# FINAL REPORT

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**Il Proponente** | **Il fruitore**

**Paolo Breber** | **Marisa Florio**

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## INTRODUCTION

Fig. 1 - Plymouth Marine Laboratory

The present report describes the research activities carried at PML, Plymouth Marine Laboratory (Fig. 1) during a period of 21 days in October-November 2008 for an International Exchange Program. This program implements Italian researchers' mobility within the international scientific community so as to increase the possible mutual cooperation. Plymouth Marine Laboratory, located in Plymouth (UK), is a Research Council Institute over thirty years old and a collaborative centre of the Natural Environment Research Council (NERC). It is an independent multidisciplinary marine research centre devoted to understanding how marine ecosystems function and their sustainability and to reducing uncertainty about the complex processes and structures that sustain life in the seas, and their role in the Earth System. It conducts world-class national and international research from the uppermost reaches of estuaries to the open ocean. This is delivered through three core research themes such as biodiversity and sustainable ecosystems, marine biogeochemistry and environment and human health. PML is investing many aspects of the ecological and socio-economic importance of biodiversity at many levels of marine life: from molecular to microbial and metazoan (multicellular organisms), in both plankton and benthos, coastally and offshore, and at a corresponding range of scales, from evualuation and ecology within species to maintenance of key ecosystem functions and services at regional and global scales.

In particular, during my stay at PML I was involved in two exercises: joining the current research activities carried out by the benthos group and the processing of raw data from benthic samples taken by Lesina laboratory using the statistical programmes of PML so as to plan future sampling campaigns to be conducted in Lesina and Varano lagoons (South Italy). These campaigns aim at applying a biotic index (Λ index) developed at Lesina Laboratory (Breber *et al*., 2007). The purpose of this stay, therefore, was to adopt the advanced statistical methods developed at PML which will allow more and better information to be extracted from the past and future samples.

Regarding the first point, I was able to follow the mesocosms experiments, for the study of ecological processes. The term mesocosm (Giesy Jr., 1980) refers to experimental units designed to contain important components and to exhibit important processes occurring in a whole ecosystem.

METHODS

During the first week in Plymouth I got down to analyse my raw data of benthic samples collected in Lesina and Varano lagoons.

For a number of years the ISMAR-CNR laboratory of Lesina (S. Italy, province of Foggia) has been studying the benthic macrofauna of two coastal lagoons, Lesina (5500 ha) and Varano (6500 ha) (Fig. 2), in S. Italy (province of Foggia). The rationale of the work has been to use the benthic macrofauna as an indicator of environmental quality. This has with time brought about the development of a biotic index (Λ) for assessing the ecological status of coastal lagoons. This index grades the increasing quality of the environment from 1 to 10. The data required for applying the index, i.e. spatial distribution, biomass (g ww/m$^2$) and number of species of the benthic macrofauna, are obtained with sediment grabs (quadrat size 15 x 15 cm) taken according to a grid of systematically distributed stations over the entire surface of the lagoon. The samples are taken in the intermediate seasons of spring and autumn in order to register the effects of winter and summer on

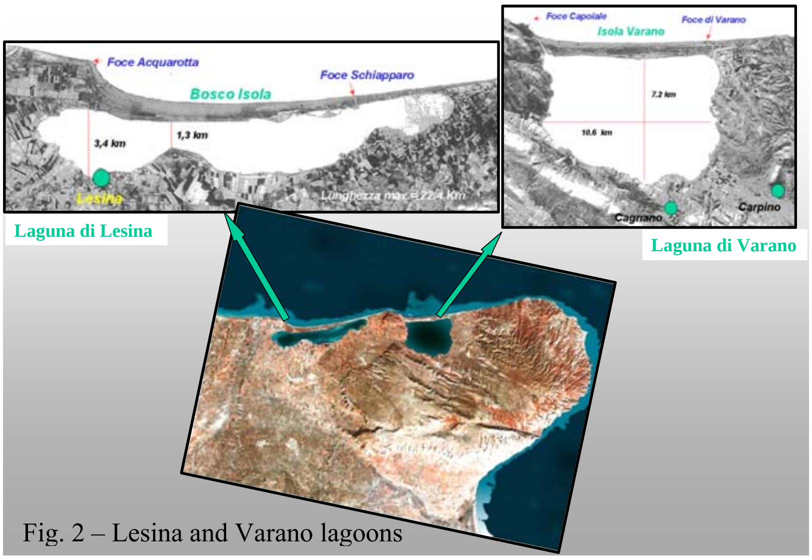


Fig. 2 – Lesina and Varano lagoons

the biocoenosis. Summer especially may be critical since it can be the occasion of harmful microalgal blooms, low levels of oxygen and formation of $H_2S$.

