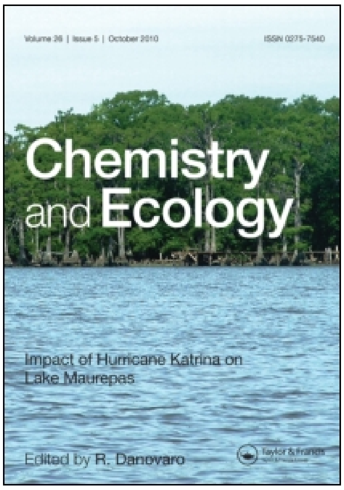

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BIOLOGICAL BENTHIC TOOLS AS INDICATORS OF COASTAL MARINE ECOSYSTEMS HEALTH

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Coastal marine ecosystems are increasingly subjected to environmental stress and degradation due to pollution. Several research programmes have addressed this problem and produced relevant data sets for specific areas, often including consistent sets of environmental and biological variables. The value of existing information gathered from these types of data can be largely increased by combining them into a common data set to determine globally applicable relationships. To perform this exercise, the Intergovernmental Oceanographic Commission (IOC) of UNESCO has recently formed the *Ad hoc* Study Group on Benthic Indicators (http://www.ioc.unesco.org/benthicindicators) with the aim of developing robust indicators of benthic health. In this paper, initial products and ongoing activities of this international initiative are described and discussed. An expansion of initial IOC/UNESCO research on benthic fauna-organic carbon relationships is also presented. As part of this follow-up research, the relationship between total organic carbon concentrations of sediment and abundance, biomass and species diversity of benthic macrofauna was evaluated using data sets from 2 different regions of the world comprising 3 different coastal marine environments. The ability of identifying threshold levels in selected variables that could serve as indicators of related adverse environmental conditions leading to stress in the benthos is envisaged within the frame of a larger joint analysis, carried out by the IOC/UNESCO Study Group on Benthic Indicators, of merged data sets from several coastal regions worldwide.

Keywords: Benthic macrofauna; Total organic carbon; Biodiversity; Bioindicators; Sediments

1 INTRODUCTION

The assessment of indicators of the ocean health is a relevant issue of Chapter 40 of UNCED (United Nations Conference on Environment and Development) Agenda 21, which calls for a sustainable use of the marine environment and the coastal zone. To cope with this objective, the Intergovernmental Oceanographic Commission (IOC) of UNESCO has launched several programmes and initiatives such as the Global Ocean Observing System (GOOS) and the Global Investigation of Pollution in the Marine Environment (GIPME). Particularly, the HOTO (Health of The Ocean) module of GOOS, formed under the auspices of GIPME and now existing as COOP (Coastal Ocean Observation Pattern)-IOC Ocean Science Section, embraces the idea of adopting common and integrated strategies for assessing the effect of anthropogenic activities on the marine environment. In this framework, the assessment and use of common methodologies and tools are considered a major path in capacity building, quality control and environmental impact assessment.

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Figure 1 shows an array of biological tools, ranging from molecular to organism level, from community to ecosystem level, for evaluating adverse conditions of the ocean based on: (1) their ability to predict early *vs.* late damages, and (2) their ecological relevance. A set of biological (benthic) tools relatively difficult to measure though having a high ecological relevance includes: distributional changes, altered abundance and reduced diversity (Fig. 1).

This work, carried out within the international initiative started by the Ocean Science Section of the IOC of UNESCO in 1999 within the HOTO framework and denominated the *Ad hoc* Study Group on Benthic Indicators (http://www.ioc.unesco.org/benthicindicators), will focus on these latter measures conducted on benthic macrofaunal communities and aims at assessing indicators of marine benthic health.

In shallow water, benthos dynamics are tightly related with processes occurring in the overlying water column (Cowan *et al.*, 1996; Magni and Montani, 2000; Fabiano *et al.*, 2001; Magni *et al.*, 2002). Integrated measurements of physical, chemical and biological components of the benthos may thus represent important tools in the assessment and implementation of existing observing systems.

