores. A time-temperature treatment of 5 seconds at 85°C was recommended to guarantee an excess of 12D on *P. variotti* chlamydospores.

A STUDY OF ROCKY SHORE BIOTIC ASSEMBLAGES ALONG A POLLUTION GRADIENT

Praessede Vella
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During the last decades, ever-increasing socio-economic exigencies have given rise to greater utilization and sometimes haphazard squandering of our natural resources. An urgent need is now felt to devise new ways to safeguard the fragile and precious marine environment from further deterioration caused by anthropogenic activities.

One such method involves the use of bioindicators, either in ecological monitoring of population assemblages and communities, or in the study of biological responses at molecular, cellular and tissue levels, known as biomarkers. Ecological monitoring is a relatively inexpensive method with the added advantage of permitting the study of one or more species in their natural environment, and therefore providing a better estimation of biological-chemical interactions.

This project involved the study of a community structure along a known pollution gradient. The aim was twofold, first to identify a zonation pattern on the rocky shore under study; second, to determine whether the biotic abundance and biomass data from which k-dominance curves are plotted (ABC method) could be used to indicate the state of the marine environment in the area. From this, it could be deduced whether certain species, or possibly the community structure as a whole, could be used as a bioindicator of pollution.

The area selected was that around Grand Harbour and Marsamxett Harbour, where the marine environment has been extensively studied over recent years and has been established as being heavily polluted by numerous contaminants from such activities as bunkering, ship building and repairing, tank-cleaning, yacht-berthing, and power-generation (including thermal pollution). Such contaminants have been found to cause severe deleterious effects on shore and bottom-dwelling organisms.

Abundance and biomass data were compiled for the various floral and faunal organisms encountered in the belt transects located at suitable sites on the remaining rocky shore around the harbours. Data from harbour transects were compared among themselves and compared to data collected from two control transects, using the statistical methods of hierarchical agglomerative clustering (Bray-Curtis coefficient), non-metric multidimensional scaling (MDS) and k-dominance curves (ABC comparison).

Results of cluster analysis and MDS indicated that the zonation patterns around the harbours and at the control sites generally followed those set out by Pérès and Picard.

On the other hand, k-dominance curves for the abundance and biomass of algae and molluscs produced anomalous results in respect of those obtained from studies on soft-bottom dwellers, especially polychaetes, studied in northern Europe under conditions of stress resulting mainly from pollution.

From this study, it was therefore established that the community structure encountered in the harbours consisted mainly of bioindicator species; varied along known pollution gradients, as regards species diversity, abundance and distribution; and varied along an exposure gradient, although exposure and pollution factors were greatly interlinked.

It could be concluded that stress resulting from pollution does not seem to influence the general zonation patterns as encountered on other Maltese shores. On the other hand, the ABC method does not seem to be the ideal method for detecting stress due to pollution on hard-bottom dwellers.

Hence, the state of the marine environment with respect to pollution in a certain locality can be ecologically determined through the particular community structure in terms of species diversity, abundance and distribution.

INVESTIGATIONS ON FEEDING RATES, FEEDING FREQUENCY AND COMPENSATORY GROWTH OF GILTHEAD SEA BREAM, SPARUS AURATA (L.)

Naser K El-Kebir
Supervisor: Carmelo Agius

Three growth trials were performed on *S. aurata*, which is of importance to the aquaculture industry in the Mediterranean region.

In the first investigation, a feeding trial was conducted to investigate the influence of feeding rates on the growth performance. Five feeding rates were employed, 0.5, 1.0, 1.35, 1.7 and 2.0% body weight (BW)/day. The fish increased in body weight with time for all feeding regimes. At the end of the trial, the feeding rate of 1.7% BW/day gave the best growth performance in terms of SGR, but this growth was not significantly higher ($p < 0.05$) than that observed with 1.0% BW/day. The feeding regime of 1.0% BW/day gave the best performance and improved feed utilisation overall with regard to food conversion ratio (FCR), feeding efficiency (FE), protein efficiency ratio (PER), apparent net protein utilisation (ANPU) and apparent net lipid utilisation (ANLU). This should be seen both in the light of economical as well as environmental implications. No important differences were observed in the carcass and fillet composition or condition factor (K). Histological studies showed an increased lipid deposition in the liver with increased feeding rates.

In the second experiment, a feeding trial was carried out to investigate the influence of feeding frequency on growth performance and food utilisation. Five feeding regimes were employed, once/day (morning), once/day (afternoon), twice/day, 3 times/day and 4 times/day. The best growth performance with improved food utilisation was obtained in feeding frequencies once a day (in the morning) and 4 times a day. SGR, FCR, FE, PER and ANPU were significantly similar ($p < 0.05$) for both these feeding regimes. From the point of view of food utilisation, feeding once a day in the morning may be considered the optimum feeding frequency. No important differences were noted in the carcass and fillet composition or the condition factor and hepatosomatic index. Histological studies showed an increase of lipid deposition in the liver with increased growth performance.