Up to now the ISMAR laboratory of Lesina has followed the usual basic statistical rules in planning the sampling operations and in processing the data. The first task has been to check the statistical validity of the samples taken by the Lesina laboratory in the past, i.e. number of stations, number of replicates, quadrat size. PML staff showed me main techniques that can be employed to simplify and elucidate structure in the data. Data used to statistical analysis were of abundance, biomass and presence/absence. Prior to data analysis, there are several stages of initial data processing that must be conducted. It has been created a species-sample matrix of taxon abundance which should document both quantitatively and qualitatively measured taxa. Statistical methods used for describing assemblage structure can be summarized into three categories: univariate methods, distributional techniques and multivariate methods. The unifying feature is that all data sets are reduced to an appropriate triangular matrix representing the (dis)similarity of every pair of samples, in terms of their assemblages, suites of biomarkers, particle size distributions, shape of growth curves, etc. Univariate methods include different univariate indices analysing mainly the diversity within benthic communities and including total number of individuals, total number of species, diversity, dominance, species richness and evenness. Univariate measurements include particle size analysis for water or sediment samples and size frequency distributions of organisms in cohort studies. Distributional techniques focus on plotting curves showing the dominance pattern within a sample, without reducing the information to a single summary statistic. They allow a visual interpretation of any trends and their statistical significance. Multivariate analyses are also used to determine whether biological assemblages respond to different types of disturbance by small, but consistent changes in the relative abundances of species. These changes might not be detected by comparisons of univariate indices. Multivariate analysis used were *Cluster Analysis* and MDS (*Multidimensional scaling*). The first contains a number of different alghorithms and methods for grouping objects of similar kind into respective categories where members of the groups share properties in common. It can be used to discover structures in data without providing an explanation or interpretation. The first step carried out in PML to run a cluster analysis is to create a table of relative similarities or differences between all objects and second to use this information to combine the objects into groups. The method of combining objects into groups is called a clustering algorithm. The idea is to combine objects that are similar to one another into separate groups.

MDS is a multivariate techniques used in a wide variety of fields, that provides a visual representation of the pattern of proximities (i.e. similarities or distances) among a set of objects. MDS pictures the structure of a set of objects from data that approximate the distances between pairs of the objects. Each object or event is represented by a point in a multidimensional space.

The points are arranged in this space so that the distances between pairs of points have the strongest possible relation to the similarities among the pairs of objects. That is, two similar objects are represented by two points that are close together, and two dissimilar objects are represented by two points that are far apart. The space is usually a two- or three-dimensional Euclidean space, but may be non-Euclidean and may have more dimensions. The degree of disturbance was assessed by means of the abundance/biomass comparison (ABC; Warwick, 1986; Warwick and Clarke, 1994). Finally, for the macrofauna, univariate measures included number of taxa, abundance, Shannon–Wiener diversity H' (log2 basis) and Pielou's evenness J' were applied.

During the two successive weeks at PML, I was involved in the activities of the benthos group. In particular, the staff showed me some experiments focusing on the mesocosm approach for studying both the effects of increasing ocean temperature and the impact of surface ocean acidification. The organisms used in these experiments were the benthic invertebrates and in particular, ophiuroid echinoderms and the early life stages of barnacles over a range of pH and temperature predicted by climate change models. Benthic invertebrates are organisms that live on the bottom of a water body (or in the sediment. The size of benthic invertebrates spans 6-7 orders of magnitude. They range from microscopic (e.g. micro invertebrates, <10 microns) to a few tens of centimetres or more in length (e.g. macro invertebrates, >50 cm). They live either on the surface of bed forms (e.g. rock, coral or sediment – epi benthos) or within sedimentary deposits (in fauna), and comprise several types of feeding groups e.g. deposit-feeders, filter-feeders, grazers and predators. The abundance, diversity, biomass and species composition of benthic invertebrates can be used as indicators of changing environmental conditions. The biomass of benthic invertebrates in estuaries and coastal embayments is often high. It declines if communities are affected by prolonged periods of poor water quality especially when anoxia and hypoxia are common.