A list of potential indicators of benthic condition has been compiled by the IOC/UNESCO *Ad hoc* Study Group on Benthic Indicators which identifies *Non-Disturbed vs. Heavily Disturbed* environments on the basis of environmental and biological characteristics of the benthos in unconsolidated substrates (Hyland *et al.*, 2000). They include benthic faunal condition (*e.g.* measure of community composition, structure, biomass and functional aspects), controlling natural abiotic factors (*e.g.* salinity, sediment properties, sediment organic

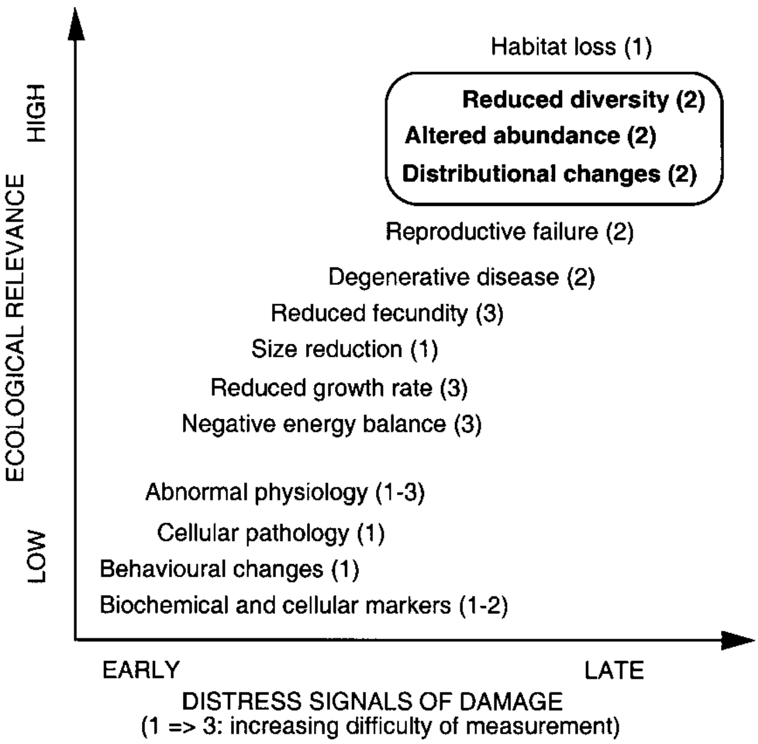

FIGURE 1 Biological distress signals of damage to ocean health (adapted from IOC/INF-1044 HOTO Strategic Plan, IOC Information Document Series, May 1996). In bold and circled: signals of major concern to this work.

matter), and sediment-associated stressors (*e.g.* sulfide, ammonia, contaminants). In this context, the IOC/UNESCO *Ad hoc* Study Group on Benthic Indicators has merged data sets containing synoptic information on environmental variables and benthic macrofaunal communities from several coastal regions worldwide. As such, it is aimed to identify threshold levels in selected variables that could serve as indicators, or "warning signals," of related adverse environmental conditions leading to stress in the benthos. Important attributes of such indicators should be: (1) reliability in their ability to detect stress and (2) ease of use and broad applicability in different ecosystems. As an initial study, a global analysis on the relationship between benthic macrofauna and TOC (total organic carbon) has provided compelling and statistically robust evidence of a marked reduction of species diversity along a gradient of organic carbon in sediments (Hyland *et al.*, submitted).

In this paper the relationships between organic matter enrichment of sediments and the structural and functional properties of benthic macrofauna are reviewed and tested. Independent data sets from 2 different regions of the world comprising 3 different coastal marine environments were merged, including additional parameters and data from a coastal lagoon in the western Mediterranean Sea (Magni *et al.*, in press) not used in the initial IOC/UNESCO analysis (Hyland *et al.*, 2000).