In the third study compensatory growth was investigated. A feeding trial was carried out to investigate the possibility of using the phenomenon of compensatory growth to improve growth and food utilisation. Five feeding cycles were employed: daily feeding; one week of starvation followed by one week feeding (1:1); two weeks of starvation followed by two weeks of feeding (2:2); three weeks of starvation followed by three weeks of feeding (3:3); and six weeks of starvation followed by six weeks of feeding (6:6). By the end of the trial, fish subjected to a feeding cycle of 3 weeks starvation followed by 3 weeks satiation feeding produced comparable results in terms of growth performance to fish that were fed daily. These showed improved food utilisation in terms of FCR, FE, PER, ANPU and ANLU compared to those on the other treatments. Carcass and fillet analysis of protein, lipid, moisture and ash showed some differences with the most notable being the relatively higher moisture levels in both carcass and fillet, as well as the relatively lower crude lipid levels in both carcass and fillet. Histological studies showed that lipid deposition in the liver of fish fed on 3:3 and on the basis of daily satiation was higher than in the rest of the fish. The studies provide evidence of the adaptation of the fish to starvation followed by what may be termed compensatory growth, once feeding was resumed.

STUDIES IN FISHERIES BIOLOGY AND AQUACULTURE

Carmelo Agius

Our research activities in the field of Marine Biology largely addressed the fields of Marine Fisheries Biology and Aquaculture, and can be considered under the following subheadings:

1. Fisheries Biology Studies previously conducted on the feeding habits of the coryphene, *Coryphaena hippurus* (Lampuka) were extended to the amberjack, *Seriola dumerlii*. Juvenile specimens of various sizes were caught from the wild in Maltese waters and their gut contents examined in detail.

2. Oyster culture Oyster growth trials were continued with the aim of establishing the viability of integrating shellfish with finfish culture, given the possibility that wastes from finfish farming could enhance phytoplankton production. In oligotrophic waters this may make it economically viable to produce shellfish on a commercial level.

3. Studies on marine macroalgae Collaboration with a private firm was continued on studies of macroalgal production for the purpose of extracting products of medicinal importance as well as for providing sources of nutrition to fish and shrimp larvae.

4. Fish nutrition Feed trials were conducted in order to establish optimal feed composition as well as feeding methods for commercially cultured species like sea bream *Sparus aurata*.

5. Fish diseases Studies on fish diseases were directed mainly at investigating fish parasites and bacterial diseases, both on wild fish as well as on commercially cultured species. Studies were also carried out on the pathology of such diseases. Some work on fish bacterial vaccines was also undertaken.

6. Environmental studies The environmental aspects of aquaculture were investigated. These ranged from

monitoring of the water in the vicinity of commercial fish farms to studies on the effect of wastes produced by such farms on phytoplankton production in the open sea.

7. Offshore fish farming Developments in offshore or exposed-site fish farming in the commercial sector continued to be followed closely. Collaboration in this field was established with a number of other institutions in several Mediterranean countries.

POLLUTION STUDIES, MARINE ECOTOXICOLOGY AND ENVIRONMENTAL MANAGEMENT IN MALTA

Victor Axiak

Malta's economic development will increasingly depend on our ability to make a judicious and wise use of our living and non-living marine resources. However, such resources are presently at risk from a number of pressures resulting from man's activities. These risks include those related to pollution. The Marine Ecotoxicology Laboratory within the Department of Biology had been set up in the early 1990s, with the main aim of providing data and information on the basis of which our environmental authorities may be able to manage properly such resources as well as to conserve and protect our marine environment from marine contamination hazards.

Since its inception, the Laboratory had actively participated in internationally funded projects dealing with a range of themes including: biological impacts of antifouling paints, chlorination by-products; oil dispersants and oil products as well as pesticides and heavy metals. Most of these projects were undertaken through programmes funded by the European Union or the Mediterranean Action Plan. It has also produced baseline information on the state of coastal water quality for a number of localities in Malta and Gozo. A number of projects have also been sponsored by the Environment Protection Department of the Ministry of Environment.

Throughout its investigations, great efforts are made to ensure that all the information produced will be translated into a meaningful format which would be of direct use to the environmental authorities and on the basis of which decisions in environmental management and policy making may be made. For example, investigations and field surveys carried out in Xlendi (Gozo) during 1996-1997, produced preliminary data on the basis of which the upper carrying capacity for the influx of tourists and bathers may be determined for this popular bay (Axiak, 1998).