Current research of this group is to study the interaction between a warming climate and a more acidic ocean on the ecology and physiology of marine invertebrates, by means of the mesocosms experiments. Mesocosms are enclosed bodies of water or sediment kept under controlled experimental conditions and is a useful way of understanding marine processes and cycles. PML has used large scale sediment mesocosm facilities in Norway to look at the effect of a high $CO_2$ content on benthic invertebrates biodiversity.

Since the benthic mesocosms experiments had only just yet been started, I could follow only the early phase of these activities, but I was provided with a good researcher who gave me detailed explanations.

Ophiuroid samples were collected from the glacial bay at Blomstrand using a hand-hauled dredge operated from one of the Polish RV Oceania's RIBs. Barnacle samples were collected immediately in front of the Marine Laboratory.

The $CO_2$ system consisted of six header tanks which had flowing seawater, three of which were at ambient fjord temperature (~5 C°) and three where at higher temperature (~9 C°). The header tanks were bubbled through with $CO_2$ with a feedback system connecting solenoid valves on the $CO_2$ cylinders to a pH computer. The computers regulated the pH of the water in each header (control 8.2, 7.7 and 7.3).

## RESULTS AND DISCUSSION

For over ten years Lesina Laboratory has been conducting, a survey on the benthos community of two lagoons near each other, Lesina and Varano. This research activity aims to assess the ecological status of two basins but in general it could be used to assess the environmental quality of the each Mediterranean lagoon. In this report only the data about Lesina Lagoon have been analysed and reported in a row. Statistical methods that are very useful for describing assemblage structure have been applied to the sets of macrofauna species counts and biomass collected in 2008. Before data matrices are subjected to either type of analysis, species abundances and biomass data were transformed and similarities between every pair of samples computed using the Bray-Curtis coefficient (Bray and Curtis 1957): $Cz = 2w/(a+b)$, where a is the sum of abundances of all species found in a given sample, b is the sum of species abundances for another sample, and w is the sum of the lower abundance values for each species common to both samples. Ranked lower triangular similarity matrices among samples were constructed using the Bray-Curtis similarity measure calculated on root-root transformed abundance and biomass data. Starting from Bray-Curtis similarity a Cluster Analysis and a Multidimensional Scaling have been carried out. The result of a hierarchical clustering is represented by a tree diagram or dendrogram, with the x axis representing the full set of samples and the y axis defining a similarity level at which two samples or groups are considered to have fused. Fig. 3. shows a dendrogram for the similarity matrix from the Lesina macrofauna abundances.

It could be possible to choose two or more (arbitrary) similarity values at a spread of hierarchical levels, each determining a particular grouping of samples. In Fig. 3, two groups are formed at around a 20% similarity level and eleven groups would be determined at around 60%. The combination of clustering and ordination analyses can be very useful for checking the adequacy and mutual consistency of both representations.