2 MATERIAL AND METHODS

Data sets containing the same environmental and biological variables were selected from a sandflat and an adjacent subtidal zone of an estuary in the Seto Inland Sea (Japan), and from the coastal lagoon of Cabras in the Gulf of Oristano, Western Mediterranean Sea (Italy). Details of sampling procedure and study site characteristics are given in Magni (1998), Magni and Montani (1998) and Magni *et al.* (2000) for the Seto Inland Sea (70 and 18 stations at the intertidal and subtidal sites, respectively), and in Magni *et al.* (in press) and De Falco *et al.* (in press) for the latter site (26 stations). Briefly, at all sampling dates duplicate sediment samples were collected at each station using grabs or stainless cores with a surface area varying from 0.01 (sandflat site) to 0.04 m^2 (subtidal site). They were subsequently sieved on a 1 mm (Seto samples) or a 0.5 mm (Cabras lagoon samples) mesh-size screen. The residue was fixed with a buffered formaldehyde solution, stained with rose Bengal, for later determination of macrofauna. In the laboratory, after macrofaunal sorting, organisms were transferred into a 75% ethanol solution. For each replicate, animals were sorted under a stereomicroscope and classified to species level where possible, and for each replicate and species or *taxon* the number of individuals was counted and the total biomass was measured. All individuals of a species or *taxon* in a given core were grouped for biomass measurements. Wet weights (WW) of polychaetes were obtained from each sample after carefully blotting off any fluid excess. Wet weight soft tissue biomass of dominant bivalves, *Musculista senhousia* and *Ruditapes philippinarum* (Seto Inland study sites), was calculated as 32% and 19% of the total weight, respectively, based on total weight *vs.* soft tissue and shell length direct measurements (Magni, 1998); that of minor/uncommon species with shells or exoskeleton was calculated as 20% of the total weight. The macrofaunal communities at each station were analysed for values of species diversity by mean of the Shannon's index, *H*' (Shannon-Weaver, 1949), and the associated evenness component, *J*' (Pielou, 1966), using the PRIMER (Plymouth Routines In Multivariate Ecological Research) programme (Clarke and Warwick, 1994). Total organic carbon (TOC) concentration of sediment was determined from dried subsamples using a Fisons CHN analyzer, following the procedure described by Froelich (1980) modified by Hedges and Stern (1984).

3 RESULTS AND DISCUSSION

3.1 Benthic Fauna-Organic Matter Relationship: General Patterns and Conceptual Model

According to a widely used model for benthic fauna-organic matter relationship (Pearson and Rosenberg, 1978), a given biological variable (*e.g.* macrofaunal abundance, biomass, species richness) would increase to varying extents in relation to increasing organic matter up to a certain threshold level, and then would decline (Fig. 2). Population increase below the threshold level reflects a combination of the nutritional value of moderate amounts of organic matter present in the sediment and other favourable environmental conditions (*e.g.* high levels of dissolved oxygen and low levels of ammonia and sulfide). An excessive organic load, on the other hand, exposes the benthos to physiological stress (Diaz and Rosenberg, 1995; Gray *et al.*, 2002), and the biological variable starts to decline as an evidence of a degraded environment.

Although this relationship has been widely used over the past three decades to describe distributional and spatial changes of macrofaunal communities along a gradient of organic enrichment, it must be considered that such a model is descriptive rather than predictive (Gray *et al.*, 2002). In particular, quantitative relationships (*i.e.* with an indication of real values and/or thresholds) between measures of community condition and organic matter of sediment or any related environmental variable are still lacking. Several research programmes have addressed the problem of organic matter enrichment in coastal marine ecosystems and produced relevant data sets for specific sites, often including consistent sets of environmental and biological variables. The value of existing data can be largely increased by combining them into a common data set to determine large-scale relationships. The results presented in this work are intended to give an example of the potential of merging independent data sets containing comparable environmental and biological information into common analyses. In particular, it is assumed that an evaluation of the distribution of macrofaunal communities along a gradient of organic enrichment would be enhanced by using data sets from different study areas characterized by marked differences in the organic carbon

