Intergovernmental Oceanographic Commission
technical series

57

Ad Hoc Benthic Indicator Group Results of Initial Planning Meeting

Paris, France
6-9 December 1999

Organised in co-operation with:

U.S. Department of Commerce, National Oceanographic and Atmospheric Administration

Institute of Marine Biology of Crete

Kagawa University, Miki, Japan

National Academy of Sciences of Ukraine, Institute of Biology of the Southern Seas

Harvard University, Harvard School of Public Health

UNESCO

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Ad Hoc Benthic Indicator Group* Results of Initial Planning Meeting

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*Formed under the auspices of UNESCO/IOC's Global Investigation of the Marine Environment (GIPME) program

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SC-2000/WS/60

UNESCO 2000

For bibliographic purposes, this document should be cited as follows:
Ad hoc Benthic Indicator Group – Results of Initial Planning Meeting.
IOC Technical Series No. 57, UNESCO 2000 (English)

Printed in 2000
by the United Nations Educational, Scientific and Cultural Organization
7, place de Fontenoy, 75352 Paris 07 SP

Printed in France

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SECTION I: MEETING SUMMARY

1. Introduction

UNESCO/IOC's Global Investigation of Pollution in the Marine Environment (GIPME) program is aimed at assessing the presence of contaminants and their effects on human health, marine ecosystems, and marine resources and amenities (both living and non-living). Under the auspices of GIPME, a new initiative is being pursued to develop indicators for assessing the condition (or "health") of marine benthic communities. This initiative is pursuant to chapter 40 of Agenda 21, which calls for the development of indicators for sustaining healthy ocean ecosystems. This new "benthic health" initiative also relates to the Health of the Oceans (HOTO) module of IOC's Global Ocean Observing System (GOOS). The work will support a major goal of the HOTO module, which is to provide environmental health criteria and indices that can serve as early warning signals of change in the quality of the world's ocean environment.

Efforts commenced during the fall of 1999 to form a small committee of scientists, hereafter referred to as the *Ad Hoc* Benthic Indicator Group (BIG), to provide the expertise and data needed to carry out this initiative. Though the committee is still in the process of being formed, a partial group of members assembled at UNESCO headquarters in Paris, December 6-9, 1999, to describe the kinds of data that they could provide in support of this initiative and to begin identifying overall goals, approaches, products, and milestones. Results of this initial planning meeting are presented in the following report. Overall scope and objectives, preliminary recommendations on possible indicators and analysis approaches, and a listing of potential products and milestones are included in the present Section I. Summaries of meeting presentations are given in Section II. Contact information on committee members and other meeting participants is listed in Section III. A copy of the meeting agenda is provided in Section IV. Lastly, Section V shows preliminary results on the examination of benthic-TOC relationships and their potential use as indicators of anthropogenic stress in test data sets from various committee members.

2. Overall Scope and Objectives

An important goal of this initiative is to develop recommendations for benthic health indicators that are: (1) reliable in their ability to detect stress where stress should be occurring (e.g. due to high contaminant levels); (2) powerful in their ability to discriminate between anthropogenic vs. natural sources of stress; and (3) easy to use and broadly applicable in different parts of the world. To address this goal, potentially useful indicators will be identified and evaluated across several test data sets containing synoptic information on benthic faunal condition (e.g. measures of community composition, structure, biomass and functional aspects); controlling natural abiotic factors (e.g. salinity, sediment grain size, sediment organic matter); and levels of contaminants and other anthropogenic stressors in sediments.

Test data sets consisting of such information will be obtained from various coastal regions around the world, including among others: northern Black Sea, eastern Mediterranean, coast of Japan, North Sea, coastal areas of Great Britain and Scandinavia, Chilean coast, and estuaries along the U.S.A. Members of the Benthic Indicator Group (BIG) will bring together these data sets and look for consistent patterns of response in selected indicators. A goal will be to identify variables and corresponding thresholds that could serve as indicators of high versus low risks of benthic impacts and other related adverse environmental conditions. There is a significant amount of information in the open literature on benthic indices and analysis methods. The present UNESCO initiative will build upon knowledge gained from such previous efforts and seek to identify high-impact, easy-to-measure, and robust indicators of benthic condition that are applicable across different parts of the world's oceans. The initiative does not involve a new data-collection effort. Rather, available information will be gathered, examined, and used to develop recommendations that hopefully will provide useful guidance for future coastal research and management applications.

The objectives (terms of reference) of this initiative are:

1. Develop recommendations for a suite of globally applicable indicators and techniques to use in measuring the state ("health") of marine benthic communities.
2. Demonstrate the effectiveness of these indicators through application in test data sets from selected coastal regions of the world.
3. Help to promote the use of these indicators, by as broad of a user community as possible, through the presentation of results in reports, publications, symposia, Internet-based web sites, or other effective forums.

3. Initial Recommendations on Indicators and Approaches

A preliminary list of indicators for assessing the state (or “health”) of benthic communities was developed at the December 1999 planning meeting (Table 1). An attempt also was made to rank these indicators with respect to the level-of-difficulty involved in measuring them (Table 2). The committee believes that in order to make reliable and meaningful assessments of benthic health, it is best to base such judgements on a variety of measurements, including pollutant concentrations and their associated toxicity, condition of resident benthic fauna, and controlling natural abiotic factors. Thus, the committee embraces the idea of developing monitoring strategies that include as many of the indicators in Table 1 as possible. Similarly, Chapman (1990, also see Chapman et al. 1991) recommended using a “sediment quality triad” approach (combining information on sediment contamination, toxicity, and condition of resident benthic fauna) to support weight-of-evidence judgements about contaminant-induced degradation of the benthos.

Table 1. Potential indicators of benthic stress.

Non-Disturbed Environments:

Environmental Characteristics	**Biological Characteristics**
- Low Total Organic Carbon (<1%)	- High number of species
- High RedOx potential (positive Eh values)	- High Diversity (H′)
- Low porewater sulfide (< 0.01 mg/L unionised H_2S)	- High total faunal abundance
- Low porewater ammonia (< 0.2 mg/L unionised ammonia N)	- High biomass
- High bottom water dissolved oxygen (> 5 mg/L)	- High species evenness/low dominance
- Low measures of sediment contamination:	- High Nos. of long-lived/equilibrium & pollution- sensitive species
- Low chloroform extractable bitumen (< 1 mg/g)	- Low Nos. of opportunistic and pollution-tolerant species
- Individual chemical concentrations less than sediment quality guideline values (ERM, PEL values).	- Higher ratio of filter feeders to carnivores and deposit-feeders - Higher multi-metric benthic index score - Diverse age-class structure
- Low mean ERM quotient (< 0.01)	- Low incidence of morphological anomalies
- No sediment toxicity in standard bioassays with ambient amphipod	- Higher ratio of crustaceans to polychaetes and molluscs - Low abundance/biomass ratio - Low abundance/ species ratio - Low incidence of internal parasites (esp. molluscs)

Heavily Disturbed Environments:

Environmental Characteristics	**Biological Characteristics**
- High Total Organic Carbon (> 3%)	- Low number of species
- Low RedOx potential (negative Eh values)	- Low Diversity (H′)
- High porewater sulfide (> 0.05 mg/L unionised H_2S)	- Low total faunal abundance
- High porewater ammonia (> 0.4 mg/L unionised ammonia N)	- Low biomass
- Low bottom water dissolved oxygen (< 2 mg/L)	- Low species evenness/high dominance
- High measures of sediment contamination:	- Low Nos. of long -lived equilibrium & pollution-sensitive species
- High chloroform extractable bitumen (>10 mg/g)	- High Nos. of opportunistic and pollution-tolerant species
- Individual chemical concentrations greater than sediment quality guideline values (ERM, PEL values).	- Lower ratio of filter feeders to carnivores & deposit-feeders - Lower multi-metric benthic index score - High incidence of morphological anomalies
- High mean ERM quotient (> 0.1)	- Lower ratio of crustaceans to polychaetes and molluscs
- Significant sediment toxicity	- High abundance/biomass ratio - High abundance/ species ratio - High incidence of internal parasites (esp. molluscs) - Higher incidence of younger forms - Presence of beggiatoa-like mats - High incidence of imposex - Abnormal occurrence of infauna relative to sediment depth (high density of deep burrowing fauna on surface).

Note: Other features to consider include:

1. Abiotic factors to help interpretation of data on biological and environmental (stressor) variables such as grain size, C/N ratios, chlorophyll a/phaeopigment ratios, and acid volatile sulfides.
2. Seasonality.

Table 2. Ranking of indicators with respect to measurement level-of-difficulty.

	Less Difficult	More Difficult
Environmental Indicators:	Total Organic Carbon	Multiple contaminant analyses
	RedOx potential	ERM quotient analysis
	Chloroform extractable bitumen	Bioassays
	Dissolved Oxygen	
	Porewater sulfide, ammonia, other reduced compounds	
Biological Indicators:	Number of species	Multi-metric benthic indices
	H′	Incidence of imposex
	Biomass	Incidence of internal parasites
	Evenness/Richness	
	% equilibrium/pollution-sensitive vs. opportunistic/pollution-tolerant species	
	Age-size structure	
	Incidence of morphological anomalies (once reference range established)	
	% filter feeders vs. carnivores & deposit feeders	
	% crustaceans vs. polychaetes & molluscs	
	Abundance/biomass ratios	
	Abundance/species ratios	
	Visual presence of Beggiatoa-like mats (where direct observations are possible)	

However, an important goal of the present initiative is to identify a subset of specific indicators that are sensitive and reliable in their ability to detect stress where stress should be occurring (e.g., due to organic over-enrichment or sediment contamination) in addition to being easy to measure and applicable in different parts of the world. Along these lines, the committee agreed that looking at relationships between simple measurements of organic-carbon content in sediments and infaunal community variables (e.g., numbers of species, H′ diversity, abundances, biomass) might produce such an indicator. Lists of measurements recommended for the analysis and interpretation of these relationships are given in Table 3 (“Tier 1” optimal measurements) and Table 4 (“Tier 2” supplemental measurements).

Table 3. A short list of optimal measurements (first tier) needed to assess benthic-TOC relationships in unconsolidated substrates.

Abiotic variables:

- Total organic carbon and nitrogen
- C/N ratios and/or chlorophyll a/phaeopigment ratios in surface sediments
- Turbidity/water clarity measurements (e.g., secchi disk depth)
- Sediment grain size (minimum of % silt-clay vs. coarse fraction)
- Near bottom water dissolved oxygen concentration
- RedOx potential in the surface layer of the sediments (0-5 cm)
- Temperature and salinity in near-bottom waters [Salinity will be especially important in estuarine and coastal habitats].

Biotic variables:

- Measures of infaunal community structure, including number of taxa (at least to the family level), and abundance by taxa (including total abundance).
- Biomass in wet weight by lowest possible taxon.

Table 4. List of supplemental (secondary tier) information helpful in interpreting cause-effect relationships.

Abiotic variables:

- Concentrations of toxic contaminants such as PAHs, pesticides, or heavy metals
- Concentration of ammonia and sulfide in the sediment porewater
- Ancillary measurements to assess the bioavailability of stressors
- Levels of other stressors

Biotic variables:

- Measurement of ATP or other biomarkers in sediments that provide links among cellular, individual and community responses to stress.

A conceptual model for benthic-TOC relationships is presented in Figure 1 (modified from Pearson and Rosenberg 1978). In accordance with this model, benthic variables such as numbers of species, abundance, and biomass would increase in relation to increasing organic carbon (TOC) content of the sediment up to a certain critical level, and then begin to decline. Population increases below the TOC critical level reflect a combination of the nutritional value of moderate levels of organic matter present in the sediment and other favourable environmental conditions, such as sufficiently high levels of dissolved oxygen and low, unharmful levels of sediment-associated stressors (contaminants, ammonia, sulfide). As TOC exceeds a certain critical level, the benthos is exposed to increasing amounts of physiological stress resulting from oxygen depletion and increasing concentrations of contaminants or other sediment-associated stressors. A more detailed illustration of how specific infaunal variables are expected to respond in relation to TOC is provided in Figure 2. Note that the maximum values for these various biotic variables may not correspond to the same TOC concentration. For example, the abundance maximum may occur at a higher level of organic enrichment due to the contribution of opportunistic pollution-tolerant species. Also, there is often a secondary biomass maximum associated with the abundance maximum.

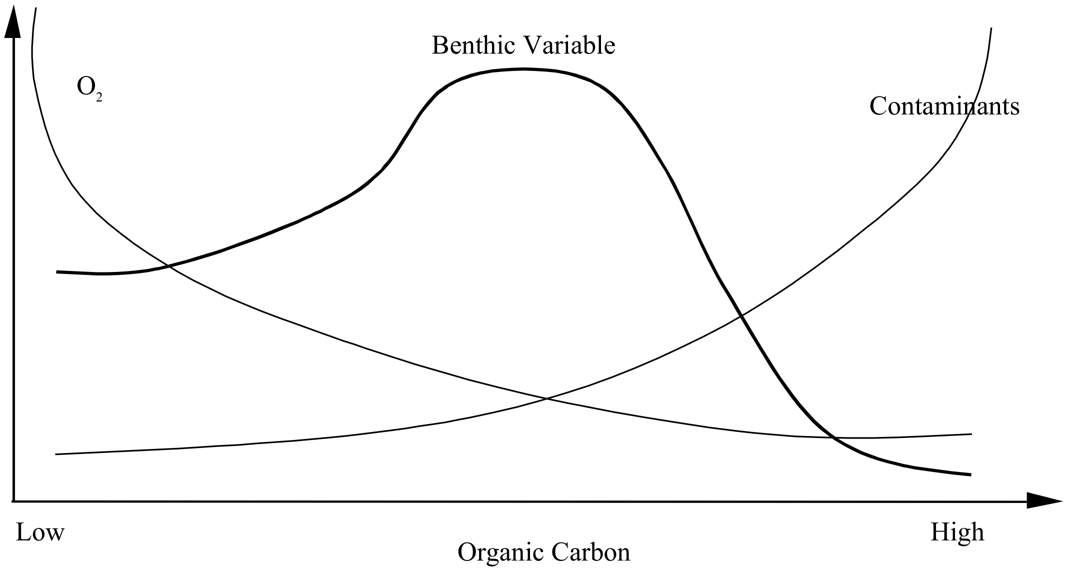

Figure 1. Conceptual diagram describing a benthic-TOC relationship, including how potential stressors (contaminant levels or anoxia in the sediments) may covary with sediment TOC.

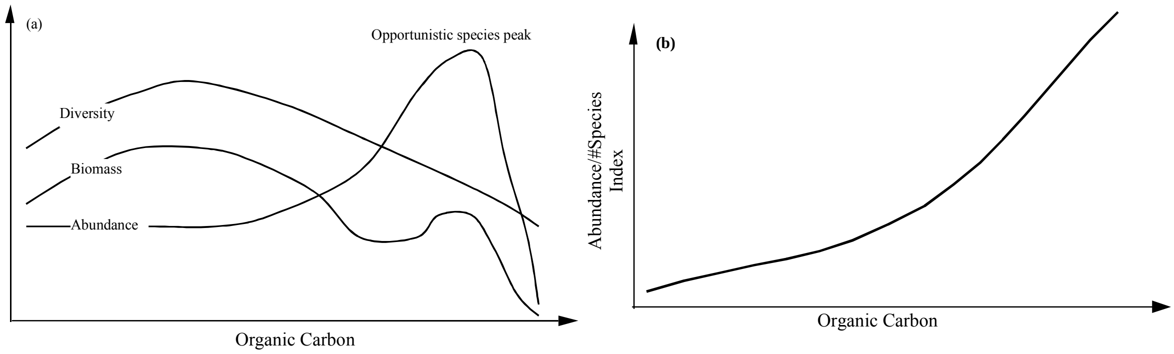

Figure 2. (a) A more specific conceptual diagram describing the potential relationship of TOC with diversity, biomass, and abundance in marine sediments. (b) A more specific conceptual diagram describing the relationship of TOC with the abundance/number of species in marine sediments.

An important goal of the committee is to demonstrate the effectiveness of various indicators through application in test data sets from selected coastal regions of the world. To help fulfill this goal, the committee will perform a series of analyses to examine benthic-TOC relationships and their effectiveness as potential indicators of anthropogenic stress using data sets provided by the various members. At a minimum, data will be tested from the following regions: eastern Mediterranean Sea, northern Black Sea, North Sea, coastal areas of Great Britain and Scandinavia, coast of Japan, and coastal regions of the U.S.A. Initially, analyses will be performed for each region using all samples (regardless of sampling time or location) that provide synoptic data on benthic community structure and composition, stressor levels, and natural controlling abiotic factors. Thus, each data set may contain information representative of multiple sampling places and periods, and ignores potential variation due to such factors. If clear patterns cannot be detected with data lumped in this fashion, then subsequent analyses may be performed with subsets of data to account for such influences.

Initial work with several of the above data sets is underway and preliminary results are included in Section V of this report. These results look very promising and suggest that it may be possible to identify TOC thresholds that could serve as indicators of high vs. low risks of benthic impacts and other related adverse environmental conditions (though values of such thresholds may vary somewhat among regions or habitat types). Subsequent applications in other regions will be performed as additional data sets are identified and accessed. To the extent possible, data sets will be obtained from both developing and developed countries. Once these analyses are completed, a manuscript will be prepared for publication provided the final results are successful and make a significant contribution to the scientific literature.

4. Products and Milestones

- Summary report on December 1999 meeting — Draft: March 2000, Final: April 2000
- Second committee meeting — May 2000
- Journal manuscript on relationships between TOC concentration and incidence of stress in the marine benthos — Draft by approximately 4th quarter 2000 to 1st quarter 2001
- Third committee meeting — Fall/Winter 2000-2001
- Develop web site to facilitate information transfer and promote use of resulting data products — Discuss plans at May 2000 meeting
- Identify other indicators to evaluate and appropriate courses of action — Discuss at May 2000 meeting.

5. Acknowledgements

The contributors of this report would like to thank Dr. Umit Unluata (Head, Ocean Sciences Section, UNESCO/IOC) for promoting the benthic health initiative and sponsoring the initial planning meeting. We also thank Patricio Bernal (Executive Secretary & Assistant Director-General, UNESCO/IOC) and Anna Widen (Consultant, UNESCO/IOC) for their overall support and help with the organization of the initial planning meeting. Funding support for the preparation of this report was provided by the Center for Coastal Monitoring and Assessment (CCMA), of the National Centers for Coastal Ocean Science (NCCOS), NOAA National Ocean Service, U.S. Department of Commerce. Special appreciation is extended to Ms. Cindy Cooksey and Dr. Len Balthis, both members of Dr. Hyland's staff at NOAA in Charleston South Carolina, USA, who assisted in the preparation of this report and related materials for the meeting.

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SECTION II: PRESENTATION SUMMARIES

THE INDICATORS ISSUE AND ASSOCIATED IOC INTERESTS AND EFFORTS

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1. Background: IOC'S Overall Interests in Indicators for the Marine and Coastal Environment

Chapter 40 of Agenda 21 calls for the development of indicators for sustainable development. In particular, it requests countries at the national level, and international governmental and non-governmental organizations at the international level, to develop the concept of indicators of sustainable development in order to identify such indicators. The topic has direct relevance to IOC because of the endorsement of GOOS by UNCED Agenda 21.

Indicators are pointers regarding condition. Indicators are quantitative representations of the forces that drive a system; of responses to forcing functions; or of previous, current, or future states of a system. When they are used effectively, indicators are expected to reveal conditions and trends that help in development planning and decision making. Policy makers set the targets and measurable objectives, while scientists determine relevant variables of the marine and coastal environment, monitor its state and develop models to make projections of future state. In this sense indicators are intended to close the gap between the fields of policy making and science. Once they are selected, indicators are expected to give direction to monitoring and research programs. The choice of a core set of indicators requires cooperation among policy makers and scientists.

International conventions and other multilateral environmental agreements, in particular, require indicators of country performance and overall effectiveness. This will require an intellectual exercise in each case to identify the most appropriate variables for selection as indicators. It will also require a major data-collection effort including the adoption of common methodology, capacity building, harmonization, quality control, and an assessment process. The IOC in general and the Global Ocean Observing System, in particular, could assist the various agreements and their scientific advisory bodies to design and implement a supporting information system to generate the necessary indicators and to use them for management purposes.

The following principles have been set forward in relation to indicators by the UN Commission on Sustainable Development:

1) In order for indicators to be effective and successful they should:

- Quantify information so that its significance is apparent;
- Be user-driven (to help summarize information of interest to the intended audience);
- Be scientifically credible;
- Be responsive to changes in time and/or space;
- Be simple and easily understood by the target audience;
- Be based on information that can be collected within realistic capacity and time limits;
- Be limited in number, remaining open-ended and adaptable to future developments; and
- Be representative of an international consensus, to the extent possible.

2) In developing indicators, it is important to address eventually the challenge of fully integrating the social, economic, environmental and institutional aspects of sustainable development. Much further work, primarily by the scientific community, is needed in order to understand and explain these linkages.

Social indicators have been developed during recent years and are used all over the world. Economic indicators have also been used for many years at national, regional, and international levels.

It is feasible to select from among these social and economic indicators some that capture the specific issues most relevant to sustainable development.

However, the status of development of environmental indicators for oceans and coastal areas leaves much to be desired.

The following five indicators have been proposed initially for the marine environment:

- Population growth in coastal areas;
- Discharges of oil into coastal waters;
- Releases of nitrogen and phosphorus to coastal waters;
- Maximum sustained yield for fisheries; and
- Algae index.

Of these indicators, the first two and the last one are listed as being in the development stage. However, not much progress has been made since the identification of these environmental indicators. Clearly, a significant gap exists in the identification of indicators for the marine and coastal environment.

2. IOC's Specific Interests Regarding Indicators for the Marine and Coastal Environment and the Current Status of Efforts

IOC's specific interest in indicators reflects GOOS design requirements that are about to be completed through its Health of the Ocean (HOTO), Living Marine Resources (LMR), and Coastal (C-GOOS) Modules. It is expected that the efforts related to the development of indicators should be strongly coupled to the development of observing systems that also involve the development and adoption of common methodologies, capacity building, harmonization, quality control, and assessment processes. The observing systems will provide, in turn, supporting information systems that can assist in the generation of new indicators or the modification of previously adopted ones.