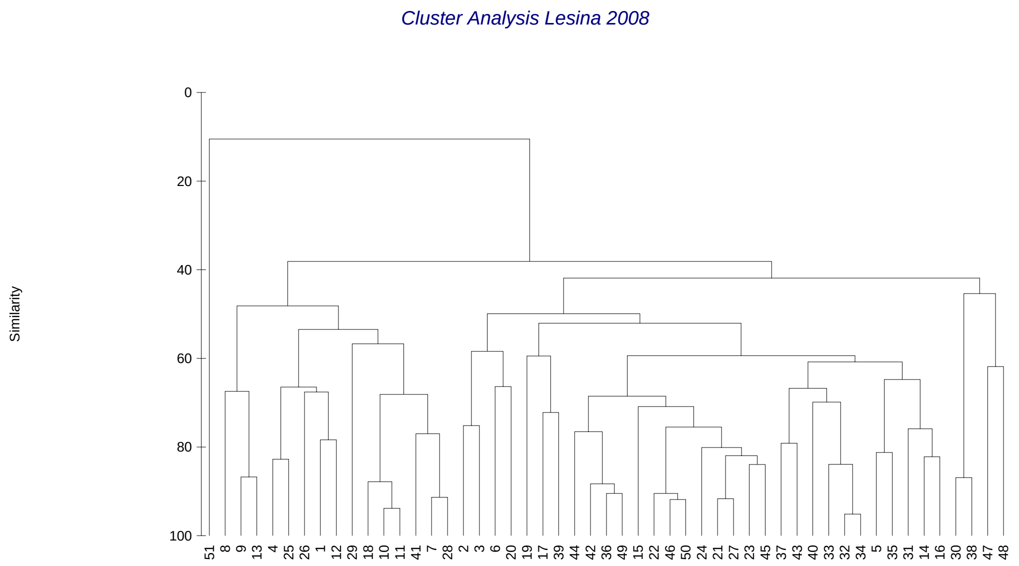



Figure 3 - Dendrogram for hierarchical clustering of fifty-one sites, based on the Bray-Curtis similarity matrix.

The Figure 4 shows a plot MDS from the same data previously used and in order to make a comparison with the cluster analyses the data matrix was treated in exactly the same way prior to analysis.

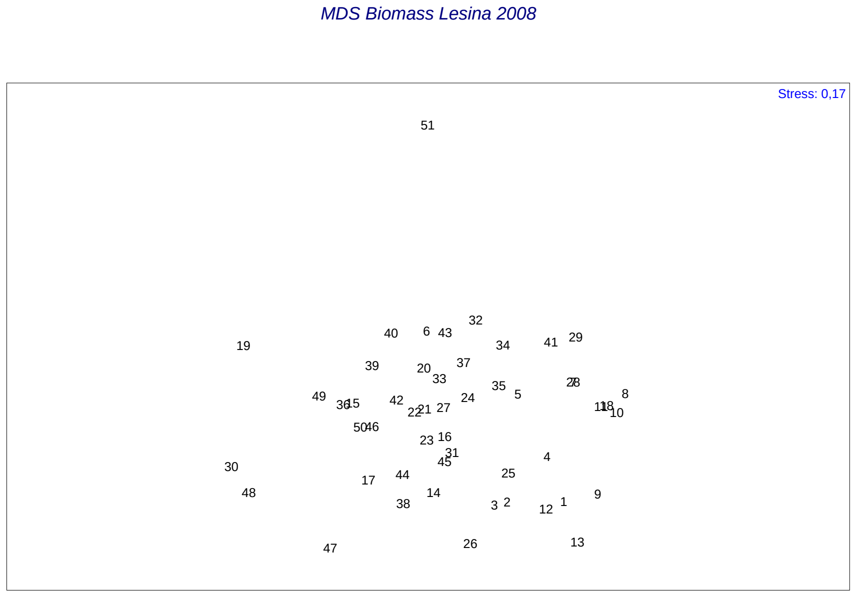
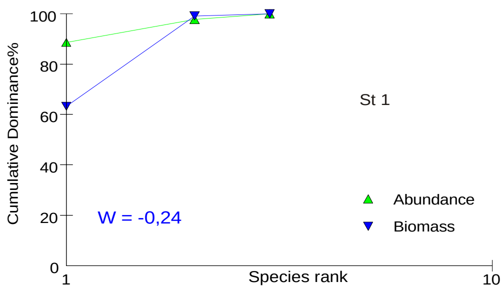
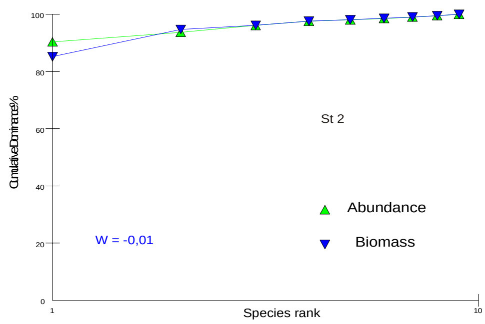


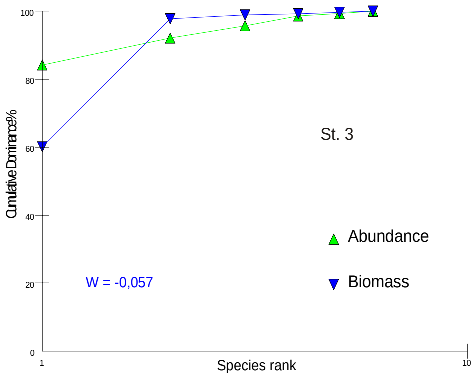

Fig. 4 – MDS plot for 51 station in Lesina Lagoon in 2008.