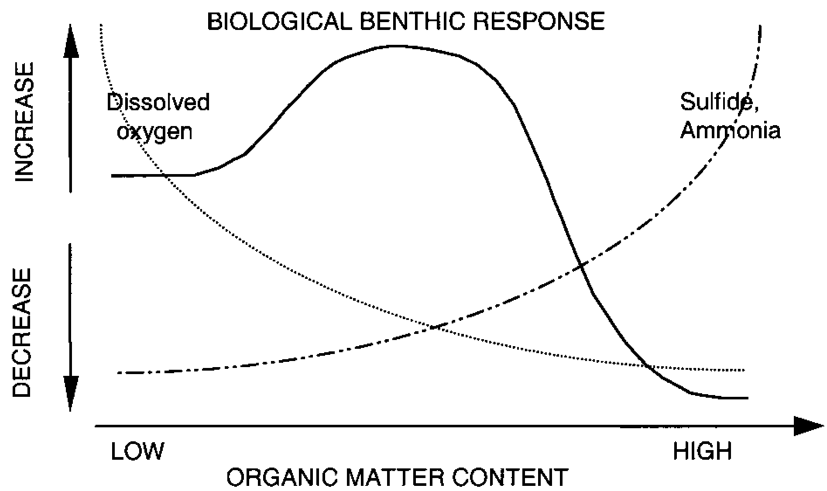

FIGURE 2 Conceptual model describing the relationship between a given biological benthic variable, organic matter content of sediment and potentially co-varying sediment stressors (modified after Pearson and Rosenberg, 1978).

concentrations of sediments, *i.e.* covering a wide range of TOC values. As a follow-up research of initial investigations on benthic fauna-TOC relationships (Hyland *et al.*, 2000), simple measures of macrofaunal condition (*i.e.* abundance, biomass and species diversity) are considered in this study in order to assess and test dominant patterns of macrofaunal distribution according to the earlier conceptual model (Fig. 2; Pearson and Rosenberg, 1978). Additional univariate (*e.g.* Hurlbert, 1971) and multivariate (Clarke, 1993; Clarke and Warwick, 1994) diversity measures or more recently developed taxonomic indices (Warwick and Clarke, 1995; Clarke and Warwick, 2001) are recommended and envisaged in future analysis of larger data sets from more locations.

3.2 Benthic Fauna-TOC Relationship: The Use and Potential of Data Set Merging

The data sets merged in this study highlighted a marked separation of the three study areas along a gradient of sediment organic carbon, especially between the intertidal flat of the Seto Inland Sea of Japan and the coastal lagoon in the Mediterranean Sea (Fig. 3). This was consistent with major differences between a highly dynamic and self-purifying ecosystem, typical of the intertidal zone where sediments are daily exposed during low tide (Montani *et al.*, 1998; Magni *et al.*, 2002), and a eutrophic basin characterized by poor water mass exchange with the coastal sea, such as the lagoon of Cabras (Magni *et al.*, in press). The high macrofaunal biomass at relatively low to moderate TOC concentrations (Fig. 3b), mostly belonging to