As reflected in its Strategic plan, the HOTO Module specifically addresses the ways and means of developing integrated mechanisms for observing, assessing, and forecasting the effects of anthropogenic activities on the marine environment. The strategic plan in particular calls for biological indices of contaminant stress to be identified at molecular, organism, population and community levels of biological organization. In addition, the plan stresses the fact that relating contaminant loads to biological effects will require measurements that are multi-disciplinary, multi-scale and multi-purpose.

Figure 1 displays the biological stress signals of damage in terms of difficulty of measurement and levels of ecological significance. Biochemical and cellular biomarkers, behavioral changes, cellular pathology, and abnormal physiology form a group, which constitute early distress signals that are relatively easy to measure but have the disadvantage of not easily being interpreted in terms of ecological significance. GIPME/HOTO is considering this group of stress indicators under a program called "Rapid Assessment of Marine Pollution" (RAMP).

An additional group encompasses stress signals that are more difficult to measure but of obvious ecological significance. These indicators include measures of change at population and community levels (Reduced Diversity, Altered Abundance, and Distributional Changes in Figure 1). This group of stress indicators is the focus of our new benthic health initiative and present planning meeting.

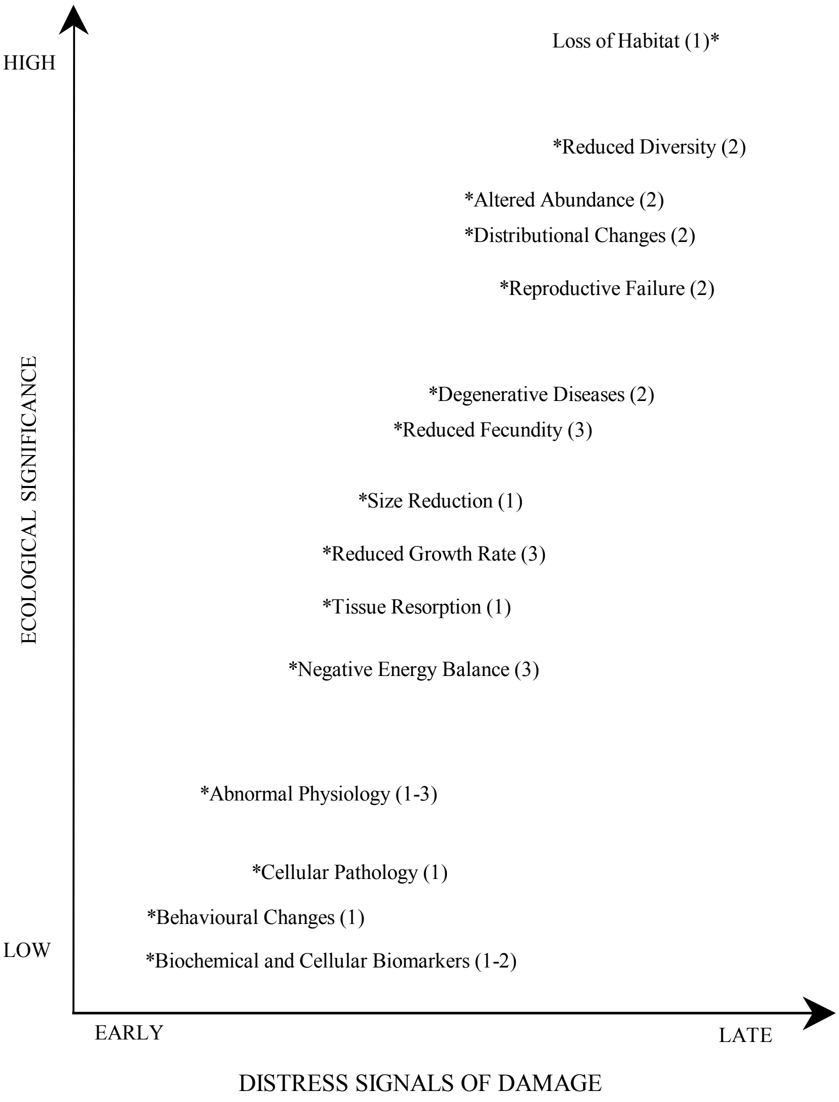

Figure 1. Biological distress signals of damage to ocean health.

The new IOC initiative involves the study and evaluation of indicators of benthic-community health that are:

- Reliable in their ability to detect stress where stress should be occurring (e.g., due to high contaminant levels);
- Powerful in their ability to discriminate between anthropogenic versus natural sources of stress; and
- Easy to use and broadly applicable in different parts of the world.

The initial stage of this endeavor will be based on existing databases that contain synoptic measurements of benthic faunal condition (e.g., measures of community composition, structure, biomass, and functional aspects); controlling natural abiotic factors (e.g., salinity, sediment grain size, sedimentary organic matter); and the levels of contaminants (and other anthropogenic stressors) in sediments. The fundamental aim is to seek globally applicable methods. This initial stage will be followed by design of appropriate monitoring systems, training, and pilot studies.

Another related issue being addressed by GIPME is the increasing risk to human health posed by anthropogenic mobilization of artificial and natural toxic agents in the environment. Such risks exist as a result of coastal degradation, climate variability, and increased industrialization. On the other hand, the world’s oceans provide great health benefits to humans from food and nutritional resources to recreational opportunities and new cures of human disease. These associations between the ocean and human health are important to design and implementation of the GOOS HOTO module. This program is expected to address indicator issues at molecular, organism, and community levels in the context of links between environmental changes and stresses on human health. It will be a collaborative effort with the BBSR’s International Center for Ocean and Human Health. A preliminary framework of a program was defined at a meeting jointly sponsored by IOC and the National Institutes of Environmental Health Sciences (NIEHS) (BBSR, 16-19 October 1999). The program will comprise four components: research modeling component; a biomarkers developmental resource component; an informatics component; and a training component.

In contrast, the C-GOOS panel requires that indicators should be selected in such way that they measure status or vulnerability to change in response to natural perturbations or anthropogenic stresses. Three types of indices are envisioned related to the risk of an event occurring, the vulnerability of a system or region to natural perturbations or stress (hazards), and the health of the ecosystem. The following indices are under consideration:

- Risk Index — To assess the probability that a particular event will occur or have a certain impact;
- Vulnerability Index — To assess the capacity of an ecosystem or region to "bounce back" following natural perturbation or anthropogenic stress; and
- Ecosystem Health Index — To assess the extent to which an ecosystem or region has been degraded or stressed by anthropogenic activities.

The LMR Module is yet to consider the indicators issue fully. It is expected that the LMR Panel will be guided to a large extent by indicators being developed for the Secretariat of the Convention on Biodiversity (CBD) as well as within FAO. IOC plans to participate in the CBD efforts in which the LMR Panel could play a pivotal role.

IOC’s further involvement in indicators encompasses the sediment-quality guidelines work being carried out jointly with IMO and UNEP under the GIPME Program. It examines various approaches and scientific elements for use in assessing anthropogenic influences on marine sediments and associated risks to marine life and human health.

A MULTIVARIATE ENVIRONMENTAL DATABASE FOR ESTUARIES OF THE SOUTHEASTERN USA: OVERVIEW AND IMPLICATIONS FOR THE NEW UNESCO INITIATIVE ON DETERMINING GLOBAL INDICATORS OF BENTHIC HEALTH

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1. Database and Program Overview

In 1993, the National Oceanic and Atmospheric Administration (NOAA) and the U.S. Environmental Protection Agency (EPA) initiated a joint study of the quality of estuaries along the southeastern U.S.A. by coordinating two nationwide environmental monitoring efforts, EPA's Environmental Monitoring and Assessment Program (EMAP) and NOAA's National Status and Trends Program (NS&T). The study region, known as the Carolinian Province, is one of 12 national EMAP estuarine regions and extends from Cape Henry, Virginia, through the southern end of Indian River Lagoon, Florida (Figure 1). The study was implemented through partnerships among a large network of federal, state, academic, and private research institutions. Over a hundred scientists, from about 25 to 30 different research institutions have been involved in various phases of the work.

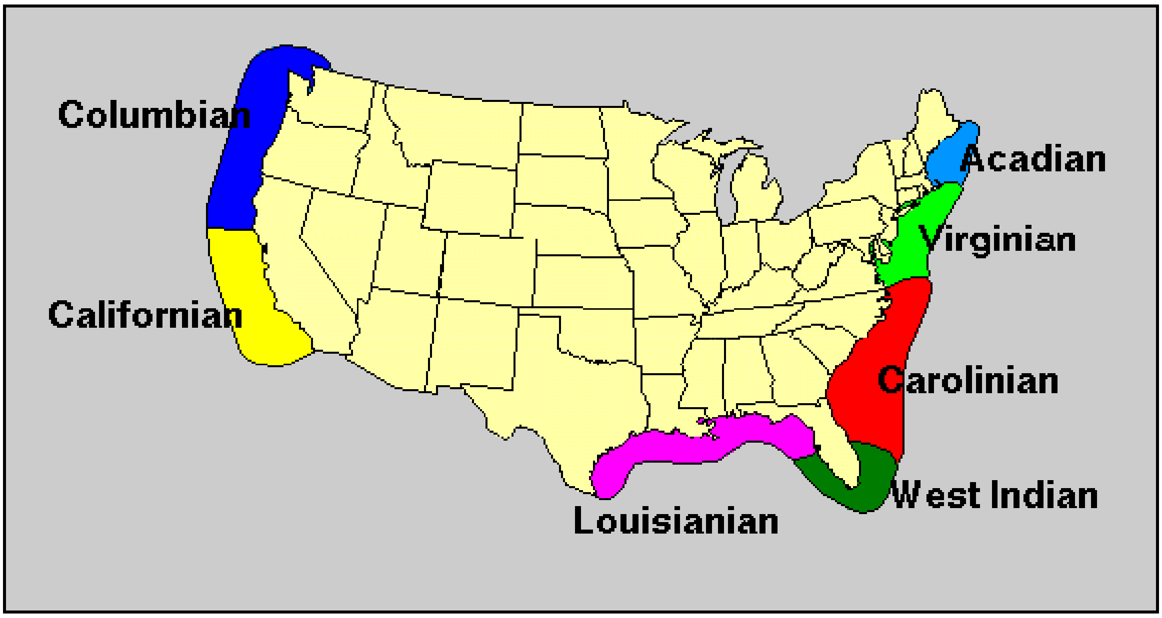

Figure 1. EMAP-Estuarine Provinces (sampling regions).

Estuaries of the Carolinian Province encompass an estimated 12,000 km^2 of the coastal zone and contain natural resources of both high economic and ecological value. There has been an increasing need for effective management of these systems given an enormous influx of people and businesses predicted to occur in southeastern coastal states over the next few decades and the ensuing pressures of human activities in these areas. The Carolinian Province study was initiated to help support such management needs by providing year-to-year estimates of estuarine condition based on a variety of biological, chemical, toxicological, and aesthetic indicators. Prior to this study, there had been no comprehensive region-wide baseline of ecological information available to support management needs for these systems.

One important goal of EMAP has been to make statistically unbiased estimates of ecological condition with known confidence. To approach this goal, a probabilistic sampling framework was established among the overall population of estuaries comprising the Carolinian Province. Under this design, each sampling point is a statistically valid probability sample. Thus, percentages of total estuarine area with values of selected indicators above or below suggested environmental guidelines can be estimated based on conditions observed at individual sampling points. Statistical confidence intervals around these estimates can also be calculated. Further details on sampling design and statistical methods are given in Hyland et al. (1996, 1998a).

Surveys to estimate condition province-wide were conducted during the summers of 1994 and 1995 (see Hyland et al. 1996, 1998a, 1998b). Probabilistic sampling was conducted again during summers of 1996–97, but due to funding constraints, was limited to the North Carolina portion of the province. The continued monitoring in this area, however, allowed completion of a four-year cycle of probabilistic sampling covering the entire population of North Carolina estuaries identified under the original EMAP sampling design. North Carolina estuaries contribute 76% of the total estimated surface area of Carolinian Province estuaries and include several large coastal sounds (e.g., Currituck, Albemarle, and Pamlico), extensive river systems (e.g., Pamlico and Neuse Rivers) draining substantial portions of the state, and numerous smaller tributaries and coastal embayments. The Albemarle-Pamlico-Currituck Sound system is the second largest estuarine system in the United States (second to Chesapeake Bay). An analysis of the quality of North Carolina estuaries, based on all four years of data, has been completed and submitted for publication (Hyland et al. In Review).

Table 1. Stations sampled in the Carolinian Province study (includes only sites with benthic data).

Year	Base Sites	Supplemental Sites
1994	82	10
1995	86	19
1996	42 (NC)	—
1997	59**	10 (Chowan R., NC)
1998*	—	20 (Neuse R., NC)
1999*	—	24 (Neuse R./Pamlico S.)

* Data not yet available.
** 43 sites in North Carolina and 16 former (province-wide) sites.

To date, benthic data are available from samples collected at 308 sites throughout the Carolinian Province, during summers of 1994-97 (Table 1). Included are samples from 269 random “base” stations that comprise the probability-based sampling design and 39 additional “supplemental” stations. Supplemental stations include sites selected non-randomly in areas for which there was some prior knowledge of ambient environmental conditions. Such sites, which consist of a combination of both non-degraded reference areas and places with histories of anthropogenic disturbance, were used to help test the discriminatory power of new ecological indicators developed during the program (e.g., new sediment bioassays). Supplemental sites also include 10 random stations sampled in the Chowan River, North Carolina, during the summer 1997 as part of a separate site-intensive survey of this system. Table 1 also lists additional sites sampled in 1998 and 1999, but for which data are not yet available.

At each station, samples and in-situ measurements were obtained (wherever possible) for characterization of: (1) general habitat conditions, including physical and chemical properties of water (depth, dissolved oxygen, salinity, pH, temperature, water clarity) and sediment (% silt-clay vs. coarser-fraction, % moisture, organic carbon content); (2) potential pollution exposure (sediment contaminants, sediment toxicity, ammonia and sulfide in sediment porewater, and low dissolved-oxygen conditions in the water column); (3) biotic conditions (diversity and abundances of macroinfauna and demersal fishes and invertebrates); and (4) aesthetic quality (presence of anthropogenic debris on sea surface and sea floor, visible oil on sea surface and sea floor, noxious sediment odor).

Salinity, pH, temperature, dissolved oxygen, and water depth were recorded electronically with a Hydrolab, "Datasonde 3" (DS3) multiprobe data logger. Instantaneous Datasonde measurements were taken at near-surface and near-bottom depths at each station. Near-bottom, time-series measurements also were recorded over a 24-h period (30-min intervals) at all stations during the summers of 1994-95. Contaminant concentrations, sediment toxicity, % water content of sediments, % silt-clay, and % total organic carbon (TOC) were measured at each station in subsamples of composited surface sediment (upper 2-3 cm). For contaminant analyses, sediments were measured for a total of 16 metals, four buty-tins, 27 aliphatic hydrocarbons, 44 polynuclear aromatic hydrocarbons (PAHs), 20 polychlorinated biphenyls (PCBs), and 24 pesticides. Sediment toxicity was measured using up to four different assays: (1) Microtox® solid-phase assay (Bulich 1979; Microbics 1992a,b); (2) 10-day, solid-phase test for survival of the marine amphipod *Ampelisca abdita* (ASTM 1993); (3) a similar amphipod test with the congeneric species *Ampelisca verrilli* (Ringwood et al. 1997); and (4) a one-week, solid-phase test for sublethal effects of sediment exposure on growth of juvenile clams *Mercenaria mercenaria* (Ringwood and Keppler 1998). Analysis of benthic macroinfauna (> 0.5 mm) was performed on replicate samples collected with a 0.04-m^2 Young-modified Van Veen sampler. Benthic fauna were identified and enumerated to lowest possible taxon (usually species). Biomass was not measured.

Further details of methods for these various analyses can be found in the original references cited above or in previous publications on results of the Carolinian Province study (e.g., Hyland et al. 1996; Hyland et al. 1998a,b; Hyland et al. 1999; Van Dolah et al. 1999; Hyland et al. In Review). Data and descriptions also are available on the Internet (data and metadata descriptions:

> http://www.epa.gov/emap/html/dataI/estuary/data/cp9497; related reports:
> http://www.epa.gov/emap/html/pubs/docs/estuary/ssum.html).

In summary, as a result of these various analyses, synoptic measurements of benthic and a variety of key environmental variables have been obtained, thus far, at 308 stations sampled in estuaries along the southeastern U.S. coast, from 1994-97. Many of these data (especially the measurements of benthic community structure, stressor levels, and other controlling abiotic factors) would be appropriate for the present UNESCO benthic-health initiative and can be made available for this purpose. In addition, as indicated in Table 2, similar data have been generated from numerous stations in three other EMAP estuarine regions (see Figure 1): (1) the Virginian Province, which extends from Cape Cod Massachusetts to the mouth of Chesapeake Bay; (2) the West Indian Province, which extends from the southern end of Indian River Lagoon to Tampa Bay, on the east and west coasts of Florida respectively; and (3) the Louisianian Province, which extends from Tampa Bay westward along the Gulf of Mexico coast to the U.S.-Mexican border. The possibility of obtaining data from these other companion EMAP efforts for the present UNESCO benthic initiative should be explored. The collective data sets from these four EMAP regions would contribute, at a minimum, data from 1,424 sites in estuaries along a majority of the U.S. Atlantic and Gulf of Mexico coasts. Additional data from subsequent sampling periods will be available soon as well.

Table 2. Number of benthic stations and sampling periods in various EMAP-Estuarine regions.

EMAP Region	No. Stations	Sampling Period
Virginian	511	1990-93
Carolinian	308	1994-97
West Indian	68	1995
Louisianian	539	1991-94
Total	1,424	—

2. Implications for the UNESCO Benthic-Health Initiative: A preliminary look at Benthic-TOC Relationships

An important goal of the new benthic health initiative of UNESCO is to develop recommendations for a suite of reliable and globally applicable indicators for assessing the condition (or "health") of marine benthic communities. Ideally, these indicators should be: (1) reliable in their ability to detect stress where stress should be occurring (e.g. due to high contaminant levels or other pollutants); (2) powerful in their ability to discriminate between anthropogenic vs. natural sources of stress; and (3) easy to use and broadly applicable in different parts of the world. Initial efforts to address this goal will be focused on examining benthic-TOC relationships in test data sets provided by members of the UNESCO benthic committee. Synoptic measurements of macroinfauna, TOC concentrations, pollutant levels, and other controlling abiotic variables will be examined across the various data sets to look for consistent patterns of association and to attempt to identify TOC thresholds that could serve as indicators of high vs. low risks of benthic impacts and other related adverse environmental conditions.

Preliminary results from the EMAP Carolinian Province data set look promising. Table 3 provides a comparison of TOC concentrations and selected infaunal variables for three categories of stations (polluted, marginally polluted, and highly polluted) grouped on the basis of the level of sediment contamination. Sediment contamination was based on the mean ERM quotient approach and associated benthic-bioeffect thresholds described by Hyland et al. (1999). The mean ERM quotient is calculated as the mean of the ratios of individual contaminant concentrations in a sample relative to corresponding "Effects-Range-Median" sediment quality guideline values (from Long et al. 1995, MacDonald et al. 1996). This measure provides a way of quantifying potentially harmful mixtures of contaminants present at varying concentrations in a sample. Table 3 shows that mean TOC concentrations vary significantly among the three station categories, with the lowest levels (mean of 3.16 mg/g) occurring at non-polluted sites and the highest levels (mean of 40.56 mg/g) occurring at highly polluted sites. All three benthic community variables (# of species, H′ diversity, and abundance) also decrease successively from non-polluted sites, to marginally polluted sites, to highly polluted sites.

Table 3. Comparison of TOC concentrations and selected infaunal variables at non-polluted vs. polluted sites throughout southeastern U.S. estuaries (EMAP Carolinian Province data, 1994-97, n=299 sites). Mean and range (in parentheses) are shown for each category.

	Non-Polluted Sites (Mean ERM Quotient ≤ 0.02) n=145	Marginally Polluted Sites (0.02 < Mean ERM Quotient ≤ 0.058) n=70	Highly Polluted Sites (Mean ERM Quotient > 0.058) n=84
TOC (mg/g)	3.16 (0.05-28.00)	15.93 (0.60–47.00)	40.56 (0.46–175.20)
# Species (per 0.04m^2)	19.33 (3.50–77.00)	9.89 (0.00–46.00)	6.30 (0.00–42.50)
H′ (per 0.04m^2)	2.86 (0.75–4.78)	2.26 (0.00–4.36)	1.44 (0.00–3.75)
Abundance (per 0.04m^2)	179.32 (6.50–1577.50)	85.86 (0.00–422.50)	60.38 (0.00–721.50)

Table 4 provides a further comparison of selected infaunal and environmental variables within four ranges of TOC concentrations: low (0.5 mg/g), moderate (0.5 - 10 mg/g), high (10 - 30 mg/g), and very high (> 30 mg/g). Mean values of all three benthic community variables (# of species, H′ diversity, and abundance) increased from the low to moderate TOC categories and then decreased successively with increasing TOC concentration across the remaining categories. Moreover, the percentage of sites with an unhealthy benthos, based on the benthic index of biotic integrity (B-IBI) for southeastern U.S. estuaries (Van Dolah et al. 1999), was very low (< 10%) at sites with low to moderate levels of TOC (< 10 mg/g), and was very high (81%) at sites with high levels of TOC (> 30 mg/g). Note that the percentage of sites with contaminated sediments also increases with increasing levels of TOC. About 90% of sites with TOC above 30 mg/g had high sediment contamination. Degraded condition of the benthos is probably the result of the presence of these contaminants (or other stressors such as dissolved ammonia and sulfide) rather than the TOC itself. However, TOC appears to serve as a reliable indicator of such effects.

Table 4. Comparison of selected infaunal and environmental variables within four ranges of TOC concentrations based on data from southeastern U.S. estuaries (EMAP Carolinian Province data, 1994-97; n = 299 sites). A = Mean and range (in parentheses among sites within each category; B = Percentage of sites within each category that showed specified symptom).