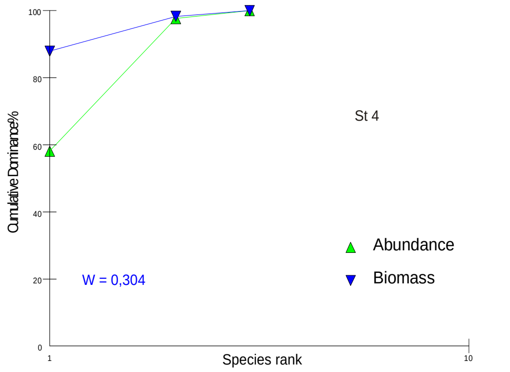
Multidimensional Scaling is popular because it is dependent only on rank information rather than quantitative values, using statements in the form ‘Sample 1 is more similar to Sample 2 than it is to Sample 3. The extent to which these relations can be adequately represented in a 2 dimensional map is expressed as the ‘stress coefficient’ statistic, low values indicating success (e.g. <0.1). In this case the stress for the 2-dimensional MDS configuration is moderately high (at 0.17) indicating some difficulty in displaying the relationship between these 51 samples in two dimension. However, the distance of the sample 51 from all other points. MDS plot agrees the results obtained from the cluster analysis, infact the stations 51 represents a distinct group separated from all others and the stations 30, 38, 47, 48 form another isolated group distinguished from the rest.

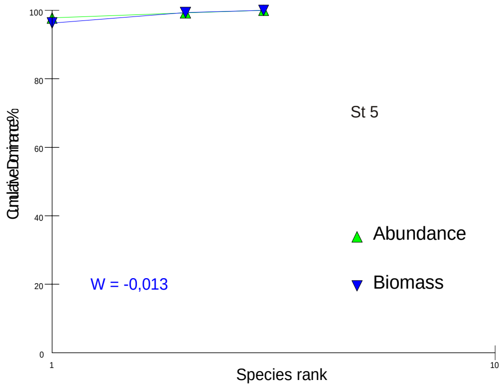
In Fig. 5 are shown ABC curves for the macrofauna at 10 stations in Lesina Lagoon in 2008. This method is based on the assumption that, in the event of environmental disturbance, the distribution of numbers of individuals among species in macrobenthic assemblages behaves differently from the distribution of biomass. Under stable undisturbed conditions, the biomass will become increasingly dominated by one or a few large species, each represented by rather few individuals which are in equilibrium with the existing resources. However, the numerical dominants are smaller species which are out of equilibrium with resources and thus an undisturbed state is indicated if the biomass k- dominance curve falls above the abundance curve throughout its length. As disturbance becomes more severe, macrobenthic communities become increasingly dominated numerically by one or a few very small species, and few larger species are present although these will contribute proportionally more to the community biomass in relation to their abundance than will the small numerical dominants.