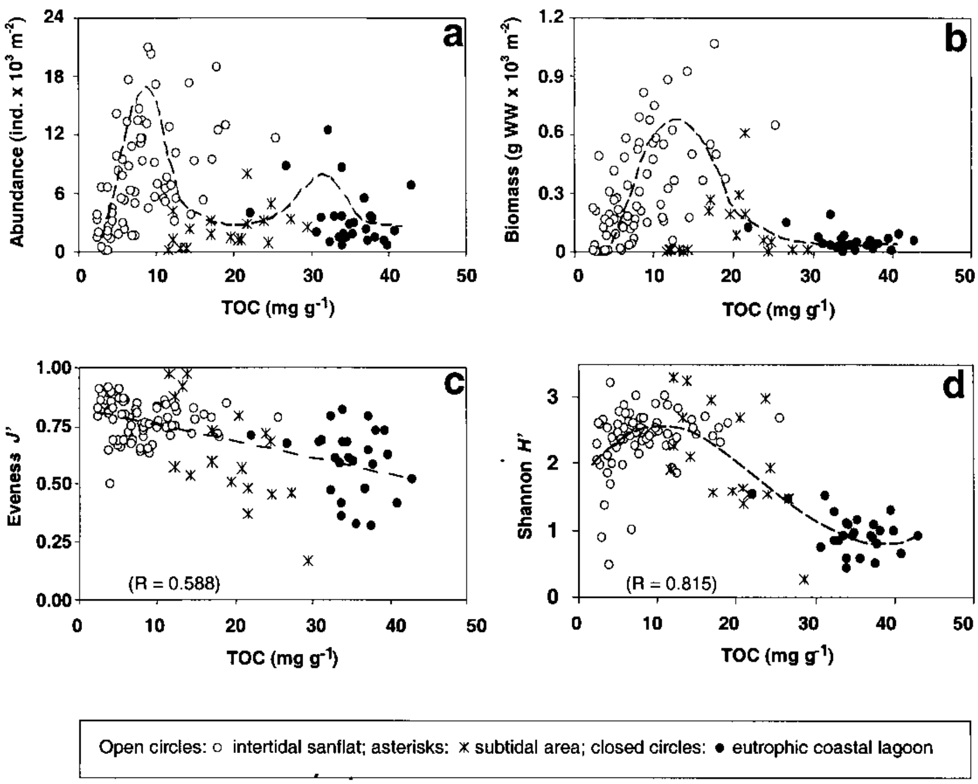

FIGURE 3 Distribution of various measures of benthic macrofaunal condition along a TOC gradient: (a) abundance, (b) biomass, (c) evenness J' and (d) Shannon diversity H' (curves and regression lines: see explanation in the text).

the sandflat samples dominated by the filter-feeding bivalves *Musculista senhousia* and *Ruditapes philippinarum* (Magni *et al.*, 2000), was an indication of a healthier ecosystem with a high potential for both primary and secondary production (Magni *et al.*, 2000; Montani *et al.*, 2003). Intermediate TOC concentrations were representative of the subtidal study site (6–10 m high tide water depth), characterized by relatively large variations of macrofaunal abundance and biomass distribution (Fig. 3a and b), also due to local and seasonal differences (Magni, 1998). At the same time, the subtidal samples, although limited to a few ones, thus not broadly applicable individually, contributed to the trend of decreasing diversity values at increasing TOC concentrations (Fig. 3c and d). By contrast, the coastal lagoon of Cabras is a shallow (*ca.* 1.7 m mean depth) microtidal basin suffering from an excessive organic load of sediments, apparently increased over the past few decades (De Falco *et al.*, in press), with TOC concentrations homogeneously high, up to $>40\,\mathrm{mg\,g^{-1}}$. This situation determines severe reduced conditions of sediments in most part the lagoon (Magni *et al.*, in press), with negative Eh values persisting throughout the year, up to $> -400\,\mathrm{mV}$ during the warm period, and the tendency to distrophic events (Magni *et al.*, unpubl.). These constraints matched with very poor macrofaunal communities dominated by organic pollution-tolerant species, such as the polychaetes *Polydora ciliata* and *Neanthes succinea*, and Tubificidae, as an evidence of a highly degraded ecosystem (Magni *et al.*, in press).

The relationships between benthic macrofaunal communities and TOC obtained from the present data sets also demonstrated some general trends of macrofaunal distribution along a gradient of sediment organic enrichment, to varying extents for the different measurements (Fig. 3). The distribution of macrofaunal abundance and biomass well fitted in arbitrarily depicted curves, which were conceptually modeled in accordance with patterns indicated by classical studies (Rhoads, 1974; Pearson and Rosenberg, 1978). In particular, a bimodal curve describing a major peak at low to moderate TOC concentrations and a secondary, minor peak at relatively high TOC concentrations consistently represented macrofaunal abundance (Fig. 3a). The primary peak indicated a marked increase of abundance at TOC concentrations between <5 and $10\,\mathrm{mg\,g^{-1}}$. The secondary peak appeared to be consistent with the expected increase in the number of individuals of a fewer opportunistic (resistant) species, accompanied by a progressive decrease of the total number of species (Pearson and Rosenberg, 1978). In contrast, biomass showed a single and less sharp peak, slightly shifted towards TOC concentrations relatively higher than those corresponding to the primary peak of abundance, *i.e.* between 10 and $15\,\mathrm{mg\,g^{-1}}$ (Fig. 3b). Such a curve demonstrated a drastic reduction of macrofaunal biomass at TOC concentrations $>25\,\mathrm{mg\,g^{-1}}$.