		TOC (mg/g)			
		0.5 n=13	> 0.5 – 10 n=161	10 – 30 n=58	>30 n=67
A.	Mean # Species (per 0.04m^2)	16 (4.0 – 52.0)	18.0 (1.5 – 54.0)	9.2 (2.0 – 34.0)	4.3 (0 – 14.0)
	Mean abundance (per 0.04m^2)	110 (6.5 – 321.0)	169.0 (5.5 – 1578.0)	86.0 (2.5 – 398.0)	46.0 (0 – 350.0)
	Mean H′ (per 0.04m^2)	2.7 (1.6 – 4.8)	2.8 (0.4 – 4.5)	2.2 (0.6 – 3.6)	1.2 (0 – 2.8)
	B-IBI	3.6 (3.0 – 4.5)	3.9 (1.0 – 5.0)	2.9 (1.0 – 5.0)	2.0 (1.0 – 4.5)
B.	% sites with unhealthy benthos (B-IBI < 3)	0	9.3	48.0	81.0
	% sites with high contamination (mean ERM quotient >0.058)	8.0	6.8	21.0	90.0

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DISTRIBUTION OF ORGANIC MATTER IN A TIDAL ESTUARY OF THE SETO INLAND SEA, JAPAN, AND ITS RELATIONSHIP WITH THE MACROZOOBENTHIC COMMUNITIES

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1. Overview of Program

This work is part of a long-term and multidisciplinary project which aims at quantifying the cycling of biophilic elements (C, N, P, Si) in a tidal estuary of the Seto Inland Sea, Japan, and to assess the role of primary producers (microphytobenthos) and consumers (macrozoobenthos) in this cycling. As an initial phase of this project, we carried out a bi-weekly survey lasting 13 months to investigate the environmental factors affecting the development of microphytobenthic assemblages at two intertidal stations differing in distance from the shore-line, elevation and grain-size composition (Magni and Montani 1997). In parallel, we assessed the short-term (24 h) and seasonal (2 year) variability of the water chemistry along the estuary to verify the sources of the different nutrients: from inland, outside or within the estuary (Montani et al. 1998, Magni & Montani 2000). In parallel, we conducted monthly field surveys on the spatial and seasonal distribution of intertidal and subtidal communities of the macrozoobenthos (Magni 1998, Magni & Montani 1998). Subsequently, as a link between primary producers and consumers, we quantified the magnitude and temporal variability of the contribution of macrozoobenthos on the upward flux of inorganic nutrients within the intertidal zone (Magni et al. 2000). By extrapolating laboratory experiments on animal nutrient excretion to a field community situation, we found that the dominant bivalves *Ruditapes philippinarum* and *Musculista senhousia* play a major role in recycling the inorganic forms of nitrogen and phosphorus available to primary producers (Magni et al. 2000). Additional work is now in progress to examine the influence of major physical (i.e. grain size, water content) and chemical (i.e. Chlorophyll *a*, pheo-pigments, total organic carbon and acid-volatile sulphide) parameters of the sediments on the spatial arrangement and the seasonal change of macrozoobenthos along the estuary. In this report, I will present major results on the relationship between organic matter and macrozoobenthos along the estuary (i.e. the intertidal and the subtidal zone), with emphasis on the role and ecological significance of total organic carbon (TOC) in the sediments.

2. Study Area and Sampling Activities

Our sampling strategy included five surveys carried out at all four different seasons (April, July, October 1994, and January and April 1995) on three parallel transect lines (transects A, B, C) located on the lower part of the intertidal zone (Figure 1). Each transect consisted of 5 sampling stations (Stns. 5, 4, 3, 2 and 1) set at regular interval of 25 m (Figure 1). The across- and along-shore distribution of the macrozoobenthic communities were investigated and the spatial/temporal distribution of major chemical parameters of surface sediments (i.e. Chlorophyll a, pheo-pigments, total organic carbon and nitrogen, and acid-volatile sulphide) were simultaneously examined.

A parallel and similar work was carried out on a subtidal zone adjacent to the intertidal zone under investigation (Figure 1). At this site, the macrozoobenthic communities were investigated quarterly from July 1994 to July 1995 at three sampling stations (Stns. Y3, Y2 and Y1) set along a transect line of 800 m (Figure 1). At each station and season, the same chemical parameters as those considered for the intertidal sediment samples were examined simultaneously.

Additional surveys were carried out both on the intertidal and the subtidal zone, which included the analysis and quantification of suspended and sinking particulate matter in the water column (Montani et al. 1997, Magni 1988, Magni & Montani 2000).

3. Data-set Presentation

Based on the results of several associated pieces of work, the tidal estuary under investigation was divided into four major compartments: the intertidal zone (A, B, C transects), the inner subtidal zone (Stn. Y3), the intermediate subtidal zone (Stn. Y2) and the outer subtidal zone (Stn. Y1; Figure 2). For each compartment, the annual mean values of major chemical parameters of surface water and surface sediments, and the abundance and biomass of the macrozoobenthic communities are presented in Figure 2.

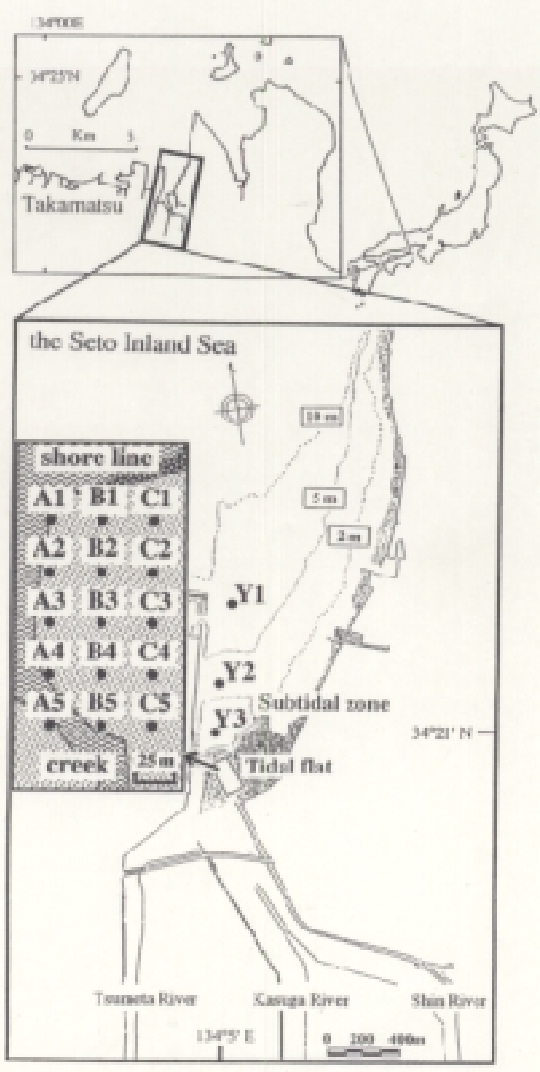

Figure 1. Study area and location of sampling stations.

In the water samples, the content of Chlorophyll *a* (Chl *a*), pheo-pigments, particulate organic carbon (POC) and nitrogen (PON) was highest on the intertidal zone. Accordingly, POC and PON collected by sediment traps progressively decreased from the intertidal to the subtidal zone (i.e. Stn. Y3), indicating a considerable amount of organic matter being removed within the intertidal zone. Accordingly, at this site the filter feeding bivalves *Ruditapes philippinarum* and *Musculista senhousia* were dominant, and the values of abundance and biomass of macrozoobenthos were highest. Both in the water and the sediments, pheo-pigments, but not Chl *a*, correlated significantly with the organic carbon and nitrogen pools, further suggesting grazing pressure and algal degradation by macrozoobenthos. The pheo-pigment/Chl *a* ratio was also used as an indicator to evaluate the fraction of living and refractory algal material within the microalgal standing stock. In the water, this ratio was much higher on the intertidal zone than on the subtidal zone. In addition, similar ratios were found in the water and surface sediments on the intertidal zone (i.e., closer water-sediment coupling), while it was markedly lower in the water than in the sediments on the subtidal zone (i.e. much higher fraction of refractory algal material in the sediments, highest at Stn. Y1).

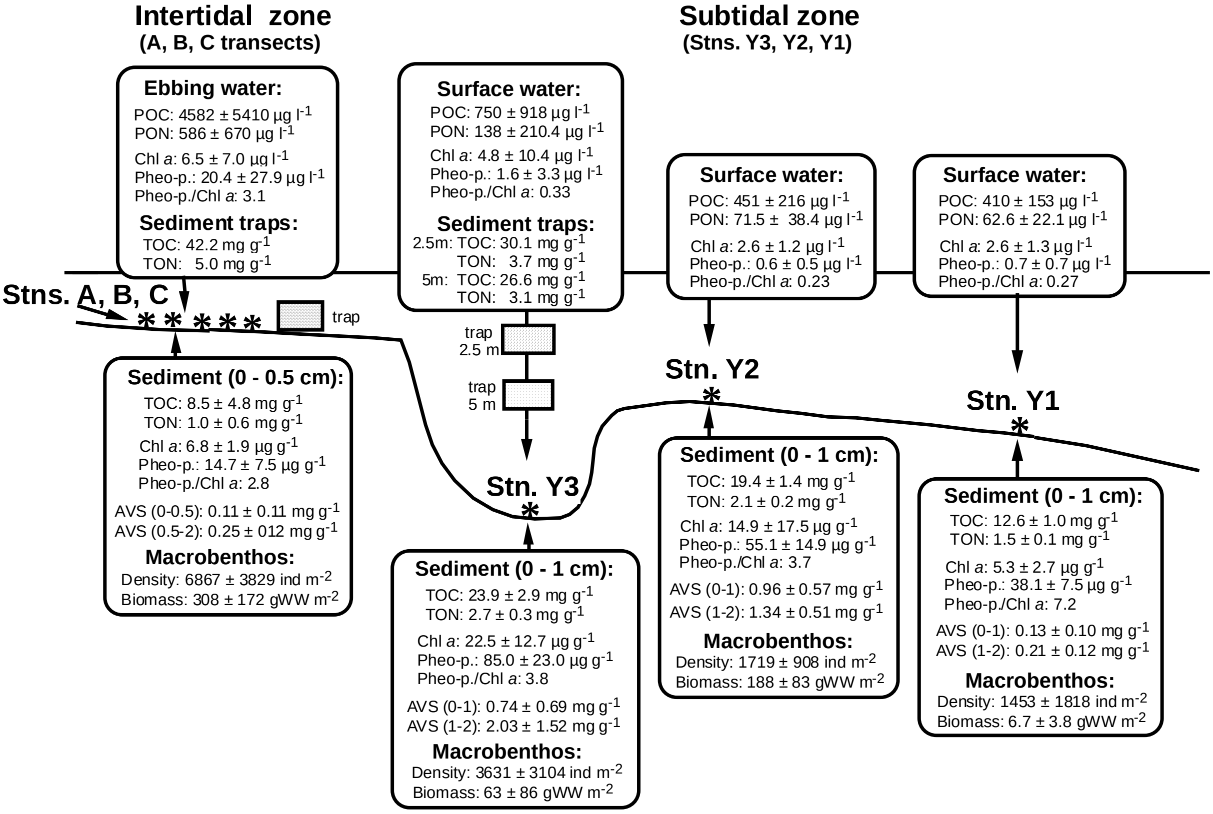

Figure 2. Schematic view of four major compartments in which the tidal estuary under investigation (tidal range *ca.* 2 m) was subdivided. They include the annual mean values of major suspended and sedimentary compounds/parameters, and their relationship with intertidal and subtidal communities of macrozoobenthos (adapted from Magni 1998).

At the sediment level, the total organic carbon (TOC) and nitrogen (TON) tended to be much lower on the intertidal zone than on the subtidal zone (i.e. Stns. Y3 and Y2). At this site, the TOC, TON, Chl *a* and pheo-pigments contents markedly decreased from Stn. Y3 to Stn. Y1. Along this gradient, the abundance of macrozoobenthos was relatively higher at Station Y3 than at Stations Y1 and Y2. In contrast, the biomass was highest at Stn. Y2, intermediate at Stn. Y3 and much lower at Stn. Y1. At Stn. Y3, the largest accumulation of labile organic matter went together with a high abundance of polychaeta. Whereas at this station, seasonal enhancement of decomposition processes of algal-derived labile organic matter led to a sharp increase of the acid-volatile sulfide (AVS) content, particularly in sub-surface (1 - 2 cm) sediments. This caused periodical massive benthic mortality and a drastic reduction of biomass which was accompanied by a shift from large polychaeta (i.e. *Thelepus* sp.) to small opportunistic ones (i.e. *Cossura coasta*). At Stn. Y2, characterized by intermediate TOC levels (19.4 ± 1.4 mg g^{-1}), *M. senhousia* was dominant, as on the intertidal zone, reaching a biomass up to 600 gWW m^{-2}. At Stn. Y1, the TOC, TON, Chl *a*, pheo-pigment and acid-volatile sulfide contents in the sediments were much lower. Accordingly, the limited extent of organic enrichment (TOC: 12.6 ± 1.0 mg g^{-1}) corresponded to AVS levels in the sediments as low as those found on the intertidal zone. At this station, the small deposit feeder *Theora fragilis*, an indicator of organic pollution, was relatively abundant, whereas the values of abundance and biomass were the lowest along the estuary, as most likely due to limited food availability in the sediments.

4. TOC-Benthic Indicators

In the intertidal zone, the relationship between TOC and macrozoobenthos, irrespective of the station and season, was described by a polynomial regression (Figures 3a and b). This indicated an increase of abundance (up to *ca.* 13000 ind. m^{-2}) in relation to increasing TOC up to *ca.* 20 mg g^{-1}, followed by a decline (Figure 3a). The lowest values of abundance (<1500 ind. m^{-2}) were found at TOC < 5 mg g^{-1} (Figure 3a). The maximum TOC value (44.2 mg g^{-1}) corresponded to an abundance of 6050 ind. m^{-2}. As for abundance, the lowest biomasses (< 5 gWW m^{-2}) corresponded to TOC values < 5 mg g^{-1} (Figure 3b). At the highest biomass (1058 gWW m^{-2}) the TOC was 17.8 mg g^{-1}.

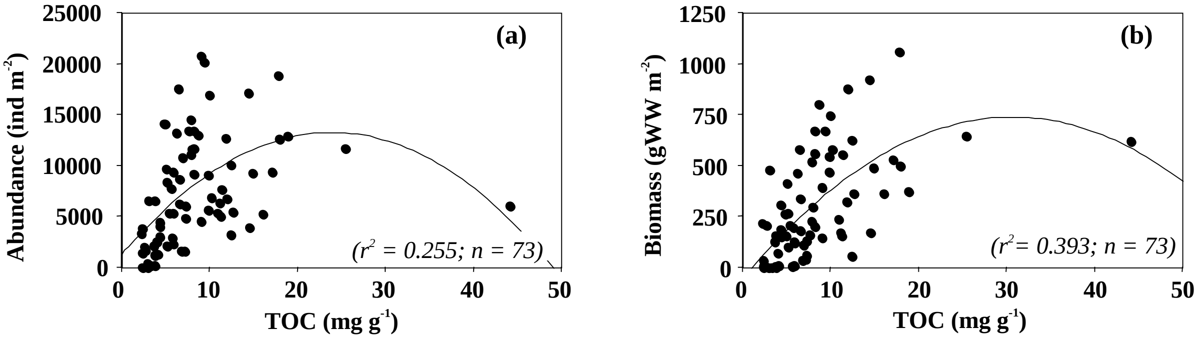

Figure 3. Relationship between total organic carbon (TOC) in intertidal surface sediment (0 - 0.5 cm) and (a) abundance and (b) biomass of macrozoobenthos.

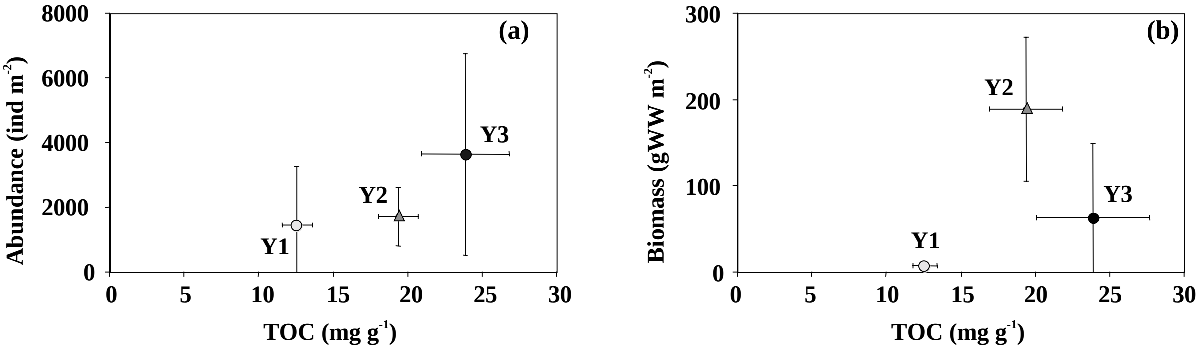

Figure 4. Relationship between the annual mean of TOC (surface sediment: 0 - 1 cm) and (a) abundance and (b) biomass of macrozoobenthos at Stns. Y3, Y2 and Y1.

In the subtidal zone, the range of TOC (11.6 - 28.2 mg g^{-1}) and macrozoobenthos values (Figures 4a and b) was more limited than that observed in the intertidal zone, demonstrating the higher variability of the latter ecosystem. At Stns. Y3, Y2 and Y1, the relationship between TOC and macrozoobenthos appeared to be mainly regulated by the ecological events described in the previous section. Yet, similar patterns were observed at the intertidal and subtidal sites. Also on the subtidal zone, the lowest abundances (< 350 ind. m^{-2}, Stn. Y1) corresponded to the lowest values of TOC (< 14 mg g^{-1}). The highest abundance (8075 ind. m^{-2}, Stn. Y3) and biomass (605.6 gWW m^{-2}, Stn. Y2, not included in the mean) were found at TOC of 21.8 mg g^{-1} and 23.7 mg g^{-1}, respectively.

Overall, these results indicated a close relationship between TOC and benthic variables (i.e. abundance and biomass of macrozoobenthos) in the coastal ecosystem of the Seto Inland Sea under investigation. They will be integrated with other benthic community variables (e.g. number of species and H′ diversity) and seem to be promising of further extrapolation and comparison with data from other areas of the world ocean.

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BENTHIC ENRICHMENT DATA FROM THE EASTERN MEDITERRANEAN AND IMPLICATIONS FOR DETERMINING INDICATORS OF ENVIRONMENTAL HEALTH

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1. Introduction

The Mediterranean has often been an obstacle for attempts to arrive at general marine biological rules (Petersen 1985). Some of its characteristics make this sea a quite peculiar environment with pronounced ecological differences even with the adjacent ocean basins. Listed below, some of these peculiarities affect the ecological processes determining the fate of wastes in the marine environment:

- High temperature (annual minimum of 12°C, reaching up to 25°C during summer) induces high metabolic rates, thus affecting the activity of microbial communities.
- The microtidal regime (tidal range is typically less than 50 cm) reduces the potential for dilution and dispersion of solute and particulate wastes particularly in enclosed bays where wind-driven currents are relatively weak.
- Oligotrophy: low nutrient content, low primary production, and low phytoplankton biomass are typical of most Mediterranean marine ecosystems, particularly in the Eastern Basin (Bethoux 1981, Azov 1986). Low phytoplankton biomass induces high transparency of the water and light penetration deeper in the water column (Ignatiades 1998) thus allowing photosynthesis at a greater depth.
- Primary production is considered to be phosphorus limited (Krom et al. 1991) as opposed to nitrogen limitation in the Atlantic and in most of the world's Oceans. In this context, eutrophication could be expected only when phosphate is released in adequate quantities.
- The biotic component of the ecosystem, i.e. the fauna and flora, is highly diverse particularly in the coastal zone and consists of a large proportion of endemic species (Tortonese 1985, Fredj et al. 1992) as a result of the dynamic geological past of the Mediterranean. It is typically of low abundance and biomass as a result of the prevailing oligotrophic conditions (Karakassis and Eleftheriou 1997).

The results presented here address a particular type of benthic enrichment which is due to sedimentation of fish farming wastes. This type of enrichment is quite relevant to the aims of the workshop since it allows for assessing the impact of organic material per se on the benthic communities without the presence of other sources of acute toxicity such as heavy metals or PCBs.

Until recently (Munday et al. 1994), there was little information available from the Mediterranean regarding the interactions of fish farming and the marine environment, despite the increasing need for such information in order to provide support for policy making.

During the last five years, there has been some progress in the understanding of these processes in the Mediterranean through a series of published papers addressing the impact of fish farming on water column chemistry and parasites (Papoutsoglou et al. 1996), the effect on nutrients and plankton (Pitta et al. 1999), the effects on seagrass (Delgado et al. 1999), the dynamics of sediment accumulation beneath fish farm cages (Karakassis et al. 1998), the recovery process of benthos after cessation of fish farming (Karakassis et al. 1999) and the effects on sediment geochemistry and benthic organisms (MacDougal & Black 1999; Karakassis et al. 2000; Mazzola et al. 2000). These studies involved monitoring environmental variables at one or more fish farms in different regions within the Mediterranean and therefore they may be used to compare results with those reported for fish farming (e.g. salmon farms) or other types of organic enrichment from other places of the world.

2. Methodology

The data presented in this workshop have been published in a series of papers (Karakassis et al. 1998, 1999, 2000) addressing fish-farming impacts on benthos. Three fish farms were visited, two in the Eastern Ionian and one in the Aegean Sea, henceforth referred to as Cephalonia, Ithaki and Sounion, respectively. All three sites are situated at shallow depth (20-30m) in the coastal zone. The coarse sediment in Ithaki and Sounion with median grain diameter (MD) 0.42 and 0.60 mm respectively, was typical of the “Amphioxus sand” biocoenose described by Pérès (1967) as “coarse sands and fine gravels under bottom currents” (with the French acronym SGCF). The seabed in Cephalonia was silty (MD=0.02 mm) and showed the typical characteristics of the biocoenose of “terrigenous mud” (or VTC) after Pérès (1967). Samples were taken at a distance of 0 m (under the cages) as well as at 5, 10, 25, 50 and 100 m from the edge of the cages downstream from the main direction of the water current. The opposite (upstream) direction was also sampled at a distance of 25, 50 and 100 m from the edge of the cages. A control station was chosen at 1 km (upstream) distance from the cages at similar depth and sediment type.