The Laboratory is also directly involved in a number of coastal monitoring programmes including those related to the coastal developments at the Hilton site, Manoel Island, Cirkewwa, and others. New monitoring programmes which have been initiated in summer 1998 include an assessment of the environmental impacts of leachates from the Maghtab landfill, which reach the surrounding coastal waters, and the assessment of long-term trends in the levels of oil pollution in Maltese waters.

A wide range of methodologies and techniques have been used in an effort to provide the necessary information on environmental quality. These range from biochemical biomarkers of pollution stresses such as induction of metallothionein in limpets and marine snails, of hepatic cytochromes dependent monooxygenase enzymes in fish, of imposex in marine snails, to classical chemical monitoring and satellite remote sensing techniques.

Since 1996, the laboratory has been conducting a long-term project in which LANDSAT satellite TM data is being used to monitor water quality in Malta. The overall aim of the project is to develop a data bank of information about water quality using satellite imagery data which may be periodically updated and which would provide the necessary information for comprehensive marine environmental management. The specific objectives of this study are to monitor four water quality parameters for local areas which are under intense anthropogenic pressures. This is being undertaken using LANDSAT-TM data suitably calibrated by sea-truth data collected from the field. Mathematical models are being developed, validated and then applied to the study areas in order to generate colour-coded maps. This project is being funded through the Italo-Maltese Financial Protocol.

The area investigated to date extends from Qawra Point to Marsaxlokk on the south-eastern coast of mainland Malta. The water quality parameters being monitored are chlorophyll a content, water temperature and water turbidity (measured in terms of Beam Attenuation Coefficient) of surface waters, and water visibility (as measured by Secchi disk). Apart from indicating water quality, these water parameters are also indicative of land-based discharges (Axiak et al., 1992). Extremely useful information is being generated as a result of this project, and the following few examples will suffice to illustrate this.

The satellite images produced so far indicate significantly reduced water transparencies in the main ports including Marsamxett, Grand Harbour as well as Marsaxlokk. For example, Plate 1 shows that Marsaxlokk Bay had a higher mean water turbidity than any other area recorded during the survey of the 8th June. Chlorophyll a levels indicate eutrophic conditions in certain areas of Marsamxett, Grand Harbour, as well as in Marsascala.

Satellite images detected significant thermal pollution within Grand Harbour as well as at Hofra z-Zghira, both due to the discharges of cooling waters from the two power stations.

Wied Ghammieq is the location of Malta's major sewage outfall, through which almost 80% of the sewage produced in Malta is discharged untreated into the sea. This wastewater is discharged through a 700-m long submarine pipe, at a depth of 30 m. Studies undertaken at the Marine Ecotoxicology Laboratory, show that when this submarine outfall is fully

functional, sewage is adequately diffused throughout the water column, and the expected environmental impact of the resultant plume would be limited in spatial extent. However, when this outfall is not functioning properly, sewage is discharged directly on the shoreline and the sewage plume which results will affect most of the coastline up to Zonqor Point. During the time of the present study, the sewage outfall was not fully operational, and the available satellite images confirm that the resultant coastal pollution extends over most of the coastline at least up to Zonqor Point.

The Marine Ecotoxicology Laboratory is presently expanding its activities in new areas and methodologies of environmental management. Recently, as part of an international consortium of research centres, it was awarded a research contract through the EU-funded ESPRIT programme. This project aims at developing an information system for the application of airborne surveillance in the detection, response and management of environmental risks as related to maritime and fisheries activities in local coastal waters.

HABITAT CHARACTERISTICS AND FAUNA OF POSIDONIA OCEANICA MEADOWS IN THE MALTESE ISLANDS

Joseph A. Borg

Seagrass meadows are one of the most important marine habitats since they occupy extensive areas of the seabed in shallow coastal waters throughout the world and are highly productive. Seagrass meadows provide a physical refuge for numerous fish and invertebrate species, food for a large number of species, an extended substratum for attached plants and animals, and support commercial fisheries through their role as nurseries. In the Mediterranean, the endemic seagrass *Posidonia oceanica* (L.) Delile forms extensive meadows at depths of c.1 to 40 m and constitutes a key component of the infralittoral zone. In spite of its importance, the role of *P. oceanica* meadows as a habitat is poorly understood.

A number of surveys and studies have been carried out locally as part of an on-going programme of research on the ecology of *Posidonia oceanica* meadows and their associated biotic assemblages. The main aims of these studies are: (i) to gather data on the distribution and morphological characteristics of *P . oceanica* meadows and compare them with ones available for other parts of the Mediterranean; (ii) to gain a better understanding of the role of P. oceanica meadows as a habitat; (iii) to assess the impact of human activities on this habitat type; and (iv) to develop new methods (e.g. more reliable sampling techniques) for ecological study.