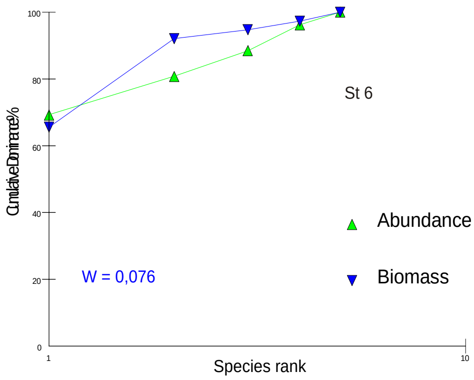


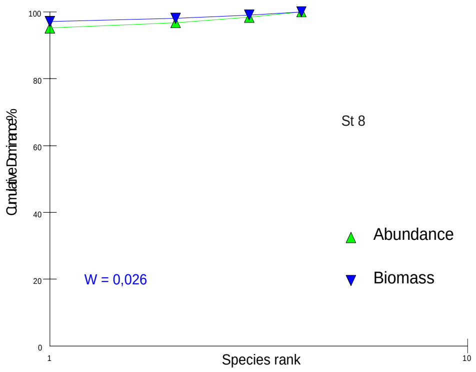



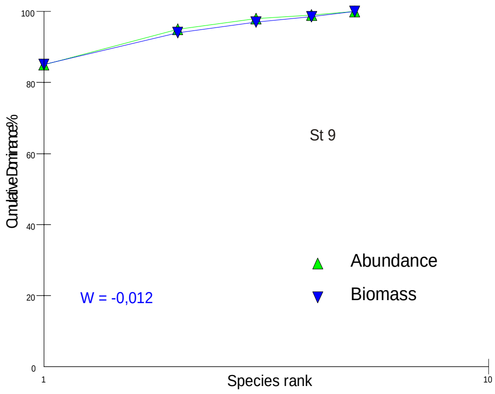


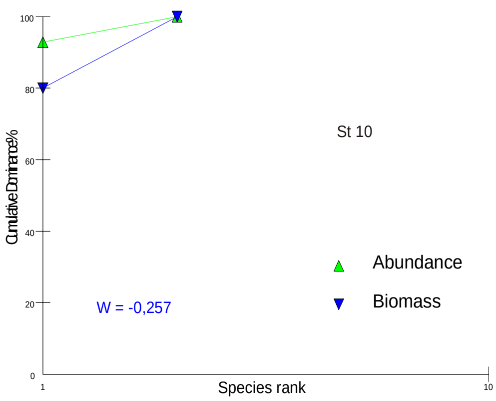




Fig. 5 – ABC curves for stations 1 to 10 in May 2008.

In figure 5, representing only a part of the results, very often k-dominance curves approach a cumulative frequency of 100% for a large part of their length, thus it may be difficult to distinguish between the forms of these curves. Stations 4 and 6 represent a case of a undisturbed community as the biomass curve lies above the abundance curve throughout its length. In contrast, site 10 shows the abundance curve lying above the biomass curve, that is the environment indicates a pollution more severe. Finally, about all other sites, both curves are closely coincident and may cross each other one or more times. In general, these curves and values of the W-statistic suggest that the lagoon, at the stations from 1 to 10, is in a moderately stressed condition. The same approaches have been applied in other Meditarranean lagoons by Riezopoulou et al (1996) who found that methods based on size changes were more sensitive than those based on changes in relative abundance. A total of 21 species were recovered and identified from bottom grab samples. Mollusca was the most abundant taxon, followed by Crustacea, Insecta and Anellida.

The variations in abundance at first sites are reported in Table 1, where it can be already observed the clear dominance of few species. Coastal lagoons typically show low benthic diversity, caused by the natural instability of the environment, which discourages the settlement of many organisms (Gray, 1974). Low values of diversity, low numbers of species and strong dominance of a few species have been found often in lagoonal ecosystems (Guelorget and Michel, 1979; Nicolaidou et al., 1985; Arias and Drake, 1994; Reizopoulou et al., 1996). These species, some exclusive inhabitants of coastal brackish habitats, have marine origin (Kevrekidis et al.,

Fig. 6 – *Abra segmentum*

Fig. 7 – *Neanthes succinea*

1996) and some represent the most abundant species, i.e. *Abra segmentum* (Fig. 6), *Neanthes succinea* (Fig. 7), *Chironomus* spp. (Fig. 9) and *Gammarus aequicauda* (Fig. 8).

Fig. 8 – *Chironomus* spp.

Fig. 9 – *Gammarus aequicauda*

Table 1 – Faunistic inventory and abundance per each station in May 2008.