Sampling for geochemical variables and macrofauna was carried out during three seasonal cruises (July and November 1995 and April 1996) aboard the RV “Philia”. In Cephalonia farm sampling was continued during the period 1996-97 on a seasonal basis using sediment profiles for the determination of the dynamics of sediment accumulation beneath the cages. In parallel, a recovery experiment was carried out at a different site in Cephalonia bay, where cages were removed and benthos was monitored over a period of 23 months.

Redox potential (Eh) was measured in core samples at 2-cm intervals from the water-sediment interface by means of an electrode standardized with Zobell’s solution (Zobell 1946). Sediment subsamples for the determination of organic carbon and nitrogen content, as well as algal pigments and ATP concentrations, were taken by means of core tubes (2.2 cm diameter). ATP subsamples were processed on board (extraction through boiling with NaHPO4 buffer for 90 sec) before being stored. Total organic carbon and nitrogen were determined in the sediment samples by means of a Perkin Elmer 2400 CHN Elemental Analyzer following the procedure of Hedges and Stern (1984). Algal pigments were extracted with 90% acetone and sediment contents in chlorophyll and phaeopigments were determined according to the method described by Yentsch and Menzel (1963) using a Turner fluorometer (model 112). The ATP content was determined in the extraction solution using the luciferine-lusiferase reaction (Karl 1980) and the luminescence was measured by means of a LUMAC Biocounter 2010. Dilutions of ATP stock solution were used for preparing regression lines to standardize the method for each sample batch separately. Chlorophyll a, organic carbon and ATP concentrations were calculated with reference to dry sediment weight.

All macrofaunal samples were sieved in situ through a 500 mm mesh and the retained sediment containing the macrofaunal organisms was preserved in 10% buffered formalin. Samples were sorted by hand into major taxa and specimens were identified to species level. Macrofauna wet biomass (g m^{-2}) was determined separately for each species and each sample.

3. Results

The results of the seasonal survey combining sediment geochemistry and macrofauna at three commercial fish farms (Karakassis et al. 2000) indicated that the impacts of fish farming on the benthos could vary considerably depending on the specific characteristics of the farming site. At the sampling stations under and near the cages, redox potential was found to decrease but reached negative values only at the silty sediment site. The organic carbon and nitrogen content of the sediment near the cages was found to increase by 1.5-5 times and ATP content by 4-28 in comparison to the respective control sites. No azoic zone was encountered in any of the stations, but the macrofaunal community was affected at a distance of up to 25 m from the edge of the cages. At the coarse sediment sites, abundance and biomass increased by more than 10 times and at all sites diversity indicated that the ecotone was in the vicinity of 25 m from the cages. Similar patterns of succession from the impacted to the normal zones were found in all three areas, although macrofaunal composition differed among the sites.

The study of sediment profiles beneath fish farm cages in Cephalonia (Karakassis et al. 1998) showed that the surface concentrations and the vertical distribution of the sedimentary parameters identified, varied substantially according to distance from the cages and season. The black-colored top layer (farm sediment) showed high concentrations of organic matter phaeopigments and total phosphorus as well as high water content, while the compact subsurface layer had concentrations close to (or lower than) those at the control site. The thickness of the farm sediment layer under the cages varied with season, while during all seasons it decreased rapidly the further the distance from the cages.

The recovery experiment, carried out at a silty sediment site in Cephalonia (Karakassis et al. 1999) showed that the environment had not fully recovered until the end of the observations (at 23 months from the cessation of fish farming). Although initially (during the first 6-10 months) there was a rapid improvement in benthic conditions, subsequently the system showed large fluctuations in the values of most variables over the 23 months. This regression was attributed to a secondary disturbance due to a benthic algal bloom, caused by the seasonal release of nutrients from the farm sediment. It is concluded that the recovery process of heavily enriched benthos in a dynamic coastal environment is subject to the influence of different factors resulting in progress and regression and therefore the succession model proposed by Pearson & Rosenberg (1978) may not be applicable in the early stages of succession.

4. Discussion

4.1 FISH-FARMING IMPACTS

Aquaculture provides a useful example for analyzing human impacts on coastal environments since it interferes directly and indirectly with different biogeochemical processes in the marine environment. In addition, it is a rapidly expanding industry with high potential for further expansion and therefore has the potential for large scale effects.

In the context of locating indicators of environmental disturbance, data from the monitoring of fish farms are also important. They represent a “pure” type of enrichment and therefore they demonstrate the effects of organic enrichment on the seabed rather than of the mixture of pollutants often encountered in sewage wastes.

The benthic survey undertaken in Mediterranean fish farms showed that:

- A large proportion of the P wastes was found to settle on the seabed in the case of sea bream and sea bass cage farming.
- The impacts on the seabed beneath the cages were found to range from very significant to relatively negligible, depending on sediment type and the local water currents.
- In silty sediments, there is higher potential for degradation of benthic conditions with bottom anoxia and H_2S emissions.
- Impacts on the sediment were not observed beyond 25 m from the footprint of the cages.
- A seasonal recovery pattern was found which is related to reduced feed supply and the environmental conditions during the winter period.

- Benthic recovery in silty sediments after the cessation of fish farming may be considerably delayed (more than two years) due to secondary disturbance and new carbon fixation.

4.2 POTENTIAL IMPLICATIONS FOR IDENTIFYING INDICATORS OF BENTHIC DISTURBANCE

The results of these studies provide some indications on the use of benthic variables as a means for assessing the state of the environment:

1. Most of the benthic variables were found to be intercorrelated showing fairly similar patterns in space and time.
2. Sediment profiles may provide a more comprehensive insight into accumulation of wastes. The measurement of surface values alone, although useful for the assessment of the size of the impacted, may not provide adequate information on the dynamic processes related to the accumulation of wasted material beneath the cages. Some of the environmental variables (and in particular concentrations) may be relatively constant in time while the depth of the farm sediment could vary considerably.
3. Traditionally benthic enrichment is monitored using TOC, Eh and macrofaunal data. In the recovery experiment the determination of pigment concentrations showed that the impact of the organic load might be amplified due to secondary disturbance induced by factors different than (although related to) the initial stressor. The nutrients, and particularly phosphate, which do not induce stress to macrofauna during fish farming, triggered benthic algal production after the cessation of aquaculture activities.

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BENTHIC MONITORING IN THE NORTHERN BLACK SEA: OVERVIEW AND IMPLICATIONS FOR THE NEW UNESCO INITIATIVE ON DETERMINING INDICATORS OF BENTHIC HEALTH

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1. Background and Objectives of the Program

In recent years, anthropogenic eutrophication and petroleum pollution have become the biggest ecological problems for the Black Sea coastal zone (mainly for the northern and northwestern coasts). These phenomena have caused major adverse impacts on species composition, structure, and condition of benthic communities and recovery capacity of the Black-Sea coastal ecosystem. To address concerns over such impacts, an ecological monitoring program was implemented about 10 years ago by the Department of Shelf Ecosystems, of the Institute of Biology of the Southern Seas (National Academy of Sciences of the Ukraine, Sevastopol Ukraine). The main emphasis of this program has been to conduct complex physical/chemical and biological studies of the nearshore zone, including comparative bioindication assessments of marine environmental quality, especially in regions with high levels of eutrophication load (i.e. urban zones and areas with developed recreational and agricultural activities).

Basic objectives of the program have been:

- To evaluate the impacts of eutrophication and petroleum hydrocarbon pollution upon biological responses (distribution, abundance, biomass, diversity, etc.) of meiobenthic and macrobenthic communities; and
- To develop appropriate indices for assessing condition of the benthos and to promote further use of these indices in bioindication monitoring of the coastal marine environment.

2. Methods

Surveys were performed from 1987-1997 by the author and other investigators (Kiryukhina and Milovidova 1988; Sergeeva and Mikhailova 1989a,b; Mironov et al. 1992; Kiryukhina et al. 1992) in 32 different regions along the Crimean and Caucasian coasts of the Black Sea (Figures 1 and 2). Data on key abiotic and biotic variables across these locations are presented in Table 1. Sampling stations were included in polluted areas affected by anthropogenic eutrophication (e.g., near municipal sewage discharges — Stations 20, 22, 23, 26, 31, 32) and/or petroleum contamination (e.g., in ports and industrial zones — Stations 7,8, 10-12, 15-17, 28-30). Additional stations were located in natural (non-polluted) parts of the Crimean coasts (Stations 2, 3, 13, 14) and within boundaries of marine reserves and other protected areas (Stations 18, 19, 21). Sampling within several of the regions was conducted along gradients of adverse environmental conditions (Table 1). For example, within large bays such as Sevastopol Bay, Kamyshevaya Bay, Balaklava Bay, and Novorossijsk Bay, samples were taken in three to four areas, from the innermost parts (usually more polluted) to the outermost mouths of the bays. The data for each of the 32 regions in Table 1 are presented as the average of several adjacent stations (minimum number of stations per location is 3-4, and maximum number is 16-18).

Benthic sampling was performed in duplicate or triplicate at each station. Macrofaunal samples were collected with a dredge or grab-corer (surface area of 0.16 m^2). Meiofaunal samples were collected with a tube sampler (diameter of 8 cm). Benthic fauna were identified to lowest possible taxon (to species wherever possible). Abundances and biomass were recorded by lowest taxon.

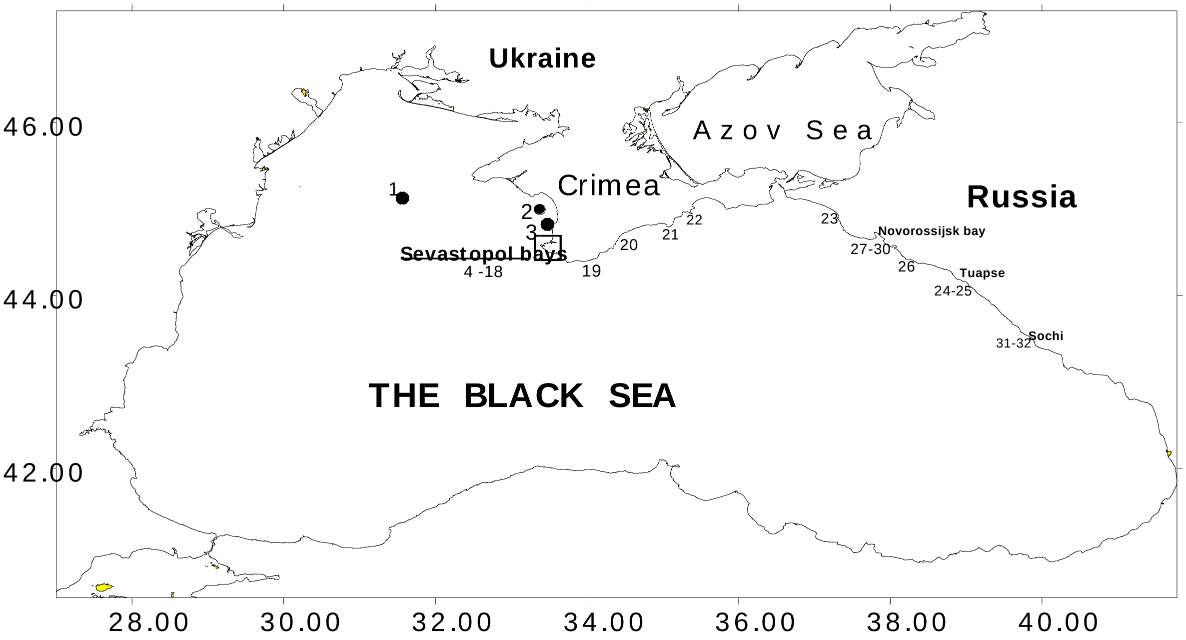

Figure 1. Map of the sampling regions along the Crimean and Caucasian coasts of the Black Sea.

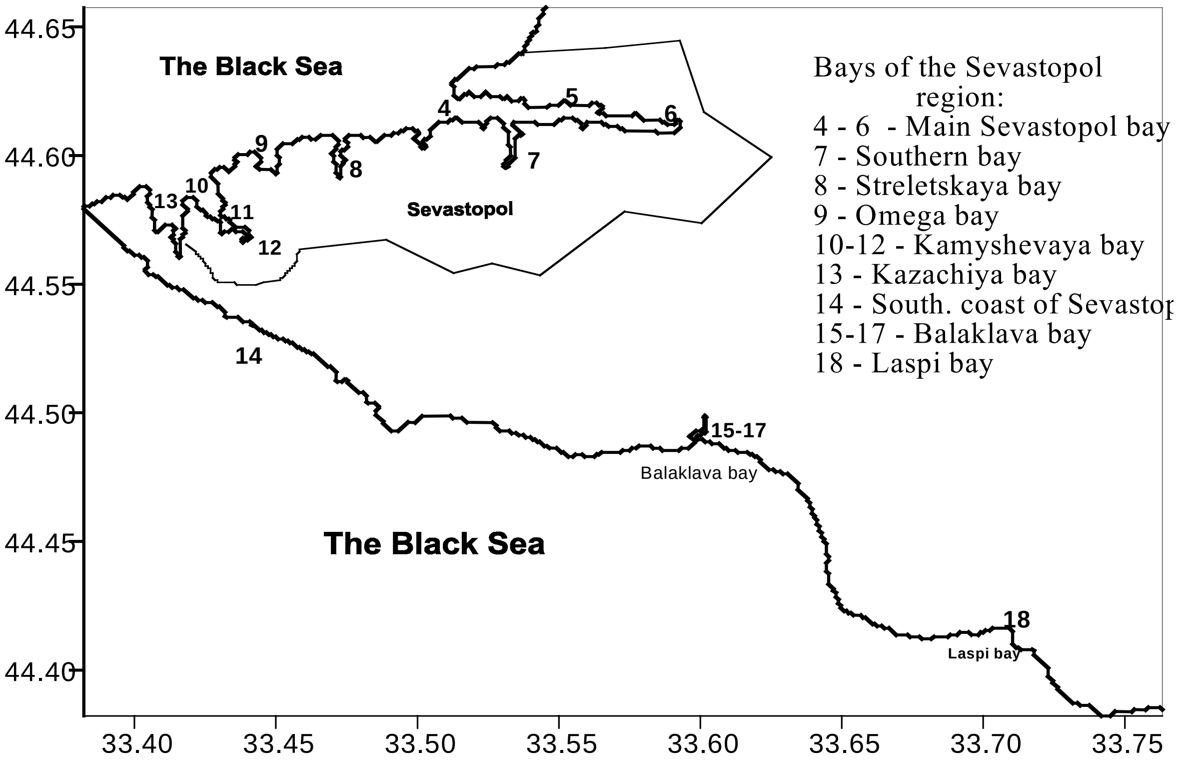

Figure 2. Map of sampling localities in the Sevastopol region.

Table 1. Average initial values of key abiotic and biotic variables used for the analysis of "Benthos-TOC Relationships." Data are presented as the average of adjacent stations (min. of 3, max. of 18) for each of 32 sampling regions, grouped into four categories of TOC load.

Level of TOC load	Region #	Regions	TOC (mg/g sed)	Eh (mv)	CEB (mg/g sed)	Number of species	Abundance (ind/m2)	Biomass (g/m2)	Shannon Index (H)	Abund/S p.N Index
LOW	3	Lubimovka coast (SW Crimea)	1.1	268	0.20	49	549	86.4	2.0	11.2
	2	Kalamitsky gulf (W Crimea)	1.4	164	0.30	26	143	117.5	2.7	5.5
	14	Southern coast of Sevastopol	2.2	140	0.50	36	292	67.8	1.6	8.1
	9	Omega Bay	3.5	313	0.30	13	277	100.8	1.8	21.3
	13	Kazachiya Bay	3.6	186	1.10	39	422	216.2	1.9	10.8
	22	Planerskoye coast (SE Crimea)	4.0	31	0.26	12	144	89.8	1.4	12.0
	1	Zemov's Phillofora Field (NW Crimea)	5.1	60	0.20	21	185	41.2	1.8	8.8
	19	Jalta coast (S Crimea)	6.0	172	0.60	20	694	59.7	2.3	34.7
MODE-RATE	20	Alushta coast (S Crimea)	6.3	244	0.40	21	1063	74.2	1.8	50.6
	24	Tuapse-Shepsi coast (25 m)	7.2	105	0.2	17	945	130.5	1.4	55.6
	18	Laspi Bay (SW Crimea)	7.7	128	0.25	52	684	269.5	2.4	13.2
	21	Cape Meganom (SE Crimea)	9.2	-20	0.10	25	1704	562.3	2.5	68.2
	31	Sochi coast (10-20 m depth)	9.5	108	0.2	20	150	73.3	1.4	7.5
	25	Tuapse-Shepsi coast (40 m)	10.3	18	0.5	9	2214	126.6	0.9	246.0
	23	Anapa Coast	13.0	176	0.15	43	507	293.4	2.4	11.8
	27	Novorossijsk Bay (entrance)	13.6	34	0.70	18	394	135.0	1.9	21.9
	26	Gelendzhik coast	13.8	159	0.30	42	412	153.1	1.6	9.8
HIGH	4	Main Sevastopol Bay (entrance)	15.4	75	4.80	16	148	12.8	1.6	9.3
	32	Sochi coast (25-30 m depth)	15.8	46	0.6	24	209	54.4	1.6	8.7
	10	Kamyshevaya Bay (entrance)	16.7	177	0.90	26	87	3.2	2.3	3.4
	28	Novorossijsk Bay (middle p.)	18.5	-45	1.20	26	233	145.3	2.3	9.0
	12	Kamyshevaya Bay (inner p.)	19.8	-10	1.70	12	206	150.7	1.6	17.2
	11	Kamyshevaya Bay (middle p.)	20.6	-102	4.50	24	35	5.3	1.9	1.5
	29	Novorossijsk Bay (inner p.)	20.8	-139	3.60	14	215	67.3	1.2	15.4
	15	Balaklava Bay (entrance)	21.4	-39	1.90	4	25	0.9	0.6	6.3
VERY HIGH	30	Novorossijsk Bay (port)	27.1	-174	8.0	5	65	5.3	0.4	13.0
	16	Balaklava Bay (middle p.)	28.6	-104	7.20	14	58	5.1	0.9	4.1
	8	Streletskaya Bay	39.7	-124	16.60	18	82	38.5	1.7	4.6
	17	Balaklava Bay (inner part)	43.8	-111	4.90	3	19	0.5	0.5	6.3
	6	Main Sevastopol Bay (inner p.)	46.2	-101	8.10	10	235	38.8	1.2	23.5
	7	Southern Bay	53.1	-139	33.60	6	70	10.1	1.0	11.7
	5	Main Sevastopol Bay (middle p.)	66.4	-147	34.30	7	130	24.6	0.6	18.6

Samples for sediment analyses were collected with the same tube corer used for meiofaunal collections. The following abiotic sediment parameters were measured and discussed herein: (1) Total Organic Carbon (TOC, mg/g sed.); (2) redox-potential (Eh, mv) in the upper 5-cm layer of sediment; (3) size-structure of soft substrate as a percentage ratio between fine (< 0.5 mm) and coarse fractions (> 2 mm) in bottom sediments; and (4) concentration of Chloroform- Extracted Bitumen (CEB, mg/g sed.) in sediments, which provides an estimate of overall oil contamination. Bitumen was extracted from air-dried sediment samples with chloroform and measured gravimetrically (Mironov et al. 1992).

3. Brief Summary of Results

Some of the monitoring results obtained for the Crimean coast were used for the presentation at the UNESCO benthic meeting held in December 1999. These results have shown that the negative influence of anthropogenic eutrophication, evaluated by comparative measurements of Eh values and TOC content in bottom sediments, has been pronounced mostly at the vicinity of Sevastopol Bay and Balaklava Bay, SW Crimea. These severely polluted locations were compared with other less impacted nearshore areas along the western coast (Kacha) and the southern coast (c. Aja) of Crimea (Figure1).

Special attention was given to the analysis of patterns in the structure and composition of benthic populations and communities. Benthic parameters included basic measures of biomass, abundance, species number, and diversity. Other conditional indices for populations and biocenoses provided additional measures of benthic “health” under varying levels of anthropogenic eutrophication stress. Examples of these indices and corresponding results are as follows:

- Mollusk Mortality Index (MMI). This is the percent ratio of the weight of shells of recently dead mollusks to the total weight of living individuals and shells of the same species (Petrov 1990, Petrov 1995). In calculating this index, only the shells of mollusks (bivalves and gastropods) that had recently died within 2-5 months of sampling were included (criteria: presence of soft-tissue remnants or intact ligament between valves, undamaged nacre on the inner surface of shell). Higher values of this index were found in polluted areas due to the higher percentage of mortality.
- Percentage of individuals per population (mussels and clams) with morphological anomalies (like delayed linear growth and deviations in the shell shape due to bulges on the external shell surface) (Petrov 1992, Petrov and Zaika 1993). Higher percentages of abnormalities were observed in the more polluted areas. Similar results were obtained for certain other benthic groups occurring in severely eutrophicated habitats (near sewage discharge outlets). For example, the percentage of nematodes with abnormal amphide disposition and number increased in such areas. Similarly, polychaete populations in such areas have shown high percentages of specimens lacking the 5th group of paragnaths (Sergeeva 1992a).
- Changes in average and maximum size of individuals and changes in number and biomass ratios in individuals belonging to certain size-age groups (Petrov 1994, 1996). In populations of common mollusk species, the size-age structure changed due to the loss of individuals of marginal size-groups, usually less resistant to negative conditions caused by hyper-eutrophication in biotope. Reduction of average and maximum size and age of animals (2-4 times for various species in comparison with the reference localities) have been recorded.
- Variations of sex-ratio in settlements of mussels and hydrobiids (Chukhchin 1992, Petrov Alyomov 1993).
- Meiobenthic Pollution Index (MPI). $MPI = \lg (H+1) + \lg (P+1) / 2 \lg N$, where H, P and N are the numbers (ind/m^2) of Harpacticoida, Polychaeta and Nematoda respectively in a certain benthic sample (Losovskaya 1983). As shown in pre- vs. post-construction studies of the marine environment near a sewage treatment facility and deep-water sewage pipe-line near Jalta, increasing impact induces the replacement of harpacticoides and polychaetes by nematodes. These shifts can be traced by changes in values of the MPI (Sergeeva and Mikhailova 1989a,b; Sergeeva 1992b). Thus, because these different taxonomic groups show different levels of tolerance to increasing organic loading (and associated oxygen depletion), changes in their relative abundances can serve as useful indicators of eutrophication stress.
- Feeding Structure Index (FSI). This is the ratio between different feeding groups of animals in bottom communities (filter-feeders usually are more sensitive to hyper-eutrophication stress in comparison to deposit-feeders and predators). In less eutrophic areas, the measured parameters of filter-feeders were 6-8 times greater than in highly eutrophic areas (Milovidova and Alyomov 1992, Petrov and Shadrina 1996).
- Changes in the relative numbers and biomass of species and taxonomic groups having different levels of tolerance vs. sensitivity to organic enrichment and oil pollution.
- Alteration in the species diversity index (Shannon index H′) and in the poly/monodominant structure of communities. Results showed that in highly impacted areas around Balaklava and Sevastopol Bays, species diversity is 2-5 times lower than in other less-polluted areas. The

community structure also showed a distinct shift towards a prevalence of one to two highly tolerant species (Petrov 1993). There were also reduced numbers and biomass of species of crustaceans and bivalve mollusks that have a low resistance to eutrophication, oxygen deficiency, and oil pollution.