The results obtained to date show that *P. oceanica* meadows attain a high level of structural complexity and occupy large areas of the bottom at depths of 1 to 43m along Maltese coasts. The species richness and abundance of the associated fauna appears to be strongly influenced by a highly complex set of environmental factors. Amongst these, changes in meadow morphology coincident with steep environmental gradients (e.g. depth), availability of food, proximity of other habitats, and biological interactions, such as predation, appear to have the greatest influence. While several macrofaunal species inhabit different microhabitats (foliar and root-rhizome strata) within the same meadow, some species appear to be abundant in both. Comparison of two popular sampling techniques used in the study of the vagile macrofauna inhabiting *P. oceanica* meadows has shown serious disadvantages in the use of both methods.

At present, the variability in community structure of macrofauna associated with *P. oceanica* meadows is being studied. This is being done through a comparison between different meadow types (e.g. patchy, reticulate and continuous), between meadows in different locations, and between different microhabitats (foliar and root-rhizome strata) within the same meadow. The impact of offshore fish-cages on *P. oceanica* meadows and their associated macrofaunal assemblages is also being studied.

THE CORALLINE ALGAE OF MALTESE WATERS

Edwin Lanfranco

Coralline algae are heavily calcified red seaweeds which belong to the order *Corallinales*. Morphologically, coralline algae fall into two groups. The geniculate forms (e.g. *Corallina, Jania, Haliptilon*) have a jointed branched thallus where calcification is weak at the joints thus conferring some degree of flexibility. The non-geniculate forms (e.g. *Lithophyllum, Lithothamnion, Neogoniolithon, Phymatolithon, Mesophyllum*), on the other hand, are completely calcified and are therefore inflexible and with a stony appearance. The latter may be either attached to a substrate or may be unattached thus forming part of the bottom sediments. In the latter case, such unattached corallines may occur in large populations giving rise to maerl communities. Some species occur in both attached and unattached forms, though most species occur predominantly in one or the other state. Of course any species may occur accidentally in an unattached state due to fragmentation or displacement caused by hydrodynamism or bioturbation.

Coralline algae are of considerable ecological importance since they create distinct communities with which numerous biota are associated and because they are responsible for rock formation. Particular communities based on coralline algae include the Mediolittoral pavements or ›trottoirs“ largely made up of *Neogoniolithon notarisii*, often associated with the Vermetid gastropod *Dendropoma petraea*; the rims or cornices, also in the Mediolittoral, constructed by *Lithophyllum lichenoides* (rare in Maltese waters); belts of *Corallina elongata* at the infralittoral fringe, often accompanied by *Phymatolithon lenormandii* and *Lithophyllum incrustans*; the lower infralittoral coralligenous or coralgal communities based on constructions involving several species including *Neogoniolithon brassica-florida* and *Lithophyllum frondosum*; and the deep sea (lower infralittoral,

circalittoral) maerl communities with free-living species, particularly *Lithothamnion minervae* and *Lithothamnion coralloides*.

Because of their calcification, coralline algae are also abundant as fossils and, in fact, two important Maltese rock strata, the Upper Coralline Limestone and the Lower Coralline Limestone, consist of remains of such algae.

MAERL ECOSYSTEMS OF THE MALTESE ISLANDS

Patrick J. Schembri

For the past three years, the Marine Ecology Research Group has been studying the maerl ecosystems of the Maltese Islands as part of an international research project on the biodiversity and structure of these habitats and the impact of human activities upon them.

The term maerl refers to biogenic sediments characterized by accumulations of live and dead unattached coralline algae. The maerl-forming algae can form rhodoliths, which are nodules or unattached branched growths made up of coralline algae. The morphology of rhodoliths varies from open branched thalli to densely branched algal nodules. Their complex structure provides a very heterogeneous habitat. Maerl grounds form a unique habitat with a high benthic biodiversity supporting many rare and unusual species.

Maerl beds have a wide geographical distribution, being known from tropical to polar environments. Their depth distribution varies geographically, mainly depending on the degree of light penetration. For the formation of maerl beds, water movement is needed to overturn the rhodoliths and to prevent burial. However, a degree of shelter is also needed to prevent dispersal of the rhodoliths into non-suitable environments. The processes leading to the development of maerl grounds are not yet fully understood. Rhodoliths can form through the fragmentation of attached branched coralline algae. These coralline algal fragments are then transported by currents and continue to grow at the redeposition sites to form rhodoliths. Rhodoliths can also form by the settlement of asexual spores on suitable sediment particles. Subsequent algal growth completely encloses the particle to form concentric free-living nodules.