| **Annelida** | St 1 | St 2 | St 3 | St 4 | St 5 | St 6 | St 7 | St 8 | St 9 | St 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| *Perinereis cultrifera* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Neanthes succinta* | 0 | 1 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Hediste diversicolor* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Nereis* spp | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Neanthes* spp. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Ficopomatus enigmaticus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Mollusca** | | | | | | | | | | |
| *Abra segmentum* | 39 | 187 | 117 | 25 | 133 | 18 | 12 | 59 | 85 | 13 |
| *Cerastoderma glaucum* | 4 | 1 | 11 | 1 | 0 | 0 | 0 | 0 | 3 | 0 |
| *Musculita senhousia* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Mytilaster minimum* | 0 | 1 | 1 | 0 | 2 | 3 | 0 | 0 | 0 | 0 |
| *Mytilaster marioni* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Ciclope neritea* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Hydrobia* spp | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Crustacea** | | | | | | | | | | |
| *Cyathura carinata* | 1 | 7 | 4 | 0 | 1 | 0 | 0 | 1 | 10 | 1 |
| *Sphaeroma serratum* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| *Sphaeroma monodi* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Sphaeroma hookeri* | 0 | 3 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 |
| *Idotea baltica* | 0 | 5 | 1 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| *Gammarus* spp. | 0 | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| *Gammarus aequicauda* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| **Insecta** | | | | | | | | | | |
| *Chironomus* spp. | 0 | 0 | 0 | 17 | 0 | 0 | 0 | 0 | 0 | 0 |

The major biological parameters (number of species *S*, total density N, species richness *d*, species diversity H' and eveness J) at each station in May 2008 are given in Table 2 and in the Figures 10 and 11. The N showed its highest values on station 2 and was minimal at station 12; S and d had also comparatively higher values at station 2 and the lowest values at station 7. H had its highest values on station 6 and was minimal at station 7, as well; J showed its highest value at station 4. In the Figure 11 it can be also observed that the diversity index show a similar trend, with a abrupt decrease at the 5 and 7 stations.

Table 2 - species number (S), number of individuals (N), species richness (d), species diversity (H) and evenness (J) of the macrobenthic fauna at the ten sampling stations in May 2008.

| Station | S | N | d | J' | H' |
|---|---|---|---|---|---|
| 1 | 3 | 44 | 0,529 | 0,374 | 0,593 |
| 2 | 9 | 207 | 1,500 | 0,221 | 0,702 |
| 3 | 6 | 139 | 1,013 | 0,356 | 0,921 |
| 4 | 3 | 43 | 0,532 | 0,701 | 1,110 |
| 5 | 3 | 136 | 0,407 | 0,109 | 0,173 |
| 6 | 5 | 26 | 1,228 | 0,636 | 1,477 |
| 7 | 1 | 12 | 0,000 | | 0,000 |
| 8 | 4 | 62 | 0,727 | 0,178 | 0,356 |
| 9 | 5 | 100 | 0,869 | 0,351 | 0,816 |
| 10 | 2 | 14 | 0,379 | 0,371 | 0,371 |

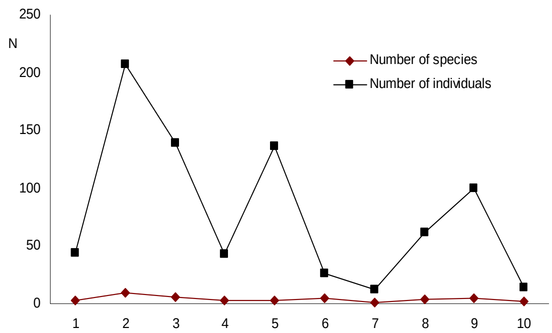



Fig 10 – Number of species and individuals at each sampling site in May 2008.

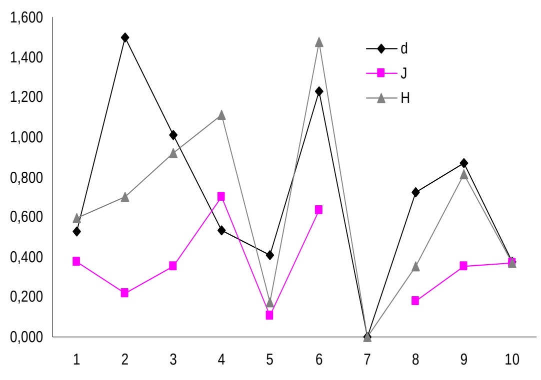



Fig 11 – Species richness, species diversity and evenness of the macrobenthic fauna at each sampling site.