- Shifts in life-history reproductive properties (*r* vs. *k* strategies).

- Shifts in forms of motility (e.g., sedentary vs. highly motile forms) and vertical positioning within sediments (e.g., deep burrowers vs. surface dwellers).

The incidence of adverse biological condition — as evidenced by the above biotic indices and other basic measures of species abundance, diversity, and biomass — was closely linked to patterns of increasing organic enrichment and associated stressors. As shown in Table 1, stations with the lowest abundances, biomass, and biodiversity occurred in areas with high organic loading (high to very high concentrations of TOC), reduced sediment conditions (negative Eh values), and high levels of oil contamination (high to very high CEB concentrations). The highest abundances, biomass, and biodiversity occurred in areas with low to moderate levels of organic loading, high oxidative potential of sediments (large positive Eh values), and low CEB concentrations (below 1 mg/g).

Results of these studies have provided a basis for understanding the impacts of eutrophication and oil pollution on macrobenthic assemblages of the northern Black Sea ecosystem and demonstrating the effectiveness of various ecological indicators as tools for monitoring future changes in the condition of these systems in relation to anthropogenic disturbances. Results also provide a basis of comparison with other regions. To this extent, the data should be useful in efforts under the new UNESCO benthic initiative to identify reliable indicators of benthic health and to compare their effectiveness in test data sets from various coastal regions of the world.

4. Implications for the UNESCO Benthic-Health Initiative: A preliminary look at Benthic-TOC Relationships

Data gathered at the 32 locations along the Crimean (Stations 1-22) and Caucasian (Stations 23-32) coasts were used to support a preliminary analysis of Benthic-TOC relationships (see Figures1-2). Most of the material considered was obtained in the warm (May - October) season of year. Benthic samples were collected predominantly at 10-30 m depths (most areas) and down to depths of 40-50 m near Yalta, Tuapse, and Sochi (Regions 19, 25, and 32, respectively). As noted above, the following abiotic sediment parameters were measured: (1) Total Organic Carbon (TOC, mg/g sed.); (2) redox-potential (Eh, mv) in the upper 5-cm layer of sediment; (3) size-structure of soft substrate as a percentage ratio between fine (< 0.5 mm) and coarse fractions (> 2 mm) in bottom sediments; and (4) concentration of Chloroform- Extracted Bitumen (CEB, mg/g sed.), which provides an estimate of overall oil contamination.

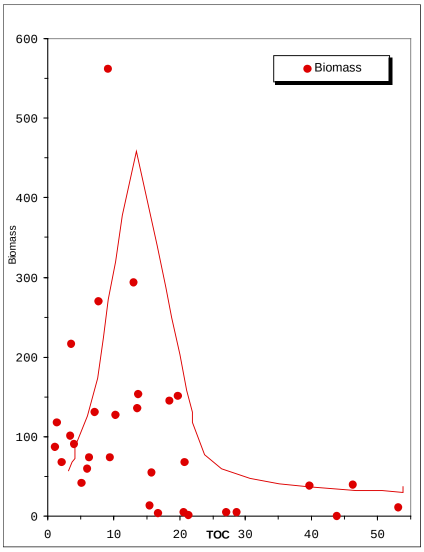

Figure 3. Relationship between TOC (mg/g sed.) and Biomass (g/m^2) of macrobenthos.

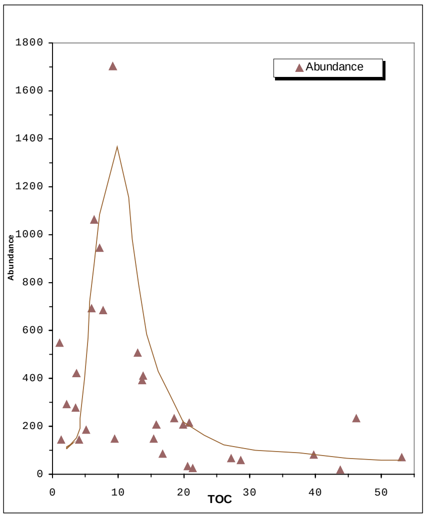

Figure 4. Relationship between TOC (mg/g sed.) and Abundance (ind./m^2) of macrobenthos.

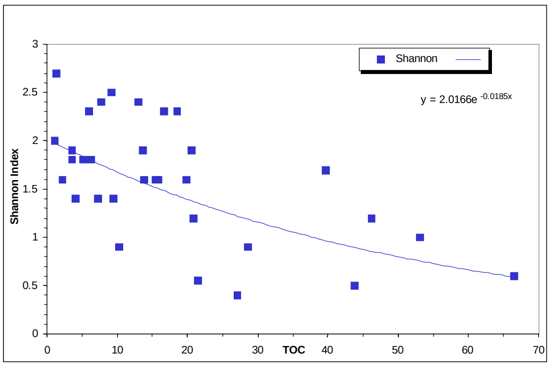

Figure 5. Relationship between TOC (mg/g sed.) and Biodiversity Shannon Index (H´) of macrobenthos.

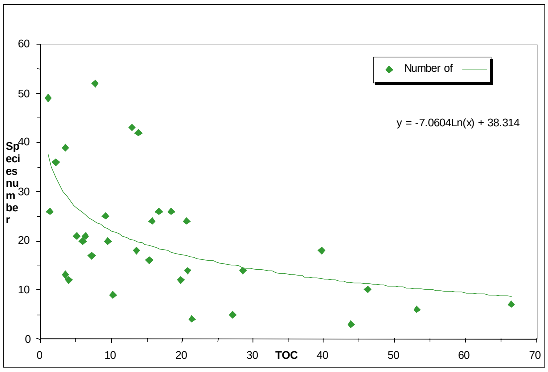

Figure 6. Relationship between TOC (mg/g sed.) and number of macrobenthos species.

Macrobenthic variables for the present analysis included: (1) total biomass (B, g/m^2), (2) abundance (A, ind/m^2), (3) number of species (S), (4) Shannon Diversity Index (H´), and (5) abundance to species ratios (A/S index).

Table 1 lists the 32 sampling regions arranged according to level of organic enrichment. TOC content in bottom sediments ranged from a minimum of 1.1 mg/g sed. (for Lyubimovka) to a maximum of 66.4 mg/g sed. (for the middle part of Sevastopol Bay). Mean values of key abiotic and biotic variables (averaged over all stations within a specific region) are reported for each coastal region. TOC was plotted in relation to each key benthic variable. For each plot, the most representative curves were drawn and regression equations formulated. The results are shown in Figures 3-6.

Using the TOC-benthic variable diagrams and data presented in Table 1, all of the 32 studied locations (regions) were subdivided into four logical categories in accordance with the alteration of TOC level in bottom sediments. The differentiation of regions into different categories was based on more pronounced change of the key abiotic parameters (primarily TOC) in the adjacent areas (see the lines in Table 1) in comparison with alteration of this parameter's values amongst localities within the same category.

The first category incorporates eight regions for which TOC values do not exceed 6 mg/g sediment (low TOC level). As a rule, these sites are in open-shore areas or bays free of anthropogenic eutrophication and other kinds of pollution. In these areas, bottom sediments are always characterized by high redox values (Eh = +160...+ 320 mv) and very low levels of oil (CEB estimates usually not higher than 0.5-0.7 mg/g). The second category comprises nine regions, where TOC content was 6-15mg/g sediment (moderate TOC level). Eight locations were included in the third category, characterized by TOC values of 15-25 mg/g sediment (high TOC level). Lastly, seven stations comprised the fourth category, which had TOC values of 25-66 mg/g sediment (very high TOC level). The last category encompasses predominantly inner areas of bays that are subject to severe anthropogenic impacts. CEB concentrations in these areas are at maximum values of up to 34 mg/g sediment (see Table 1).

Table 2. Comparison of the average values of variables distributed in 4 logical categories in accordance with arrangement of studied regions by gradients of two key parameters: TOC and Eh.

Logical categories (by TOC load)	Key parameter	Range of changes of key pameters	Average values of variables						
			Abiotic			Biotic			
			TOC	Eh	CEB	Biomass	Abund.	Sp.Num.	Sh.Index
1	**TOC -->**	1 - 6 mg/g sed	3.4	+175	0.43	97.4	338	27.6	1.94
(Low)	**Eh -->**	+320....+160 mv	6.4	+213	0.49	119.0	468	29.6	2.15
2	**TOC -->**	6 -15 mg/g sed	10.1	+88	0.31	212.0	897	27.3	1.82
(Moderate)	**Eh -->**	+160....+30 mv	10.0	+95	0.86	104.2	380	27.4	1.71
3	**TOC -->**	15 -25 mg/g sed	18.6	-5	2.47	55.0	146	18.3	1.78
(High)	**Eh -->**	+30....-100 mv	14.2	-11	0.94	214.9	754	14.8	1.56
4	**TOC -->**	25 - 65 mg/g sed	43.5	-129	14.33	17.5	94	9.0	0.89
(Very high)	**Eh -->**	-100....-230 mv	38.5	-127	13.42	21.7	101	11.2	1.04

For each of the four TOC categories, values of abiotic and biotic variables were also averaged across all component coastal areas. The results are given in Table 2 and in bar-diagrams (Figure7). Note that the division of regions into four logical categories was done in relation to TOC content and, independently, in relation to Redox-potential (Eh). Thus, TOC averages calculated for each category can be compared to a relevant Eh average, linked with TOC, but varying independently. Similar patterns in TOC and Eh among the various station categories were obtained regardless of which of these two variables were used to group the categories. For example, when regions are grouped by TOC, the mean Eh is +175 for Category 1 (low organic loading) and –129 for Category 4 (very high organic loading). If regions are grouped instead by Eh, a similar Eh pattern emerges: mean Eh of +213 for Category 1 and mean Eh of –127 for Category 4. Patterns in the CEB content and four biotic variables across the various station categories are also very similar regardless of whether TOC or Eh is used as the grouping variable.

Logical ranges of TOC (mg/g)	1 - 6	6 - 15	15 - 25	25 – 65
Level of TOC load	**Low**	**Moderate**	**High**	**Very High**
Sampling Regions	1,2,3,9,13,14,19,22	18,20,21,23-27,31	4,10-12,15,28,29,32	5-8,16,17,30
Aver. REDOX-potential (Eh), mv	175	88	-5	-117
Aver. Chl. Extr. Bitum. (CEB), mg/g sed.	0.43	0.31	2.47	14.33
Average BIOMASS, g/m^2	97.4	212.0	55.0	17.5
Average ABUNDANCE, ind./m^2	338	897	146	94
Average Number of Species	27.6	27.3	18.3	9.0
Aver. Shannon Diversity Index (H)	1.94	1.82	1.78	0.89
Abund./Spp. Number Index	14.4	29.8	9.2	11.0

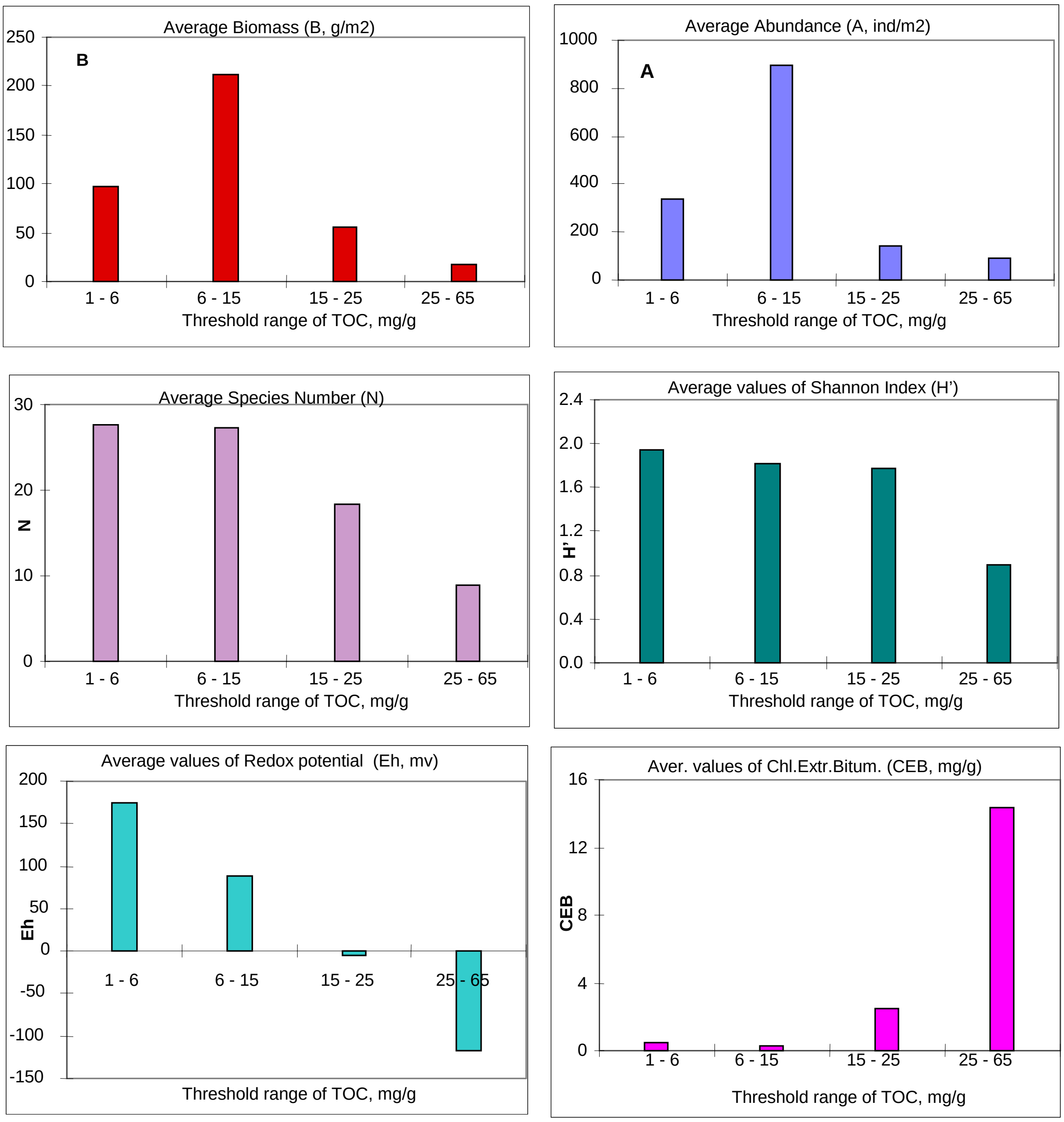

Figure 7. Bar-diagrams illustrate tendencies in changes of key variables (average values) at different threshold ranges of TOC.

Thus, the subdivision of the various localities into four categories by TOC gradient, and the determination of threshold TOC values for flagging co-occurring changes in other environmental variables indicative of healthy versus stressful conditions, can be shown to have logical grounds. These results suggest the following TOC thresholds associated with the emergence of conspicuous changes in structural and quantitative parameters of benthic communities in coastal areas of the Black Sea:

- TOC < 6 mg/g (Low). Below this threshold, diversity of macroinfaunal communities (numbers of species and Shannon H′ index) and redox potential of sediments (Eh) reach maximum values (see Figure 7). Macroinfaunal biomass and abundance are below maximum (though at moderately high values). CEB concentrations are very low.
- TOC from 6-15 mg/g (Moderate). Within this TOC range, biomass and abundance variables are highest. Species number and the Shannon diversity index are about the same as for the lower TOC category. In general, TOC values in this range may be indicative of a “conditional ecological optimum” for Black Sea benthic communities.
- TOC from 15-25 mg/g (High). Within this TOC range, oxygen content in sediments sharply declines and the oxidative state of sediments shifts from oxidizing to reducing conditions (Eh values < 0). Concentrations of CEB also show noticeable increases. The average biomass and abundance of macroinfauna decrease sharply (by a factor of 4-6) indicating that the animals are under stress. Numbers of species also show moderate declines (30-40% on the average), though changes in the Shannon index are less conspicuous (see Figure 7).
- TOC 25-65 mg/g (Very High). Finally, under a very high level of eutrophication, when TOC content exceeds about 25-30 mg/g, the structural and quantitative characteristics of benthic communities decrease 3-10 times in comparison to mesotrophic habitats with TOC in the optimum range of 6-15 mg/g. Usually such changes are accompanied by dramatic deterioration of oxygen conditions in the sediments (Eh is strongly negative with values of -100 to -230 mv). In addition, CEB concentrations reach maximum values, indicating high levels of oil pollution. Benthic communities may consist of only a few species, mainly some polychaetes and gastropods with high resistance to oxygen deficiency and hyper-eutrophication of bottom sediments.

In conclusion, by looking at the relationships between TOC and other key environmental variables at 32 coastal locations in the northern Black Sea, it was possible to divide sites into four logical categories, defined by ranges in TOC, and which reflected varying degrees of anthropogenic stress. Key benthic attributes (abundances, diversity, biomass) and other abiotic environmental variables (Eh and CEB) showed distinct differences among the various TOC categories. Additional studies should be conducted to test for consistent patterns of association between these variables in other biotopes and to attempt to identify similar thresholds for assessing the incidence of anthropogenic stress in these environments.

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CHEMICAL ASPECTS OF ORGANIC CARBON AND ECOLOGICAL STRESS IN BENTHIC ECOSYSTEMS

Dr. James Shine (Harvard School of Public Health, Cambridge Massachusetts) and Dr. Gordon Wallace (Univ. of Massachusetts, Boston Massachusetts)

1. Introduction

Contaminants discharged into aquatic ecosystems are often rapidly scavenged by particles and removed to the sediments. While this can act as an effective trap preventing the offshore transport of contaminants, it can cause high concentrations in underlying sediments leading to adverse ecological and human health outcomes. For example, in even moderately contaminated coastal ecosystems, the ratio of the concentration (or inventory) of contaminants in surface sediments to concentrations (or inventories) in the water can be as high as 100,000. Given the variability of water concentrations due to changes in factors such as tidal state or freshwater input, monitoring programs assessing the health of marine ecosystems should focus on sediments rather than water, as an individual sediment sample is a better integrator of stress over longer time periods.

The goal of this brief is to discuss the role of organic carbon on the biogeochemical cycling of contaminants in marine sediments. Organic carbon alters contaminant transport, fate, and effects in sediments. Given limited resources and technical capabilities, it is often difficult to measure all possible stressors or adverse ecological effects in sediments impacted by anthropogenic activity. In these cases, it is often desirable to determine surrogate parameters sufficiently correlated with endpoints of concern that can serve as indicators of adverse effects. There are many ways in which indicators can be used. There can be indicators of susceptibility, exposure, quantitative dose, and adverse responses. A goal of this presentation is to highlight ways in which the organic carbon content of coastal marine sediments serves as an indicator of adverse ecological effects. The presentation will focus on chemical aspects, examining the interaction of organic carbon with organic and inorganic contaminants. Specifically, this paper will discuss the role of organic carbon on the retention of contaminants in sediments, its role on contaminant bioavailability, and relationships between organic carbon and sediment-water exchange of contaminants.

2. Organic Carbon and Retention of Contaminants in Sediments

A positive correlation between the concentration of organic carbon in sediments and levels of contaminants is frequently observed in coastal marine sediments. This is due to the fact that organic carbon can react with contaminants, through processes such as hydrophobic interactions with non-polar organic contaminants, or formation of complexes at polar functional groups with heavy metals. Figures 1a - 1c show data from Massachusetts Bay and New Bedford Harbor, two coastal ecosystems receiving inputs of anthropogenic contaminants. In all cases, sediments with high organic carbon have higher concentrations of contaminants (in these cases, lead in New Bedford Harbor, and copper and PAHs in Massachusetts Bay). The slopes of the lines shown in Figure 1 are the organic carbon normalized concentration of that contaminant in the sediment. Although the sediment concentrations may vary within a location (for example, lead concentrations vary from approximately 20 g/g to almost 700 g/g in New Bedford Harbor), the organic carbon normalized concentration is fairly constant. Given these relationships, it is clear that the organic carbon content can be an indicator of the presence of contaminants. An important qualifier to this relationship is shown in figures 1b and 1c. The data in these two figures have been split into locations from different parts of Massachusetts Bay. The ‘near field’ samples were collected in close proximity to Boston Harbor, the primary source of contaminants to Massachusetts Bay. The ‘far-field’ samples were collected further offshore, in waters less influenced by inputs from Boston Harbor. The important aspect of figures 1b and 1c is that the slopes for the contaminant-organic carbon relationship are different. Because there are smaller inputs of contaminants to the far field region of Massachusetts Bay, there are smaller concentrations of contaminants per unit of organic carbon in the sediment. In general, although the organic carbon content of the sediments is an indicator of the presence of contaminants, the extent that it is predictive of adversely high concentrations depends on the nature of the contaminant-carbon relationship in a specific ecosystem. As will be shown in the next section, this relationship is related to the loading and concentrations of contaminants in the overlying water. In summary, within a specific ecosystem, there generally will be a relationship between the sediment carbon content and the concentration of contaminants in the sediments. The slope of that relationship will vary between ecosystems, such that an attempt to combine carbon-contaminant relationships from a number of locations into one universal plot will fail to yield useful results.