In order to achieve their typical concentric growth, rhodoliths need to be regularly overturned so that all their surfaces are periodically exposed to light and would thus be able to photosynthesize. Rhodolith movement can be achieved by various environmental agents including bottom currents, wave-induced bottom disturbance during storms, and bioturbation from benthic organisms during their foraging activities. It is not always clear whether the rhodoliths are being overturned or not; at great depths, bottom currents are probably not strong enough to move the rhodoliths. Some authors state that only slight oscillations are needed for the rhodoliths to survive. It has also been suggested that the lower surfaces of the rhodoliths do not need to be exposed to light, but they survive by means of transport of photosynthetic products from the upper to the lower surfaces.

Rhodoliths show a wide variety of shapes and branching densities. Although the different shapes do not have any taxonomic value, they have a high ecological significance since they provide a range of different microhabitats to benthic organisms. Some authors suggest that the different shapes of rhodoliths are a result of different adaptations to varying intensities of water energy. Thus the shape and branching densities of the rhodoliths can be correlated to the intensity of water movement. Since rhodoliths are common in the fossil record, their morphology has often been used to deduce the palaeoenvironment of the fossil maerl beds. However, other authors have suggested that rhodolith shape may not be solely dependent on the intensity of water movement, but may also depend upon other environmental factors, such as substratum type.

In the Mediterranean and in the North Atlantic, maerl habitats are threatened by various human activities including direct exploitation, demersal fishing, and eutrophication. Commercial collection of maerl is well known in most Western European countries, where it is used as a soil conditioner. Demersal fishing gears can damage maerl beds either by breaking the delicate branches of the rhodoliths or by causing burial of the rhodoliths from resuspended sediments. Being highly productive, many maerl grounds are fished; however, the impact of fishing activities on these grounds is not well documented. Whatever the source of the disturbance, human impact on maerl grounds can have profound effects on this fragile ecosystem, since rhodoliths have a very slow growth rate and cannot be replaced easily.

In Malta, maerl grounds were only recently discovered, during a UNESCO sponsored oceanographic survey in 1993. There is very little scientific information on Maltese maerl grounds and our studies are aimed at improving knowledge of the ecology of this important habitat. The most extensive maerl bed discovered to date in the Maltese Islands covers about 20km2 of the seabed and occurs at depths of between 40m and 80m off the Northeastern coast of Malta and Gozo. This maerl ground is well known to fishermen as being highly productive and in fact they refer to it as 'ramel haj' which literally translated means 'living sand', referring to its importance as a fishing ground for both demersal and pelagic fish.

Two sites within this maerl ground have been chosen for detailed study. One of the sites is impacted by trawling while the other site is not trawled due to the presence of rocky shoals and wartime wrecks which damage the fishing gear. Sampling from these two sites was carried out seasonally for two years (July 1996 - April 1998). A 0.1m2 van Veen grab and a biological dredge were used to collect six grabs and one dredge samples from each site every season. The biota and live maerl from these samples were sorted and analysed. Physico-chemical parameters of the water column were monitored at monthly intervals. The sediment characteristics of the two sites have also been analysed.

From our results, it is evident that Maltese maerl grounds have a rich biodiversity and support a wide range of taxa. However, although the species richness is high, abundances are very low. Of particular interest is the presence of two species of algae, *Flabellia petiolata* and *Polysiphonia setacea*, which bind the maerl to form a pseudo-hard bottom. Due to this binding effect, it is hard to determine whether the rhodoliths of the maerl ground studied do in fact rotate periodically.

It is evident that the impacted and control sites are different. The impacted site has larger rhodoliths and coarser sediments than the control site. This may reflect a higher degree of disturbance at the impacted site, however the source of this disturbance is not clear. Trawling at this site is likely to contribute to this disturbance but is probably not the major factor. There are also some differences in the abundances of the biota at the impacted and the control sites.

This research has been undertaken in the framework of the BIOMAERL project with support from the European Commission's Marine Science and Technology Programme (MAST III) under contract MAS3-CT95-0020. Maltese participation in the BIOMAERL project has been made possible through grants from the University of Malta, the Malta Council for Science and Technology, and the Ministry of Education, for which we are grateful.

CETACEAN RESEARCH AND CONSERVATION AROUND THE MALTESE ISLANDS

Adriana Vella

Numerous surveys have been undertaken since June 1997 with the onset of the first cetacean research project aimed at investigating the cetacean species encountered around the Maltese Islands (in the middle of the Mediterranean sea) throughout the year. The aerial and sea surveys around these Islands show the following results:

Dolphin pods of up to 50 individuals have been observed.