CONCLUSIONS

My stay in Plymouth has been very important for me because I had the occasion to know many marine ecologists involved in important research programmes at PML, and I learnt some statistical methodologies very useful in the biological field. Moreover I noticed that some topics treated at Lesina Laboratory, are studyied in depth at PML. The study of benthic invertebrates is very important, in fact they are sensitive and reliable indicators of habitat quality in aquatic environments. This is because most benthic organisms have limited mobility and cannot avoid changes in environmental conditions. Benthic organisms live in bottom sediments where exposure to contaminants and oxygen stress is most frequent. The benthos includes diverse taxa representing a variety of sizes, modes of reproduction, feeding guilds, life history characteristics, and physiological tolerances to environmental conditions. Therefore, they respond to and integrate natural and anthropogenic changes in environmental conditions in a variety of ways. The analysis of these organisms with new methods opened some as yet aspects of the two Lagoons, Lesina and Varano, and the use of multiple approaches increased the statistical robustness of our environmental assessments. These methods can be used to monitoring the two coastal lagoons, which are of considerable naturalistic and economic interest but usually characterized by marked daily and seasonal fluctuations in environmental conditions (e.g. in salinity) depending on their geomorphology and hydrological regime. Additionally, in many cases, these coastal environments suffer severe dystrophic crises during summer, being followed by mortality events. Although coastal lagoons have been the subject of extensive studies, our knowledge of the factors determining the spatial and seasonal variation in their macrozoobenthic communities, especially in the microtidal Mediterranean Sea, is restricted and mainly related to abiotic rather than biotic factors. This study allowed to understand the structure and function of coastal brackish ecosystems through the description of the distribution of the macrobenthic fauna in Lesina Lagoon and of the seasonal variation in macrozoobenthic community structure and dynamics at very low salinities (Athanassios *et al*. 2005).

Multivariate clustering methods, such as classification and ordination (Field *et al.*, 1982), are sensitive for detecting changes in community structure, but they do not show whether the changes are in the direction of a climax community (presumably positive), or attributable to natural or human-induced disturbance (presumably undesirable or negative). The Abundance Biomass Comparison (ABC) method was initially proposed by Warwick (1986) as a technique for monitoring disturbance (mainly pollution effects) on benthic invertebrate communities, by comparing dominance in terms of abundance with dominance in terms of biomass. Subsequently, it has been applied to marine benthic communities in different regions, and in most cases showed the expected changes in response to disturbance (Warwick et al., 1987; Agard et al., 1993).

ABC curves have a theoretical background in classical evolutionary theory of r- and k-selection. In undisturbed states, the community is supposed to be dominated by k-selected species (slow-growing, large, late maturing), and the biomass curve lies above the abundance curve. With increasing disturbance, slow-growing species cannot cope, and the system is increasingly dominated by r-selected species (fast-growing, small, opportunistic), and the biomass curve will be below the abundance curve. A strongly disturbed state is therefore indicated if the abundance k-dominance curve falls above the biomass curve throughout its length (Clarke & Warwick, 2001).

An advantage of the method is that an appropriate data set for any area or time should allow the status of the community to be evaluated without the need for a spatial or a temporal control against which to compare the index obtained, because the biomass is compared with the abundance for the same time and place (Clarke and Warwick, 1994). In this study the results of the ABC Curves and their corresponding W statistic for the benthic macrofauna, suggest that in Lesina lagoon assemblages have become increasingly stressed, according to the classification developed by Warwick (1986). Moreover, the low diversity, mainly at the 7, 8, 9 and 10 site, could be explained along is a result of the very variable environmental conditions in the lagoon, which are due to their shallowness and their restricted communication with the marine environment. Natural stress levels increase with increase in confinement, which results in a decrease in the variety of species and an increase in the abundance/number density of individuals of a few species (Guelorget and Perthuisot, 1983, 1992). The sites considered are also those nearest to the city and in lagoonal ecosystems, which are very complex, hydrological, sedimentological and anthropogenic activities could have a synergistic effect on biodiversity. Conseguently, the evaluation of the ecological status in lagoons is difficult because, in such extreme natural environment, it is not easy separate the effects of natural man-induced effects.

Thus, the study of macroinvertebrate population dynamics and natural production rates is necessary for informed decisions about the conservation and management of this lagoon system. Further research in comparative studies of various lagoonal systems is needed to identify the spatial and temporal patterns in such complex ecosystems. This could be helpful, not only for a better understanding of lagoonal systems, but also to point out a general model governing lagoons, without excessive emphasis on local variability, thus focusing toward developing large-scale management and conservation policies. This first contact with the PML researchers, may be seen as the beginning of a possible fuller collaboration between the two institutions involved.

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