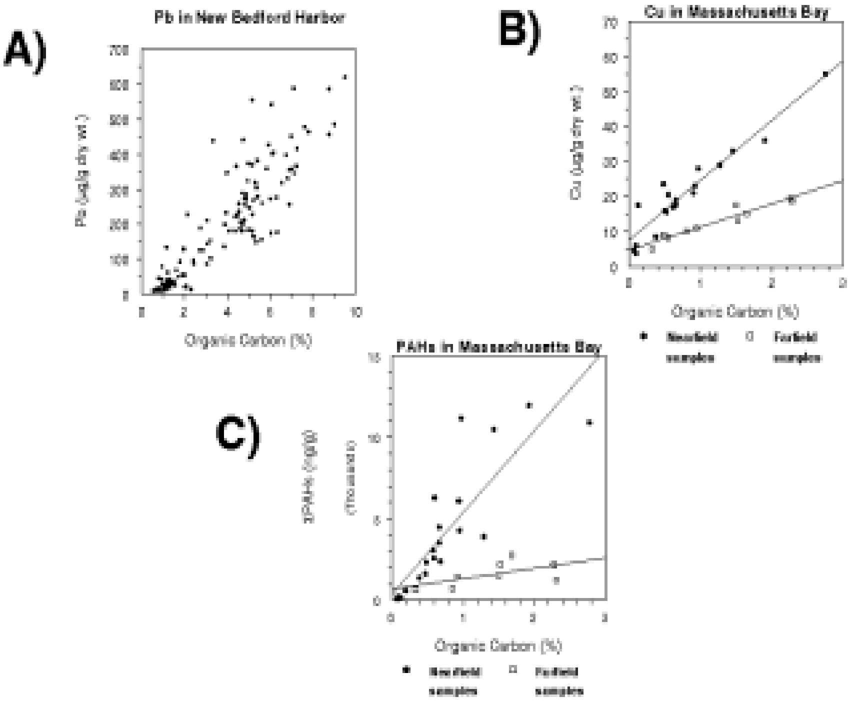

Figure 1. Relationship between organic carbon content and levels of contaminants in coastal marine sediments. Shown are data for (a) lead in New Bedford Harbor, USA, (b) copper in Massachusetts Bay, USA, and (c), total PAHs in Massachusetts Bay, USA.

3. Organic Carbon and Bioavailability of Contaminants in Sediments

To a large extent, contaminants are present in sediments due to sorption onto sinking particles. A key question concerning the availability of these contaminants to benthic organisms is the strength the reaction between the contaminant and the particle. For example, if contaminants are irreversibly bound to sediment particles, then none of the contaminants will be available for uptake by benthic organisms. Adverse effects would not be predicted for any concentration. In reality, there are a number of biogeochemical processes occurring in sediments altering the form and therefore bioavailability of contaminants. Without a thorough understanding of these reactions, it will be impossible to understand or predict the adverse effects of contaminants present in sediments.

For metal contaminants, both organic carbon and sulfides can be important reactants reducing the availability of metals to benthic organisms. In particular, sulfides in anaerobic marine sediments have been identified as a key reactant binding metals, rendering them less available for biological uptake. As a result, metal toxicity in sediments is generally not observed when the concentration of sulfides available for reaction (acid volatile sulfides, AVS) exceeds the concentration of metals potentially available for uptake by benthic organisms (simultaneously extracted metals, SEM). That is, toxicity is only seen when the SEM/AVS ratio exceeds one. The concentration of AVS in sediments has in fact been proposed as a normalizing phase against which to estimate the toxicity of metals in sediments. However, toxicity is not always observed in sediments when the SEM/AVS > 1. This implies that other binding phases, namely organic carbon, also contribute significantly to reduction of metal availability. Our current research is showing that the metal binding capacity of organic carbon in sediments from New Bedford Harbor is equal to the binding capacity of the sulfides. Relying solely on sulfides to estimate toxicity, therefore, is inappropriate. Organic carbon plays an equal role in these sediments in reduction of metal availability to benthic organisms. There are also data to support the hypothesis that since carbon is an electron donor and sulfides a major terminal electron acceptor in sediments, that the concentration of AVS is in fact related to the organic carbon content of the sediments. This relationship is likely modified by the lability of the organic carbon and the temperature of the sediments.

For hydrophobic organic contaminants such as pesticides, polynuclear aromatic hydrocarbons (PAHs), and polychlorinated biphenyls, organic carbon has been demonstrated to be the major phase binding these contaminants and reducing availability to benthic organisms. The partitioning of hydrophobic organic contaminants can be predicted from their octanol-water partition coefficient (K_{OW}). The U.S. Environmental Agency has used this approach to develop numerical criteria for five non-polar organic compounds in sediments. A crucial assumption with the Equilibrium Partitioning approach is that all contaminants in the sediments are available for equilibrium partitioning. This is not always the case. Studies in Boston Harbor have shown that not all of the PAHs in the sediment are available for partitioning into the porewater. The presence of soot carbon, a byproduct of combustion, has been identified as a reactant in sediments such as these that binds contaminants much more strongly than ‘regular’ organic carbon. A hypothesis is that soot carbon may be present at higher levels if the source of the PAHs is pyrogenic (from combustion sources) rather than petrogenic (from an oil spill). Thus, the extent of partitioning of organic contaminants to organic carbon may be related to both the source of the contaminant and the quality of the organic carbon. In short, for both metal and organic contaminants, organic carbon is a key binding phase capable of reacting with these contaminants, perhaps reducing their availability and subsequent adverse effects in benthic organisms.

4. Relationship Between the Concentration of Contaminants in the Water Column, Concentrations in Sediments, and Sediment Organic Carbon Content

As seen in figure 1, the concentration of contaminants in sediments is related to the organic carbon concentration. However, figures 1b and 1c show how the slope of that relationship differs between locations, and is likely related to the loading of contaminants to a particular ecosystem. In fact, an equilibrium relationship can be shown between the concentration of contaminants in the water column and the organic carbon normalized concentration of contaminants in the sediments. Figure 2 shows the average water column concentration of lead in the water column of New Bedford Harbor, Buzzards Bay, Boston Harbor, and two locations in Massachusetts Bay with the organic carbon normalized concentration of lead in the sediments at those locations. The plot indicates a linear relationship between the two parameters indicating (i) that water column and surface sediments are in equilibrium with respect to the distribution of contaminants between water and sediment, and (ii) the organic carbon normalized concentration of contaminants in sediments can be predicted by knowing the loadings and subsequent concentrations of contaminants in the overlying water column.

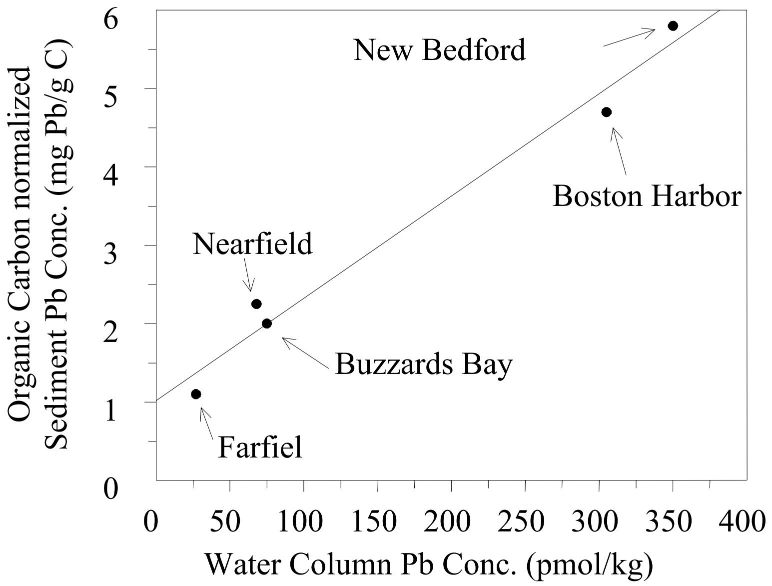

Figure 2. Relationship between average overlying water concentrations of dissolved lead with organic carbon normalized concentration of Pb in underlying sediments.

5. Organic Carbon Respiration and the Return Flux of Metals from Sediments Back to the Overlying Water Column

Given the above relationships, another question concerns the fate of the large reservoir of contaminants sequestered in sediments. If the input of contaminants to the overlying water ceases, will contaminants remobilize from the sediments to re-establish the water-sediment equilibrium relationships previously described? Will this delay the anticipated improvements in water quality after cessation of contaminant input? Also, as organic carbon is respired in the sediments, does this release contaminants from particles into the pore water, creating a diffusion gradient and subsequent flux back to the overlying water column? If so, the sediments may only be temporary sinks of contaminants. Our study in New Bedford Harbor showed that the large reservoir of metals in the sediments has become a major source of contaminants to the overlying water column. Dissolved metal fluxes were measured in laboratory microcosms collected over different seasons and incubated in the dark at in situ temperatures. Regression of metal fluxes (normalized to the concentration in sediments expressed on an organic carbon basis), against benthic oxygen demand was significant for all metals ($r^2 = 0.58 - 0.85$, Figure 3) and showed the following relationship: Cd > Zn > Ni, Cu > Pb, indicating higher relative mobility for Cd and lower mobility for Pb. The estimated annual fluxes to the overlying water column amount to between 0.02% (Pb) to 20% (Cd) of the inventory of metals in the top 2 cm of the sediments, and can equal the inventory of metals in the overlying water column in as short as 10 days (Zn). Again, this study highlights the importance of organic carbon preservation in sediments on the biogeochemical cycling of contaminants discharged to the environment.

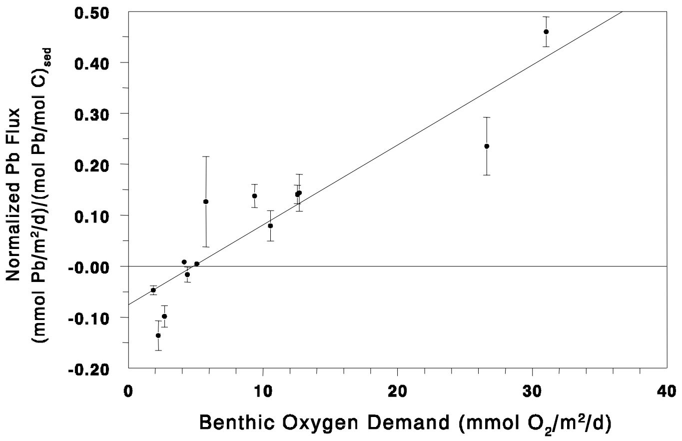

Figure 3. Relationship between sediment oxygen demand and the sediment-water flux of dissolved Pb (normalized to sediment metal content) from sediments in New Bedford harbor and Buzzards Bay, USA.

6. Conclusions

In conclusion, the cycling of organic carbon in sediments influences fate and effects of contaminants. On one hand, organic carbon can lead to retention of contaminants in sediments. In this sense, it serves as a marker of susceptibility, as the slope of the carbon-contaminant relationship will depend on contaminant loadings and concentrations to the overlying water column. Conversely, organic carbon can reduce the availability of contaminants to benthic organisms. This can occur directly, through reaction of the contaminant with organic carbon, or indirectly, through the production of sulfides during carbon respiration, which can react with and reduce the availability of metals. Depending on the endpoints of interest, organic carbon may serve as an indicator of the disposition of contaminants present in coastal marine sediments.

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SECTION IV: MEETING AGENDA

Monday, December 6

9:00 - 9:30	Assemble.
9:30 – 10:00	Introduction and welcoming remarks: P. Bernal, Jeff Hyland.
10:00 – 10:45	Overview of the background and purpose of our benthic health initiative in relation to objectives and responsibilities of UNESCO / IOC, GOOS, HOTO, GIPME Umit Unluata.
10:45 – 11:00	Coffee break
11:00 – 13:00	Case study overviews (Presentations by each committee member on the kinds of measurements that they have been collecting, and the conclusions that could be drawn from their data).
11:00 – 12:00	Hyland: *Work in southeastern U.S. estuaries*.
12:00 – 13:00	Petrov: *Work in Black Sea coastal ecosystem*.
13:00- 14:30	Lunch
14:30 - 15:30	Y. Karakassis: *Work in eastern Mediterranean/Aegean Sea*.
15:30-16:30	P. Magni: *Work in Japanese coastal ecosystem*.
16:30-17:30	J. Shine: *What a marine chemist thinks about organic carbon in sediments*.
17:30	Time for follow-up discussions.

Tuesday, December 7

Open discussions on identifying goals, approaches, products, and milestones.

9:30 - 12:30.	Morning topics:

1. Are we attempting to develop new indicators, or determine which existing ones work the best (with respect to their ability to detect human-induced stress in the benthos, their applicability in coastal systems all around the world, and how easy they are to measure and understand)?

2. How are we defining "indicators"? Do we mean specific biological and environmental attributes (e.g., species diversity as a biological attribute, concentrations of chemical contaminants as an abiotic environmental variable); or data-analysis approaches (e.g., multivariate techniques such as cluster analysis, principal component analysis, discriminant analysis, multidimensional scaling); or a combination of both.

3. What are the kinds of databases that we are looking for (e.g., multivariate databases with synoptic measurements of benthic and abiotic environmental variables, including anthropogenic stressors and natural abiotic controlling factors)? What are the minimum requirements for these test data sets? Also looking for coverage of both developed and developing countries.

4. Have we missed appropriate data sets for other regions that should be included, as well as additional committee members to represent these data?

5. What are our suggestions for potential indicators and/or data-analysis approaches to use for measuring the health of benthic communities? Begin developing, at the meeting, a list of candidate indicators/techniques.

6. Define criteria for ranking effectiveness of each indicator. For example, go over the HOTO methodology for ranking measurements (Unluata to provide overview).

12:30 – 14:00	Lunch
14:00 – 18:00	Afternoon topics:

7. Try to reach some consensus on which of these indicators/techniques hold the most promise for global applications, and thus should be tested further.

8. Discussion of the relationship between simple infaunal species variables (e.g., numbers of species, H′ diversity, abundance) and TOC concentrations. Begin examining these relationships and their effectiveness as potential indicators of stress across our different data sets [Note that, in order to facilitate this discussion, each committee member should bring a file of these data from their own data sets, and hard copies of appropriate tables and plots to illustrate the relationships resulting from their data].

9. Make commitments for each member to test the effectiveness of various other priority indicators using their respective data sets. These analyses would be done after the meeting at each member's home facility.

10. Make arrangements for comparing the test results and ranking the indicators based on these results.

Wednesday, December 8

Morning - Open - If necessary, this period could be used to continue the discussion of benthic-TOC relationships as a potential easy-to-measure and globally applicable indicator. [Note: Umit Unluata will be out Wednesday morning].

13:00 - 16:00	Remaining topics:
	1. Discussion of what our "product" should be.
	2. Discussion of follow-up action items and milestones.
16:00 - 17:00	Coffee break.
17:00 - 18:00	Final wrap-up.

Thursday, December 9

Left open to accommodate the need for any follow-up discussions of prior topics, or for individual information exchange.

SECTION V: PRELIMINARY RESULTS ON BENTHIC-TOC RELATIONS

AN ANALYSIS OF RELATIONSHIPS BETWEEN TOC CONCENTRATION AND INCIDENCE OF STRESS IN THE MARINE BENTHOS FROM SELECTED COASTAL REGIONS OF THE WORLD

1. Introduction

A new international environmental initiative is being pursued under the auspices of the Intergovernmental Oceanographic Commission (IOC), of the United Nations Educational Scientific and Cultural Organization (UNESCO), to develop recommendations for a suite of reliable and globally applicable indicators for assessing the condition (or "health") of marine benthic communities. This initiative supports two closely related programs administered by UNESCO/IOC — the Global Investigation of Pollution in the Marine Environment (GIPME) and the Health of the Oceans (HOTO) module of the Global Ocean Observing System (GOOS). Both programs are aimed at developing environmental health criteria and indices that can serve as early warning signals of change in the quality of the world's oceans.

An important goal of the UNESCO benthic-health initiative is to define indicators that are: (1) reliable in their ability to detect stress where stress should be occurring (e.g. due to high contaminant levels or other pollutants); (2) powerful in their ability to discriminate between anthropogenic vs. natural sources of stress; and (3) easy to use and broadly applicable in different parts of the world. Initial efforts to address this goal have focused on the analysis of benthic-TOC relationships in test data sets provided by participating scientists (UNESCO Benthic Indicator Group). Synoptic measurements of macroinfauna, TOC concentrations, pollutant levels, and other controlling abiotic variables are being examined across the various data sets to look for consistent patterns of association and to attempt to identify TOC thresholds that may serve as indicators of high versus low risks of benthic impacts and other related adverse environmental conditions.

A conceptual model for benthic-TOC relationships was presented in Section I of this report (see Figure 1, modified from Pearson and Rosenberg 1978). In accordance with this model, benthic variables such as numbers of species, abundance, and biomass are expected to increase in relation to increasing organic carbon (TOC) content of the sediment up to a certain critical level, and then begin to decline. Population increases below the TOC critical level reflect a combination of the nutritional value of low-to-moderate amounts of organic matter present in the sediment and other favourable environmental conditions, such as sufficiently high levels of dissolved oxygen and low unharmful levels of sediment-associated stressors (e.g., contaminants, ammonia, sulfide). As TOC exceeds a certain critical level, the benthos begins to show signs of stress due to co-occurring decreases in oxygen levels and increases in pollutant levels. Given these patterns, it should be possible to identify TOC thresholds that could serve as indicators (or "warning signals") of related adverse environmental conditions leading to stress in the benthos.

Initial efforts to evaluate the effectiveness of TOC as an indicator of benthic health are underway. Preliminary results are presented here for four coastal regions: southeastern U.S.A.; Seto Inland Sea, Japan; eastern Mediterranean; and northern Black Sea. Information on individual data sets are also discussed in further detail in the corresponding meeting-presentation summaries included in Section 2.

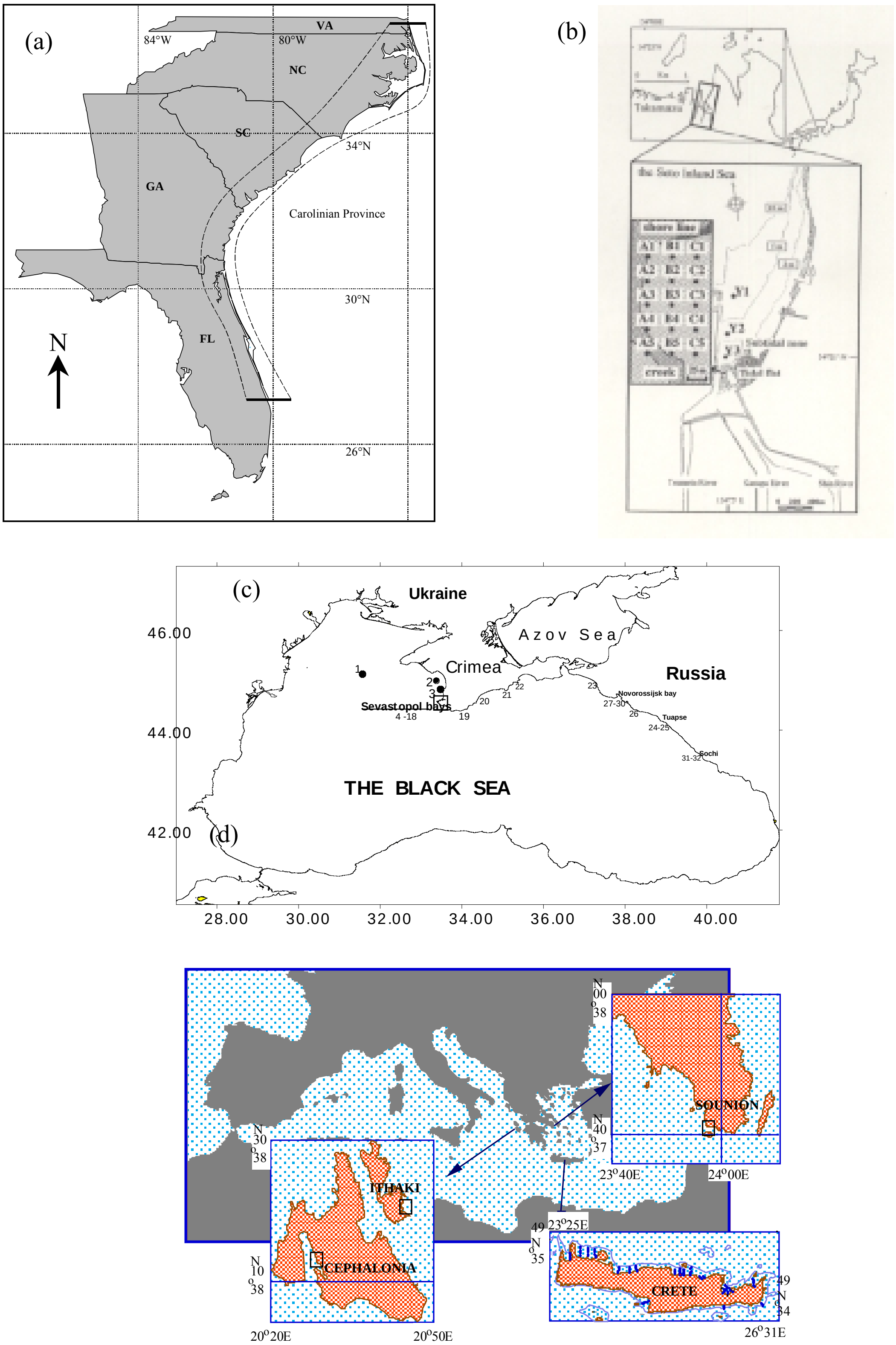

Figure 2-1. Maps of the (a) Carolinian Province sampling region (area enclosed by dashed lines) along the southeastern coast of the United States, (b) study area and location of sampling stations, Seto Inland Sea, Japan, (c) sampling regions along the Crimean and Caucasian coasts of the Black Sea, and (d) eastern Mediterranean study area.

Initial efforts to evaluate the effectiveness of TOC as an indicator of benthic health are underway. Preliminary results are presented here for four coastal regions: southeastern U.S.A.; Seto Inland Sea, Japan; eastern Mediterranean; and northern Black Sea. Information on individual data sets are discussed in further detail in the corresponding meeting-presentation summaries included in Section 2.