The expected reduction of macrofaunal abundance and biomass at increasing TOC concentrations was consistent with a major decline of macrofaunal species diversity (Fig. 3c and d). In particular, the decrease of the Pielou's index, *J'*, was described by a significant linear regression ($y = -0.007x + 0.83$; $R = 0.588$, $p < 0.001$), which clearly separated the macrofaunal communities of the sandflat, of the subtidal zone and of the coastal lagoon (Fig. 3c). Also the Shannon's index, *H'*, strongly correlated with TOC concentrations following a polynomial function ($y = 0.0002x^3 - 0.01x^2 + 0.21x + 1.56$; $R = 0.815$). This equation showed an initial increase of the curve up to TOC concentrations around $10\,\mathrm{mg\,g^{-1}}$, followed by a marked decrease having a lowest inflection at TOC concentrations around $35\,\mathrm{mg\,g^{-1}}$ (Fig. 3d). These relationships, based on best-fit regressions with the highest *R*, have to be considered with caution as they are intended to give an example of the power of evaluating general trends by merging independent data sets. Accordingly, they suggest that by using a larger number of study sites (*i.e.* larger number of data set points), and by looking at benthic fauna-stressor relationships across these sites, it may be possible to identify threshold levels in environmental variables that can serve as useful indicators of adverse conditions in the benthos.

Some methodological considerations relevant to the merged patterns and analysis of this study are also needed. Although similar procedures were used in the different studies, there were also some methodological differences, such as sampling area and sieve mesh-size. It is well known that some benthic indices are more independent of sample-size differences than others (Clarke and Warwick, 1998; Warwick and Clarke, 2001). The classical variables used in this study, including abundance, biomass, the diversity index H' and the associated evenness component J', are rather dependent on sampling effort and on subsample size. For instance, the Pielou's index, J', (reflecting evenness of abundance across species) decreases at increasing number of individuals sampled, the latter being a function of the actual surface area sampled within an individual station. Consistently, the Shannon diversity index, H', is also known to be quite sensitive to sample size (Clarke and Warwick, 1998). According to this concept, one may claim that the relationships obtained from the present merged data sets could be biased (*i.e.* underestimated) towards the samples collected from the smaller surface area and using larger mesh-size. This was the case, on the other hand, of the sandflat samples ($0.01\,m^2$ and 1 mm, respectively) which had, for instance, besides such an hypothetical underestimation, medium to high H' values (Fig. 3d). These results may lead to speculate that more marked differences may actually occur between the sandflat samples and those from the organically-enriched lagoon, which had very low H' values besides being collected from a larger surface area ($0.02\,m^2$) and using a smaller mesh-size (see Material and Methods). Besides such considerations based on the results obtained from these specific data sets, it must be considered that derived indices and data-analysis approaches taking into account for the variability due to different sampling techniques are certainly needed in order to look at more precise quantitative relationships between benthic macrofaunal communities and TOC.

It should also be acknowledged that, due to the conservative nature of organic carbon in marine sediments, the response of benthic fauna to organic enrichment may strongly differ upon the quality (*e.g.* nutritional value) of organic matter. For instance, the biochemical composition of organic matter has been shown to be a more evident index of changes in the trophic state of the sediment than the mere organic carbon or organic matter concentrations (Fabiano *et al.*, 1995; Dell'Anno *et al.*, 2002). Accordingly, the TOC concentration itself may have a limited sensibility in detecting natural *vs.* anthropogenic changes of trophic conditions (Dell'Anno *et al.*, 2002). Another source of uncertainty to a general applicability of the relationships presented in this study may be the contribution of soot (black) carbon to the total organic carbon, the former usually assumed to be chemically and biologically inert in the marine environment (Berner, 1982) and shown to be largely variable between sites, especially in organic-carbon poor sediment samples (Middelburg *et al.*, 1999).