The species which have been seen most often were the bottlenose dolphins (*Tursiops truncatus*), striped dolphin (*Stenella coerulealba*) and common dolphin (*Delphinus delphis*).
While in early summer large groups of striped dolphins have been seen in association with tuna (*Thunnus thynnus*), in autumn large numbers of common dolphins have been seen in association with dolphin-fish (*Coryphaena hippurus*).

The local strandings of a young striped dolphin in June 1997 and of a common dolphin in October 1997 further support the field observations.

Two encounters with single unidentified whales have also been recorded and one with sperm whales.

This cetacean research project has also established links with local sea-users (i.e. fishermen and yachtsmen). Local sea-users have been interviewed on specific cetacean and sea items. The results seem to suggest an increased incidence of dolphins from May to November around the Maltese Islands. Few cases of whale sightings have been recorded. The interview results also highlight the contrasting attitudes of local fishermen towards dolphin species. Some fishermen indeed go to extremes to discourage dolphins from approaching their nets and fish catch. The latter behaviour may be one important reason for the noted decrease in dolphin numbers witnessed by various experienced sea-users (> 30 years at sea).

The results highlight the importance of establishing ways and means of protecting those dolphin species associated with economically important fish populations around the Maltese Islands or in the central Mediterranean region. Further research is aimed toward obtaining photo-ID's of dolphins observed in order to distinguish between resident and transient groups of dolphins encountered. Resident groups found to associate closely with fish of economic importance must be monitored and managed for their effective protection.

Report

London International Youth Science Forum 1998 22nd July - 5th August

Lisa Pullicino

I had heard all about the London International Youth Science Forum and the great two weeks in London from past participants, but secretly thought that they were making a big fuss about it all.....until I went there myself!

I enjoyed every bit of the Science Forum - the lectures, the scientific visits, excursions, visits to museums, the student topics and last but definitely not least, the parties and the International Cabaret. What fascinated me most, however, about the LIYSF was meeting people from over sixty different countries - it's amazing how people from different cultures, from all around the world have common aspirations and goals in mind.

I shall give a brief account of my stay in London between the 22nd July and the 5th August. At the start, the Maltese participants hardly knew each other. I myself only knew two people well - Elaine Cachia who is also studying Medicine, and John Paul Tabone. However, by the end of the visit in London I got to know all the Maltese participants really well, and in fact we all make it a point to keep in contact. Actually, I must say that we Maltese had a great time wherever we went, which earned us the reputation of knowing how to have a good time!!

On the day following our arrival, the opening ceremony was held at the Institute of Electrical Engineers on the Embankment where all the general lectures were held. The opening ceremony consisted of a representative of every country carrying his country's flag, a welcome speech by George Mc Gowan, president of the LIYSF, introductory speeches by past participants of the Science Forum and introduction of all the staff members. Following this ceremony, we had our first general lecture.

I found all the lectures very interesting. Our first lecture was on Egyptian Mummies - delivered by Dr Ron Snaith. Various studies have been carried out on mummies using different techniques such as X-rays, microscopy, rehydration and chemical analysis amongst others. The average lifetime of these mummies was found to be approx. 36 years - science has definitely come a long way since then ! What really impressed me was the long and complex procedure involved in the actual mummification process - removal of brain, cutting out of entrails, treatment of viscera, dehydration of the body, stuffing anointment, treatment with resin and finally wrapping of the body.

Another extremely well-delivered lecture was that by Dr George Savage - "Living Control Systems". He tackled the topic of homeostasis - the maintenance of a constant environment in all physiological systems. The use of the extensive apparatus that the lecturer brought with him made it all the more interesting and stimulating.

Forensic medicine has always been one of my favourite branches of Science, so I found the lecture "Hello, Who Have we Here?" very interesting. Dr David Lowe showed us some slides and asked us to decide on the gender of the bodies. This was not at all that simple to do since we were only shown parts of the body. After this, the discussion was brought to a close with the showing of a number of slides on certain genetic disorders, where one cannot identify the sex of a person simply on the basis of external features - nowadays DNA - fingerprinting makes the identification of gender very simple of course.

"The Utilisation of Solar Energy" delivered by Dr Mary Archer proved to be another engaging lecture. The lecturer focused on the use of photovoltaic cells (which are able to transform light energy into electricity) and of other new technologies such as evacuated tube collection, explaining the underlying physics and chemistry. Everybody found this lecture to be very interesting since solar energy is playing a very important role in this day and age when fuel is becoming more and more scarce.

Cancer was the topic for two of the lectures - two contrasting aspects of cancer, however, were tackled. One lecture "Immunological Approaches to Cancer Diagnosis & Therapy" delivered by Professor Richard O'Kennedy. He spoke about recent advances in research on cancer, that is, studies on the role the immune system plays in the detection, development and elimination of tumour cells. He explained how scientists have now managed to manipulate the immune response so that antibodies can be produced, labelled with drugs, toxins, or pharmaceuticals and targeted to tumours.