2. Approach

Maps of the four study regions are presented in Figure 2-1. Analyses were performed for each region using all samples (regardless of sampling time or location) that provided synoptic data on benthic community structure and composition, stressor levels, and natural controlling abiotic factors. Thus, each data set may contain information representative of multiple sampling places and periods, and ignores potential variation due to such factors. If necessary, subsequent analyses may be performed with subsets of data to account for such influences.

For each region, the TOC concentration of sediments was plotted against common benthic variables (e.g., biomass, numbers of species, H′ diversity, abundance) as a basis for examining relations between the organic-carbon content of sediment and the integrity of the ambient benthic community. Best-fit curves were determined for each combination of variables by sequential testing of appropriate nonlinear regression models and selection of the ones that produced the highest R^2 values. Based on these relationships, attempts were made to identify ranges in TOC concentrations corresponding to distinct shifts in the integrity of biological attributes. Mean values of benthic variables and other abiotic environmental variables (e.g., concentrations of chemical contaminants) were compared across the different TOC ranges to determine whether the patterns were meaningful. Data plots and tables for each of the four case studies are presented below (Section 3).

Subsequent applications in other coastal regions will be performed as additional data sets are identified and accessed. To the extent possible, data sets will be obtained from both developing and developed countries. Once these analyses are completed, a manuscript will be prepared for publication in the peer-reviewed scientific literature.

3. Preliminary Results

Key data for each of the four case studies are summarized in the tables and figures of Sections 3.1 through 3.4 below.

3.1 Case Study 1: Estuaries of the Southeastern USA.

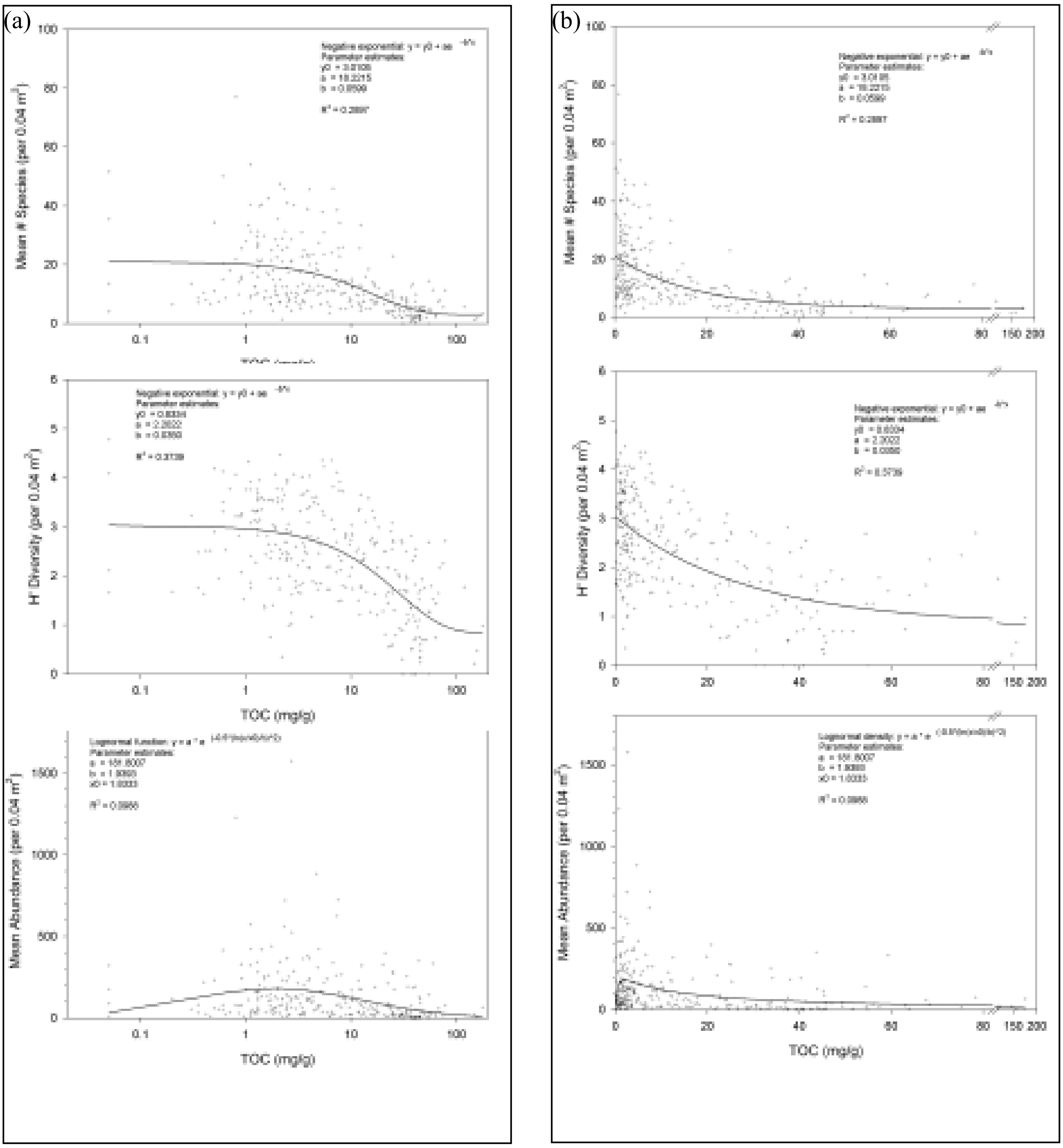

Figure 3.1-1. Plots of key benthic variables (mean number of species, H´ diversity, and abundance) vs. TOC concentration. (a) TOC plotted on log scale. (b) TOC plotted on linear scale. Nonlinear regression curves and corresponding R^2 values are included.

Table 3.1-1. Comparison of TOC concentrations and selected infaunal variables at non-polluted vs. polluted sites throughout southeastern U.S. estuaries (EMAP Carolinian Province data, 1994-97, n=299 sites). Mean and range (in parentheses) are shown for each category.

	Non-Polluted Sites (Mean ERM Quotient ≤ 0.02) n=145	Marginally Polluted Sites (0.02 < Mean ERM Quotient ≤ 0.058) n=70	Highly Polluted Sites (Mean ERM Quotient > 0.058) n=84
TOC (mg/g)	3.16 (0.05-28.00)	15.93 (0.60–47.00)	40.56 (0.46–175.20)
# species (per $0.04m^2$)	19.33 (3.50–77.00)	9.89 (0.00–46.00)	6.30 (0.00–42.50)
H′ (per $0.04m^2$)	2.86 (0.75–4.78)	2.26 (0.00–4.36)	1.44 (0.00–3.75)
Abundance (per $0.04m^2$)	179.32 (6.50–1577.50)	85.86 (0.00–422.50)	60.38 (0.00–721.50)

Table 3.1-2. Comparison of selected infaunal and environmental variables within four ranges of TOC concentrations based on data from southeastern U.S. estuaries (EMAP Carolinian Province data, 1994-97, n=299 sites). A=Mean and range (in parentheses among sites within each category; B=Percentage of sites within each category that showed specified symptom).

		TOC (mg/g)			
		≤ 0.5 n=13	> 0.5 – 10 n=161	10 – 30 n=58	>30 n=67
A.	Mean # species (per $0.04m^2$)	16 (4.0 – 52.0)	18.0 (1.5 – 54.0)	9.2 (2.0 – 34.0)	4.3 (0 – 14.0)
	Mean abundance (per $0.04m^2$)	110 (6.5 – 321.0)	169.0 (5.5 – 1578.0)	86.0 (2.5 – 398.0)	46.0 (0 – 350.0)
	Mean H′ (per $0.04m^2$)	2.7 (1.6 – 4.8)	2.8 (0.4 – 4.5)	2.2 (0.6 – 3.6)	1.2 (0 – 2.8)
	B-IBI	3.6 (3.0 – 4.5)	3.9 (1.0 – 5.0)	2.9 (1.0 – 5.0)	2.0 (1.0 – 4.5)
B.	% sites with unhealthy benthos (B-IBI < 3)	0	9.3	48.0	81.0
	% sites with high contamination (mean ERM quotient >0.058)	8.0	6.8	21.0	90.0

3.2 CASE STUDY 2: SETO INLAND SEA, JAPAN

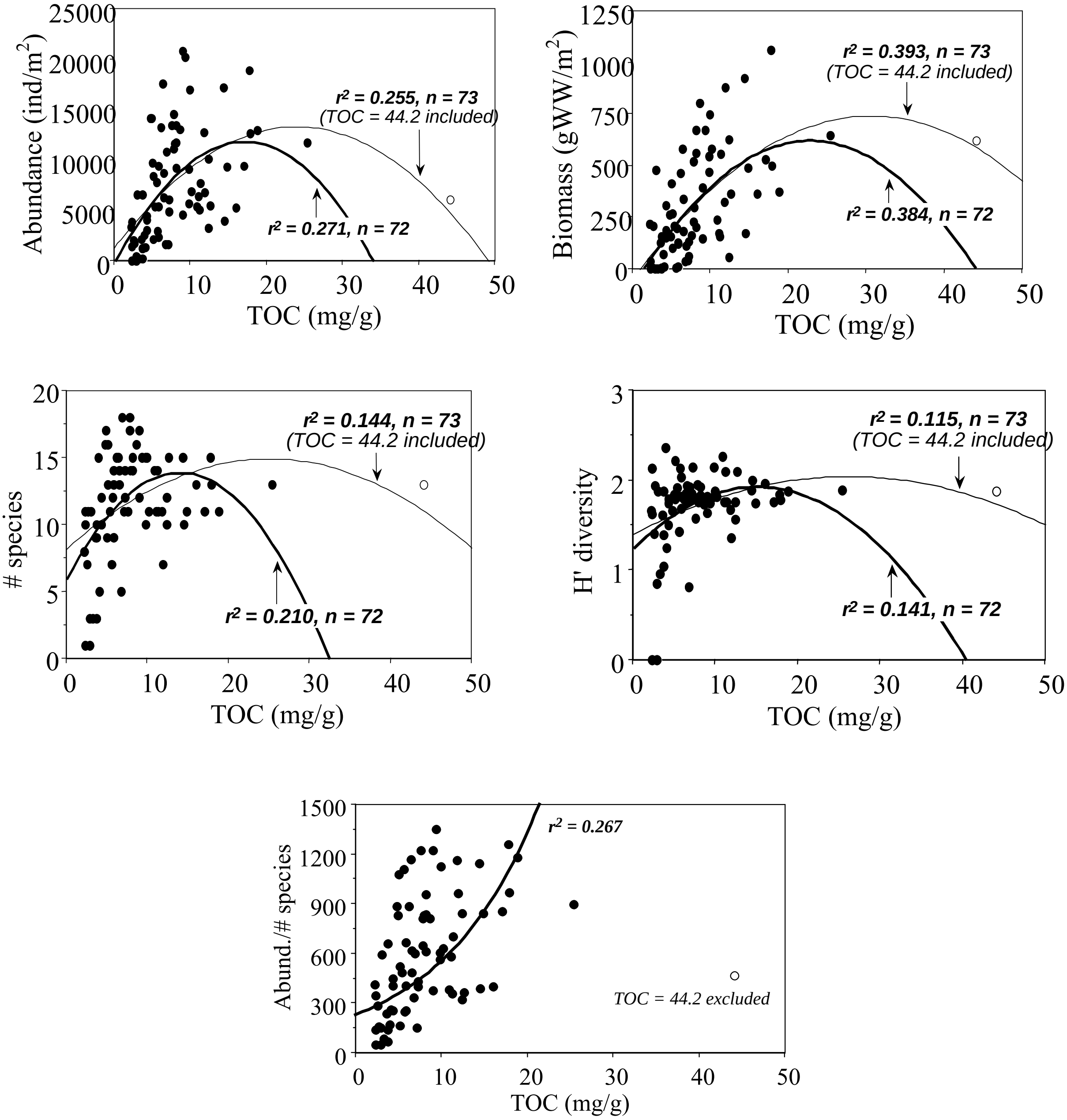

Figure 3.2-1. Plots of key benthic variables vs. TOC concentration in the intertidal zone.

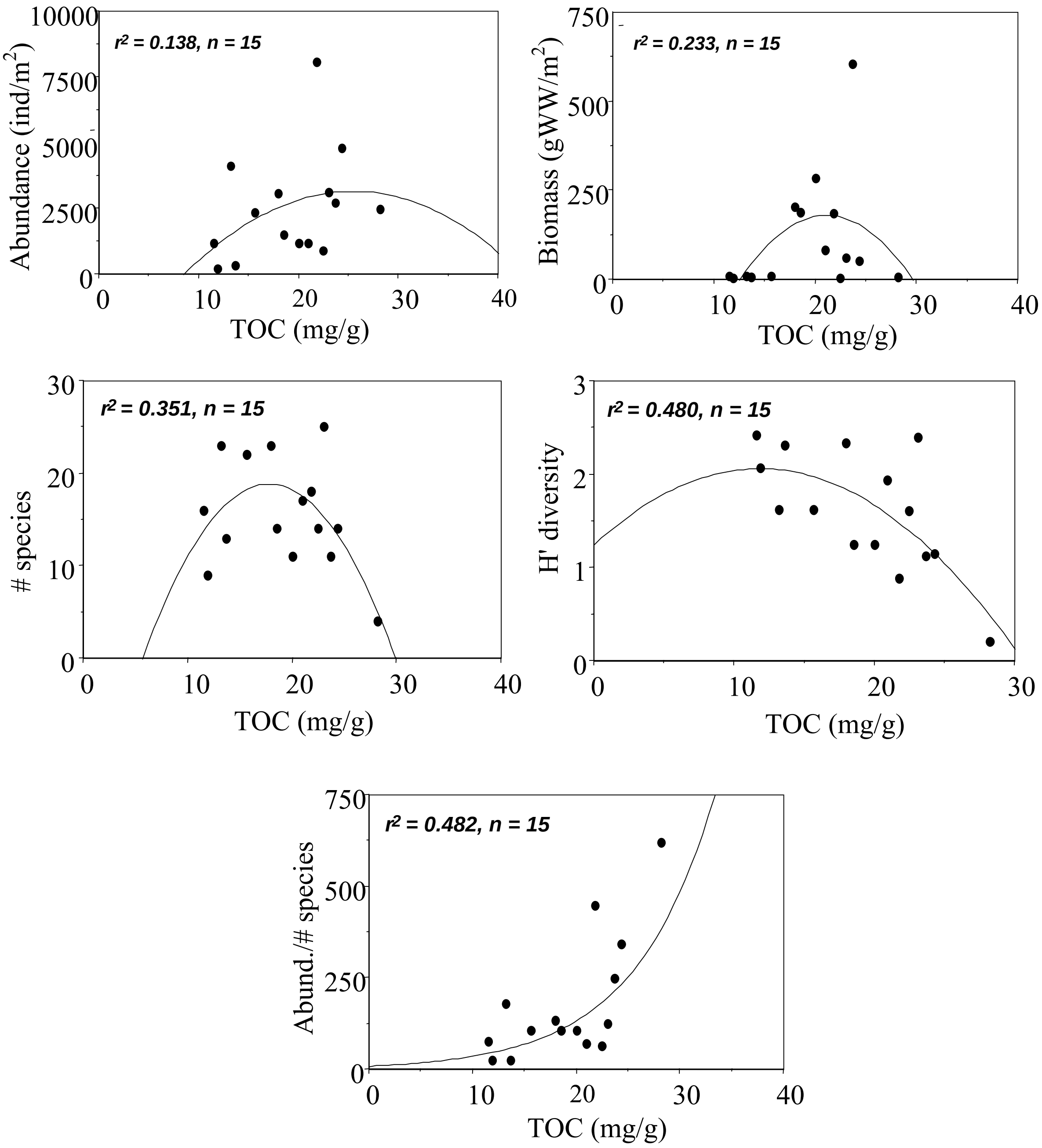

Figure 3.2-2. Plots of key benthic variables vs. TOC concentration in the subtidal zone.

Table 3.2-1. Average values of benthic variables (± SD) within three ranges of TOC concentrations. A. Intertidal zone. B. Subtidal zone.

A. INTERTIDAL	Low TOC < 6.0 mg/g	Mod. TOC 6.0 – 25.0 mg/g	High TOC > 25.0 mg/g
# species	10 ± 4.3	13 ± 2.7	13 ± 0
Abundance (ind./m^2)	4218 ± 3861	9866 ± 5022	8850 ± 3960
H´	1.5 ± 0.56	1.8 ± 0.23	1.9 ± 0.01
Biomass (g/m^2)	127 ± 130	407 ± 260	633 ± 17.0

B. SUBTIDAL	Low TOC < 16 mg/g	Mod. TOC 16 – 22 mg/g	High TOC > 22 mg/g
# species	17 ± 6	17 ± 5	14 ± 8
Abundance (ind./m^2)	1630 ± 1623	2990 ± 2949	2790 ± 1397
H´	2.01 ± 0.38	1.52 ± 0.59	1.30 ± 0.79
Biomass (g/m^2)	7.4 ± 3.6	188.1 ± 72.0	145.1 ± 258.8

3.3 CASE STUDY 3: EASTERN MEDITERRANEAN

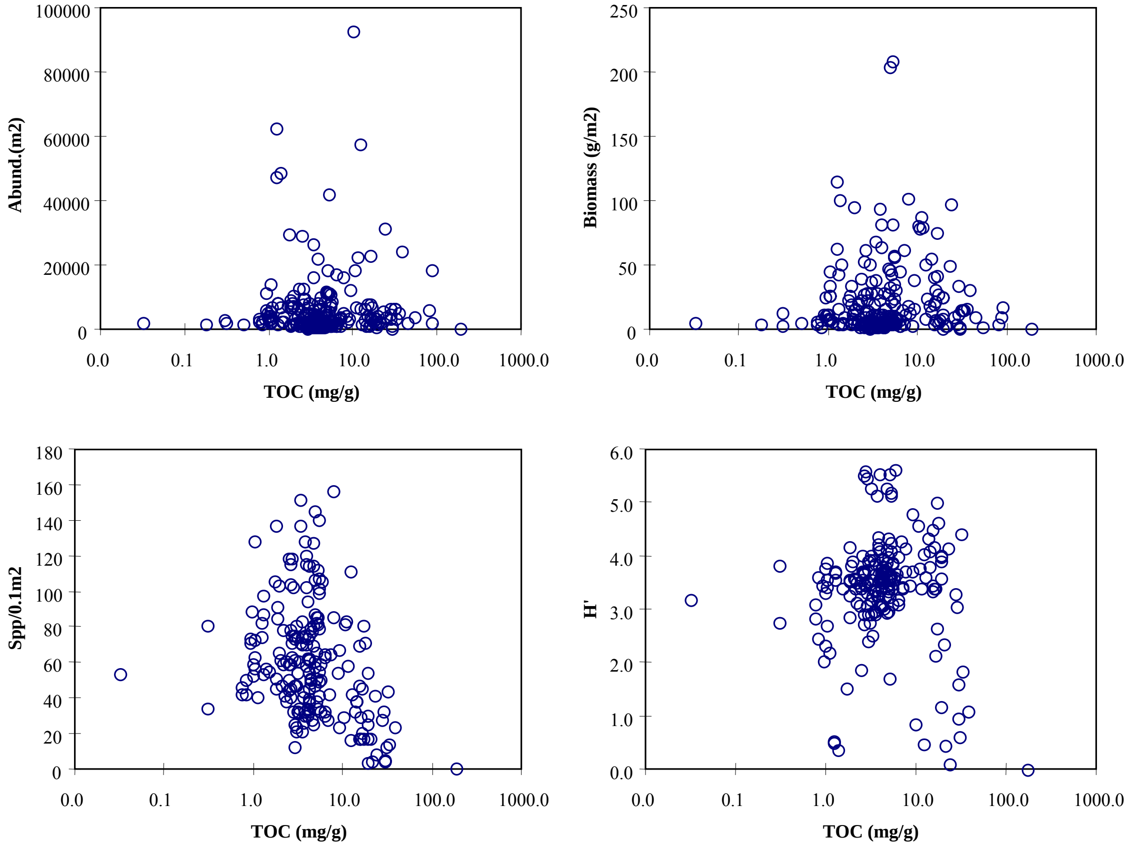

Figure 3.3-1. Plots of key benthic variables vs. TOC concentration, all TOC values included.

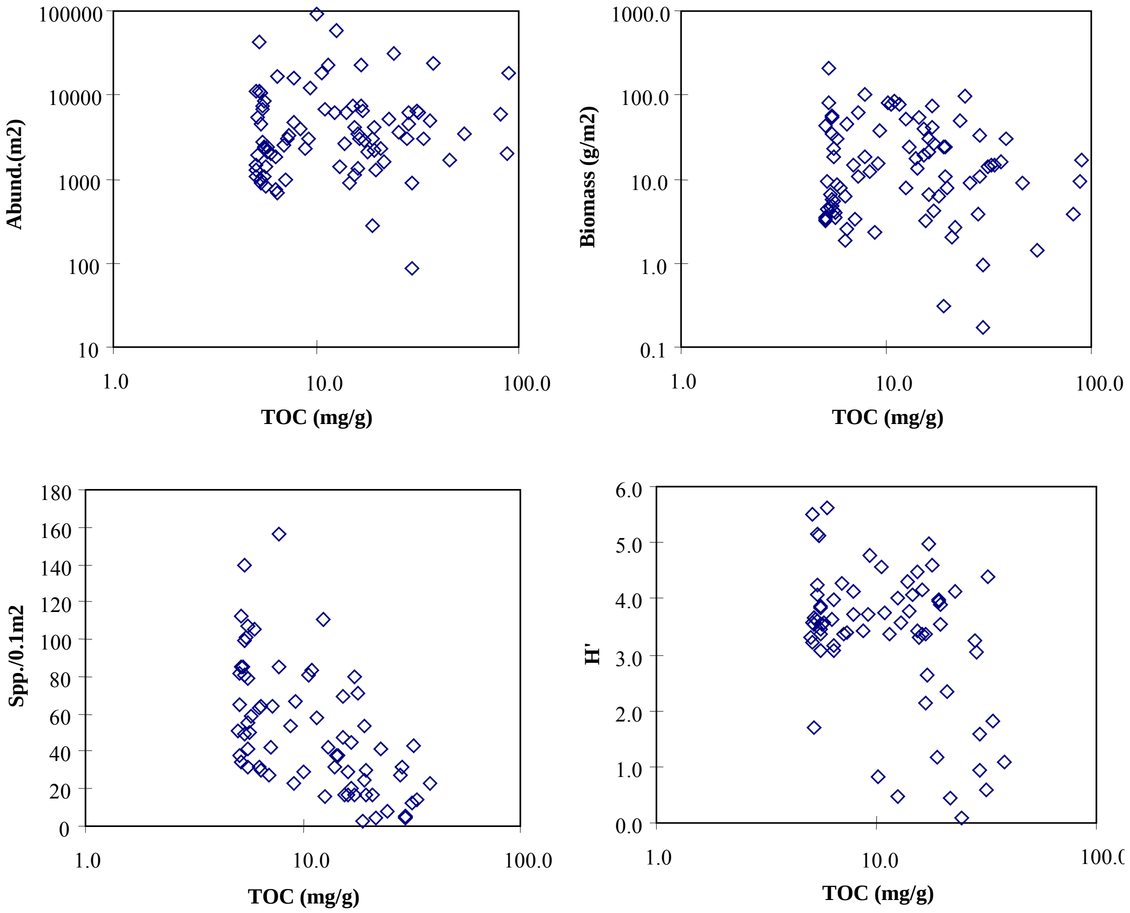

Figure 3.3-2. Plots of key benthic variables vs. TOC concentration, large TOC values (> 5 mg/g) only.