Given these clarifications, the present study provides some general indications and recommendations on the use of biological benthic tools, demonstrates a link between several measures of macrofaunal condition and TOC, and may represent a contribution to further elaboration on larger data sets. Interestingly, the results of this study (*e.g.* Fig. 3d) were consistent with the findings from independent analysis of larger data sets in which neither the intertidal samples (Seto Inland Sea, Japan) nor the lagoon samples (Cabras lagoon, Mediterranean Sea, Italy), presented here, were used (Hyland *et al.*, submitted). In particular, Hyland *et al.* (submitted) most recently demonstrated a significant inflection of a regression correlating species diversity and TOC at organic carbon concentrations > about $36\,mg\,g^{-1}$. As a follow-up of the initial recommendations by the *Ad hoc* Study Group on Benthic Indicators (Hyland *et al.*, 2000), the results of this study confirm and support the idea that macrofaunal communities and associated environmental variables, such as organic carbon concentration of sediment, are an important aid for evaluating, thus monitoring and assessing, the health of coastal marine environments.

3.3 Evaluation of Biological Benthic Tools in the Mediterranean Region

One of the major issues of the implementation of integrated coastal zone management (ICZM) is the development of guidelines for the evaluation and assessment of the health of ecosystems. Awareness on this need is growing at both international and national levels. Specific programmes and new initiatives have started, which focus on the development of sedimentary tools and promote the use of common approaches with applicable outputs to stakeholder and community end users, such as the European Land Ocean Interactions Studies (ELOISE) Network and the Italian Network for Ecological Research in Coastal Zone and Transition Areas (http://www.dsa.unipr.it/lagunet/). Recent studies have further proposed integrated benthic indices of biological and biogeochemical integrity for assessing habitat quality (Van Dolah *et al.*, 1999; Dell'Anno *et al.*, 2002; Viaroli *et al.*, submitted), representing an important contribution towards a sustainable management of the coastal zone.

This work is relevant to the first MedGOOS (Mediterranean Global Ocean Observing System) EC project MAMA – The Mediterranean network to Assess and upgrade Monitoring and forecasting Activity in the region. It is embraced the idea of the need of scientific assessment of existing ocean observing (and monitoring) systems in the Mediterranean at regional, coastal and national scales to design scientifically proven and cost effective coastal data acquisition systems, fully integrated to the basis scale systems. Within this context and in order to evaluate the potential of the use of biological benthic tools in the region, a survey is sought among the scientific community in order to evaluate the extent of existing data relevant to the benthos, including both biological and environmental variables. The existence and/or availability of *Benthic Information* in the Mediterranean region could be assessed through the compilation of an exploratory questionnaire to be distributed to relevant Institutions. Such a survey on *Benthic Information* would be collected as a spreadsheet file and subsequently organised in the form of a common database with meta-data (*i.e.* data referring to the existence/availability of different types of data without containing the actual values). The questionnaire should include major and simple features relevant to the benthic environment (Hyland *et al.*, 2000) to help, firstly, to identify (1) where such data exist, and (2) the extent to which scientists and Institutions involved are interested in sharing these data. The availability of meta-data on surveys coupling biological and geochemical sampling could be made available to the scientific community (*e.g.* by means of web/newsletter) with particular reference to the scientist in charge of the respective data sets. It is also proposed that at a later stage, scientists who are in charge of relevant data sets be contacted and asked whether they would be interested in a joint analysis (and multi-author publication) of the data which would allow detection of trends.

Through this initiative, the author aims to bring awareness to the use of biological benthic tools as an aid for monitoring the coastal marine ecosystem and an important path in capacity building, quality control and assessment processes. Within this context, a strong collaboration among countries in the entire Mediterranean basin is needed to increase efforts and resource availability, and to promote the GOOS at national and regional scales.

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