One of the lectures focused on an ethical and philosophical issue - Euthanasia. The lecture: "To die or not to die?" was delivered by two speakers - Dr Frances Calman and Dr John Ellershaw. At the beginning of this lecture there was a role play where a terminally ill patient was asking the doctor to relieve her from her suffering by giving her an injection that would kill her. After this role play, we were asked who was in favour of euthanasia and who was against it. We were shown a couple of slides and extracts of some TV programmes and we were split up into groups of six. Every group had two case studies to discuss and after this, towards the end of the lecture we were once again

asked the question we had been previously asked. Surprisingly enough, the outcome this time was different.

The two lectures that I enjoyed most, however, were "Musical Squares", delivered by Dr Mike Gluyas and "Chemistry and Light", delivered by Professor Andrew Mills. The lecture on Musical Squares was packed with sound and visual illustrations. The lecture explored many exciting aspects of sound and it examined the vital role it plays in our lives - from communication and the production of enjoyment of music, to its medical and industrial uses. Using special apparatus we even managed to hear sounds coming from the inside of the body. On the other hand, the chemistry lecture was packed with experiments and colourful explosions. The lecture consisted of a series of illuminations to show how light may be generated electrically, thermally and chemically.

Apart from the general lectures which all participants attended, there were also a number of specialist lectures which groups of around thirty people attended. I attended two of these specialist lectures - one was called: "Ecstasy or Agony? Effects of the Psychoactive Drug MDMA". This was an extremely interesting lecture and Dr Marcus Rattray delivered well and objectively. The last specialist lecture I attended dealt with biochemistry - "Watching Proteins at Work: A Look at Molecular Machines in Biology". In this lecture was explained how motions of proteins are fundamental to a wide variety of processes such as the activity and control of enzymes.

There were also some very interesting seminars. I attended the seminar entitled "Space Astronomy" which consisted of an account of the most recent and exciting astronomical discoveries, and of some violent and exotic objects in the universe. Another seminar dealt with something more practical - "The Pleasures and Pains of Postgraduate Study".

There was much more to the Science Forum than lectures, however. We had the opportunity of visiting many scientific institutions, museums and universities both in and outside London.

On Friday, 24th July, we had a half-day scientific visit organized and I had the opportunity of going to the Department of Biophysics at King's College London. There we had a brief demonstration on X-Ray Crystallography, where were shown all the apparatus involved. The person who showed us round had been working on the same crystal for about two years in collaboration with a pharmaceutical company - Smithline & Beecham. Following this demonstration, we were given a brief talk on cell motility and the structure of eukaryotic genome in relation to expression of genetic material.

Then there was the full day scientific visit - to me, this was the highlight of the Science Forum. I was chosen to go to the Unilever Research Centre in Port Sunlight village in Merseyside. This is a very large research centre producing a wide range of products, e.g., hair products, soaps, detergents, toothpastes, perfumes, chocolates and even ice-creams. There we visited a toothpaste factory, tasted chocolate and designed our own molecules on computers. We visited a lab where we saw gas chromatography of perfumes, and also an exhibition of hair and hair-care products. At the end of the day we went round Porsunlight village which was originally built for the workers of the Unilever Research Centre.

Two days after the visit to Unilever, we spent a day in Cambridge. The first part of the morning was spent at the Department of Physiology at the University of Cambridge . There we had a demonstration on ECG which we could even try the ECG on ourselves! We then had the whole afternoon to ourselves so some of us decided to hire a bicycle (in Cambridge everyone goes round on bicycles) and tour the town.

Another highlight of the Science Forum was our visit to the Welcome Centre for Medical Science. This centre featured extensive use of the latest interactive technology. One exciting thing there is that you can walk through a cell magnified a million times!

The social programme at the Science Forum was well-organized - we had a choice of excursions on Sundays. I chose to go to Stonehenge and Salisbury but I must admit that Stonehenge was quite a disappointment! When compared to Maltese temples, the temples at Stonehenge are nothing special, but that is just my opinion. I enjoyed myself at Salisbury, however. We could also buy theatre tickets from the Science Forum organizers at a relatively cheap rate so I made the most of it and watched "Grease" and "Miss Saigon' managing to arrive late to both shows!!

Other activities organized by the Science Forum staff were the Rounders Match and the Inter Hall Swimming Gala. Unfortunately, College Hall, my team, lost every time. What I really enjoyed was The Great Train Race. This was a competition where we had to split up into groups of four and handed a list of questions to answer. This involved going to various places in London to find out some information, e.g., what are the opening and closing times at Harrods? My team did not fare too badly - we were placed amongst the first five - not bad at all!

Then there were the parties held on board TS 'Queen Mary'. At the Welcome Party we were all still trying to get to know each other and everyone was extremely enthusiastic about the Science Forum. We also had a good time at the Farewell Party but this time we were all rather sad at the thought of leaving the next day.

Science Forum is an amazing experience - the lectures, scientific visits, excursions, parties etc., all contributed to making this forum worthwhile. Sharing the same experiences and living together with three hundred people from sixty different countries around the world is a unique opportunity. I doubt I shall ever have a similar experience in my life. I learnt many things at Science Forum - learnt about the enormity of science and the unlimited capabilities man has in this field; I learnt about

various cultures and ways of thinking through those late night conversations on the lawn outside College Hall with so many people of different cultures. I am sure that the experience of the Science Forum will live with me for the rest of my life.

I would like to thank all the people who have made it possible for me to have such a wonderful experience:

* The sponsors
* Ms Rosette Fenech for her dedication and patience
* The staff of LIYSF, especially Hannah El-Khatib
* All the Participants, especially the Maltese!

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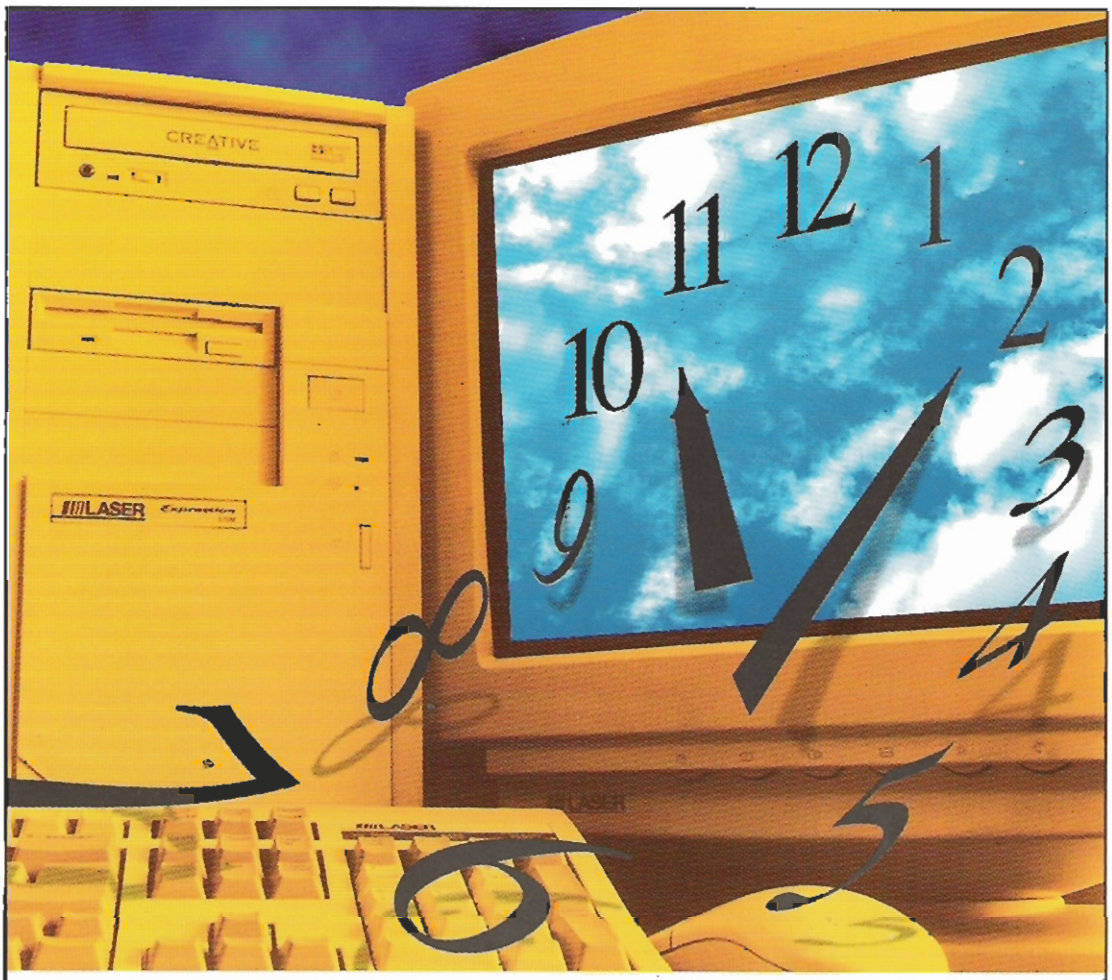

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