Table 3.3-1. Average values (plus minimum and maximum) of benthic variables within four ranges of TOC concentration (mg/g).

	<1	1-10	10-25	>25
# species (0.1 m^2)	63(34 - 128)	67(12 - 156)	39(3 - 111)	20 (4 - 43)
Abundance (m^2)	3884(1220 - 13940)	5449(170 - 62260)	10909(282 - 92364)	5876 (85 - 24125)
H´	3.1(2.0 - 3.9)	3.5(0.4 - 5.6)	3.2(0.1 - 5.0)	2.1 (0.6 - 4.4)
Biomass (g/m^2)	10.4(1.1 - 44.4)	19.9(0.2 - 207.8)	32.7(0.3 - 96.2)	11.6 (0.2 - 32.8)

3.4 CASE STUDY 4: NORTHERN BLACK SEA

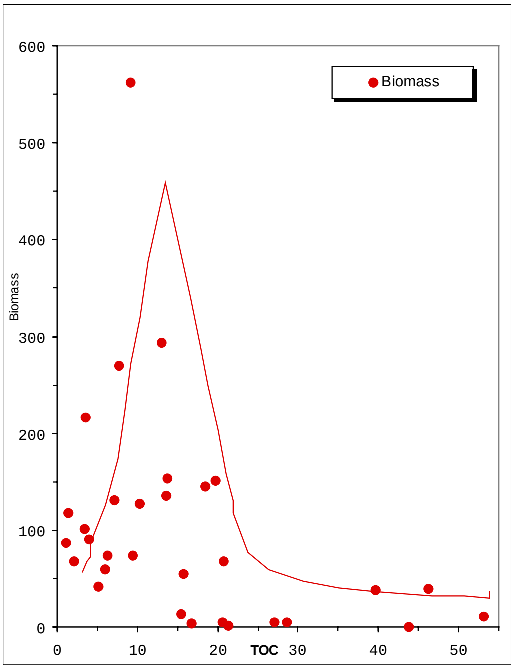

Figure 3.4-1. Relationship between TOC (mg/g sed.) and biomass (g/m^2) of macrobenthos.

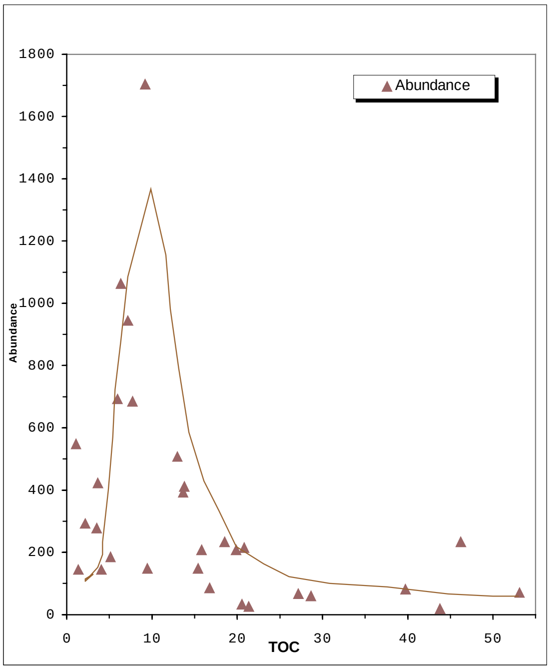

Figure 3.4-2. Relationship between TOC (mg/g sed.) and Abundance (ind./m^2) of macrobenthos.

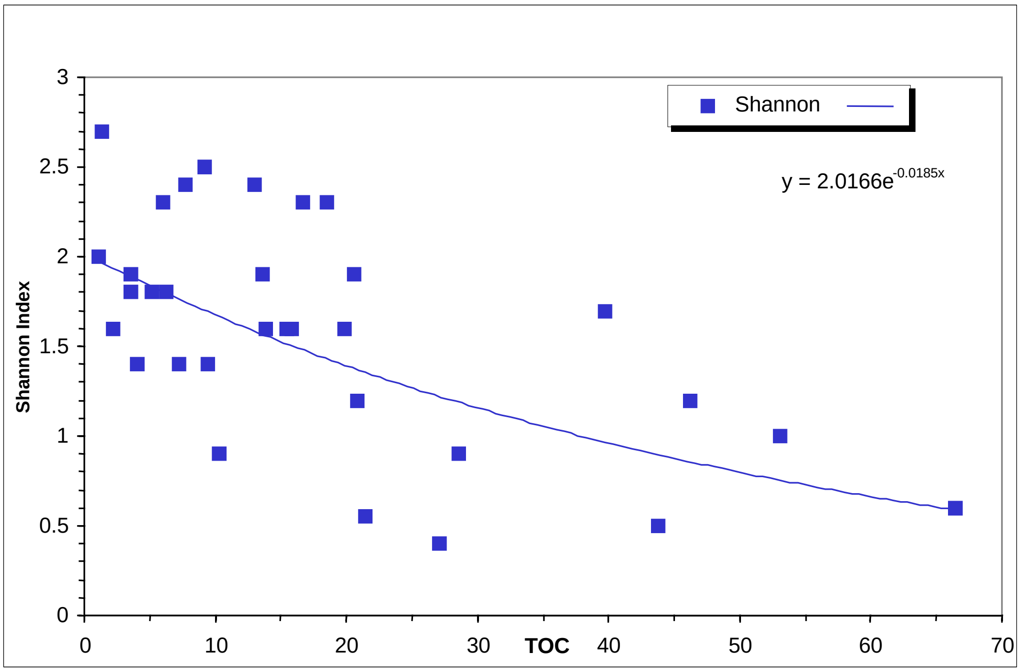

Figure 3.4-3. Relationship between TOC (mg/g sed.) and Biodiversity Shannon Index (H′) of macrobenthos.

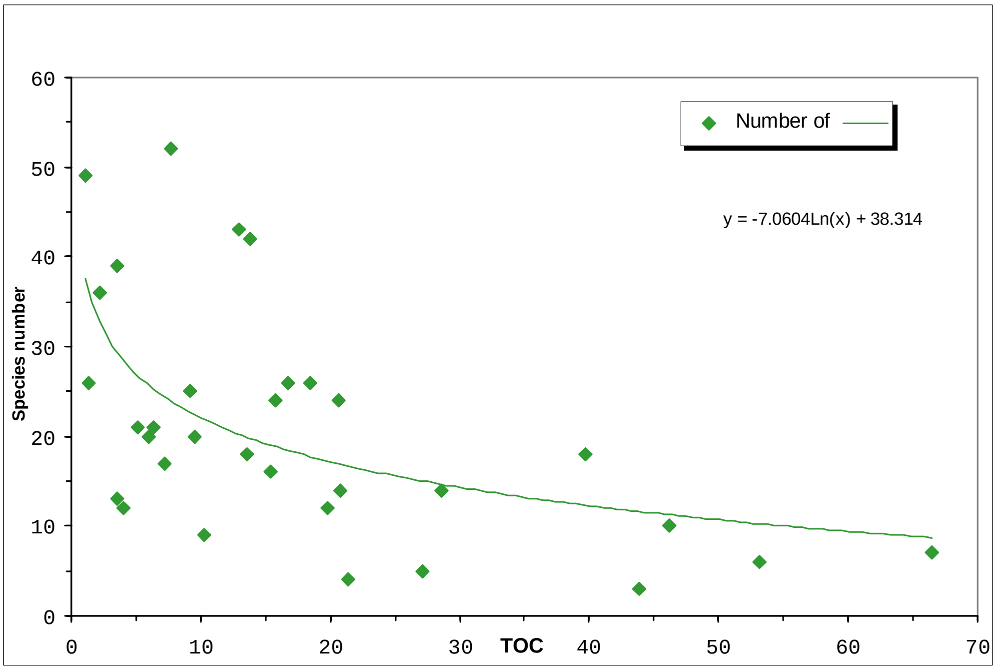

Figure 3.4-4. Relationship between TOC (mg/g sed.) and number of macrobenthos species.

Logical ranges of TOC (mg/g)	**1 - 6**	**6 - 15**	**15 - 25**	**25 – 65**
Level of TOC load	**Low**	**Moderate**	**High**	**Very High**
Sampling Regions	1,2,3,9,13,14,19,22	18,20,21,23-27,31	4,10-12,15,28,29,32	5-8,16,17,30
Aver. REDOX-potential (Eh), mv	175	88	-5	-117
Aver. Chl. Extr. Bitum. (CEB), mg/g sed.	0.43	0.31	2.47	14.33
Average BIOMASS, g/m^2	97.4	212.0	55.0	17.5
Average ABUNDANCE, ind./m^2	338	897	146	94
Average Number of Species	27.6	27.3	18.3	9.0
Aver. Shannon Diversity Index (H)	1.94	1.82	1.78	0.89
Abund./Spp. Number Index	14.4	29.8	9.2	11.0

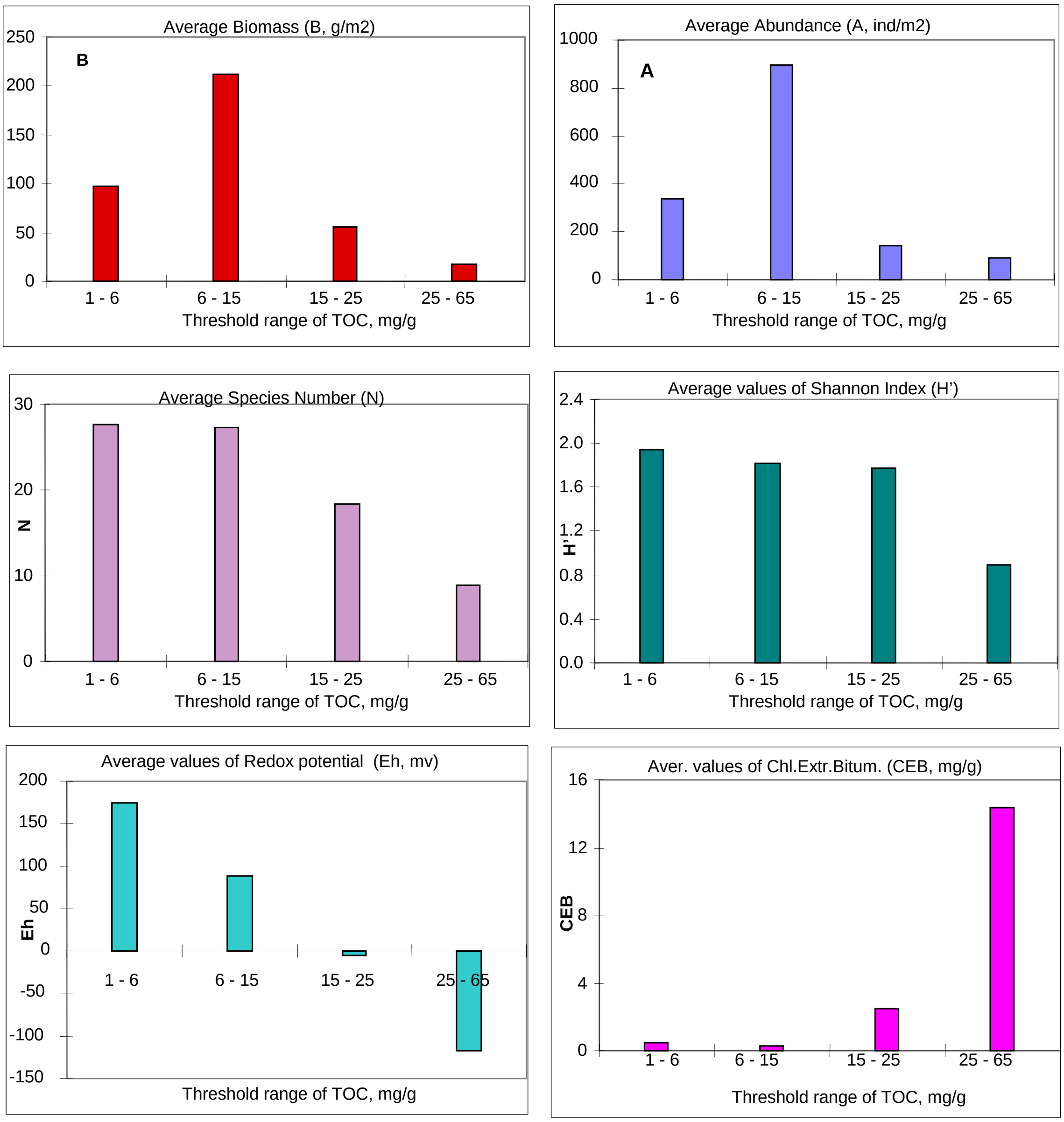

Figure 3.4-5. Bar-diagrams illustrate tendencies in changes of key variables (average values) at different threshold ranges of TOC.

Table 3.4-1. List of the studied regions (Crimean coast: NN 1-22; Caucasian coast: NN 23-32) and main abiotic variables (average data) used for analysis of "Benthos-TOC Relationships."

Sampling Region Number	Regions, depth (m)	Bottom Temp. (ºC)	Salinity (‰)	Diss. O_2 (ml/l)	Corg.max (mg/g)	%Fine fr./%Coarse fr.	CEB max (mg/g sed)
1	Zemov's Phillofora Field (NW Crimea), 10m	19.2	16.6	4.8	18.0	56,6/24,0	0.4
	20m	14.5	17.2	4.2	18.0	56,6/24,0	0.4
	30m	9.8	17.6	3.8	18.0	56,6/24,0	0.4
	40m	6.7	18.2	3.0	18.0	56,6/24,0	0.4
2	Kalamitsky gulf (W Crimea), 10m	22.0	17.3	4.9	6.2	88,8/3,2	0.4
	20m	17.4	17.3	4.9	6.2	88,8/3,2	0.4
3	Lubimovka coast (SW Crimea), 10m	22.0	18.0	6.1	0.9	46,3/38,8	0.3
	20m	17.0	18.0	5.7	0.9	46,3/38,8	0.3
	30m	12.0	18.0	5.7	0.9	46,3/38,8	0.3
4	Main Sevastopol Bay (entrance), 15m	19.0	18.2	7.7	34.5	62/18	10.6
5	Main Sevastopol Bay (middle p.), 10m	22.4	17.9	7.2	71.8	99/0,5	57.0
6	Main Sevastopol Bay (inner p.), 8m	22.8	17.5	6.7	39.7	96,4/1,2	14.1
7	Southern Bay, 14m	16.5	17.8	7.2	70.4	98,5/0,3	49.2
8	Streletskaya Bay, 12m	14.8	17.4	6.7	54.2	63/26	27.8
9	Omega Bay, 8m	24.1	17.2	5.8	5.2	24/58	0.4
	15m	22.8	17.2	5.8	5.2	24/58	0.4
10	Kamyshevaya Bay (entrance), 20m	21.5	18.0	6.9	36.6	22/70	2.6
11	Kamyshevaya Bay (middle part), 15m	20.8	17.6	4.9	25.2	67/26	7.4
12	Kamyshevaya Bay (inner part), 10m	23.1	16.8	6.4	33.4	51/39	4.2
13	Kazachiya Bay, 10m	20.3	18.0	6.7	4.3	18,2/76,5	0.7
	20m	18.2	18.1	6.2	4.3	18,2/76,5	0.7
14	Southern coast of Sevastopol, 20m	18.8	17.9	7.4	2.9	11,5/85,8	0.9
	30m	13.2	18.0	6.5	2.9	11,5/85,8	0.9
15	Balaklava Bay (entrance)	20.5	18.3	6.6	24.6	28/56	2.1
16	Balaklava Bay (middle Part)	21.9	17.7	5.8	36.4	94/0,6	8.3
17	Balaklava Bay (inner part)	23.8	16.3	4.3	50.2	77/22	5.9
18	Laspi Bay (SW Crimea), 10m	20.6	17.8	8.2	10.6	69/14	0.5
	20m	15.8	18.4	7.4	10.6	85/6	0.5
	25-30m	13.5	18.5	5.3	10.6	95/1,5	0.5
19	Jalta (South, coast of Crimea), 10m	24.0	17.6	6.9	9.1	60,6/21,5	0.6
	20m	17.2	17.8	6.6	9.1	60,6/21,5	0.6
	30m	15.0	18.0	6.0	9.1	60,6/21,5	0.6
	40-50m	11.4	18.2	5.4	9.1	60,6/21,5	0.6
20	Alushta coast (S Crimea), 10m	22.4	17.6	6.2	11.4	65,3/25,1	0.6
	20m	18.2	17.8	6.2	11.4	65,3/25,1	0.6
21	Cape Meganom (SE Crimea), 20m	15.9	17.5	8.1	10.8	58,1/24,6	1.4
22	Planerskoye coast (SE Crimea), 10-15m	21.8	17.0	7.7	14.0	46,8/48,9	0.8
23	Anapa coast, 10m	23.2	17.2	6.6	16.6	30,0/68,5	0.2
	25m	14.1	17.4	6.6	16.6	47,8/51,6	0.2
24	Tuapse coast, 25m	18.8	17.1	6.0	8.2	52,6/39,5	0.3
25	Tuapse-Shepsi coast, 40m	13.4	17.5	5.0	11.8	92,8/2,4	0.6
26	Gelendzhik coast, 10m	22.0	17.6	6.3	15.5	18,6/52,4	0.8
	25m	15.9	17.6	6.0	15.5	18,6/52,4	0.8
27	Novorossijsk Bay (entrance), 25m	16.4	18.3	6.1	23.3	86/9,3	1.3
28	Novorossijsk Bay (middle p.), 20m	16.8	18.0	4.9	25.4	79/8,2	1.5
29	Novorossijsk Bay (inner p.), 10m	19.8	17.9	3.4	21.5	72/20,5	1.6
30	Novorossijsk Bay (port), 10m	20.0	17.7	2.8	29.1	95/2,8	8.2
31	Sochi coast, 10-20m	22.0	17.0	7.4	10.4	28,8/60,5	0.3
32	Sochi coast, 25-40m	17.6	17.4	5.8	17.6	76,3/11,2	0.7

Table 3.4-2. Average initial values of key abiotic and biotic variables used for the analysis of "Benthos-TOC Relationships." Data are presented as the average of adjacent stations (min. of 3, max. of 18 for each of 32 sampling regions, grouped into four categories of TOC load.

Level of TOC load	Region #	Regions	TOC (mg/g sed)	Eh (mv)	CEB (mg/g sed)	Number of species	Abundance (ind/m2)	Biomass (g/m2)	Shannon Index (H)	Abund/Sp.N Index
LOW	3	Lubimovka coast (SW Crimea)	1.1	268	0.20	49	549	86.4	2.0	11.2
	2	Kalamitsky gulf (W Crimea)	1.4	164	0.30	26	143	117.5	2.7	5.5
	14	Southern coast of Sevastopol	2.2	140	0.50	36	292	67.8	1.6	8.1
	9	Omega Bay	3.5	313	0.30	13	277	100.8	1.8	21.3
	13	Kazachiya Bay	3.6	186	1.10	39	422	216.2	1.9	10.8
	22	Planerskoye coast (SE Crimea)	4.0	31	0.26	12	144	89.8	1.4	12.0
	1	Zemov's Phillofora Field (NW Crimea)	5.1	60	0.20	21	185	41.2	1.8	8.8
	19	Jalta coast (S Crimea)	6.0	172	0.60	20	694	59.7	2.3	34.7
MODERATE	20	Alushta coast (S Crimea)	6.3	244	0.40	21	1063	74.2	1.8	50.6
	24	Tuapse-Shepsi coast (25 m)	7.2	105	0.2	17	945	130.5	1.4	55.6
	18	Laspi Bay (SW Crimea)	7.7	128	0.25	52	684	269.5	2.4	13.2
	21	Cape Meganom (SE Crimea)	9.2	-20	0.10	25	1704	562.3	2.5	68.2
	31	Sochi coast (10-20 m depth)	9.5	108	0.2	20	150	73.3	1.4	7.5
	25	Tuapse-Shepsi coast (40 m)	10.3	18	0.5	9	2214	126.6	0.9	246.0
	23	Anapa Coast	13.0	176	0.15	43	507	293.4	2.4	11.8
	27	Novorossijsk Bay (entrance)	13.6	34	0.70	18	394	135.0	1.9	21.9
	26	Gelendzhik coast	13.8	159	0.30	42	412	153.1	1.6	9.8
HIGH	4	Main Sevastopol Bay (entrance)	15.4	75	4.80	16	148	12.8	1.6	9.3
	32	Sochi coast (25-30 m depth)	15.8	46	0.6	24	209	54.4	1.6	8.7
	10	Kamyshevaya Bay (entrance)	16.7	177	0.90	26	87	3.2	2.3	3.4
	28	Novorossijsk Bay (middle p.)	18.5	-45	1.20	26	233	145.3	2.3	9.0
	12	Kamyshevaya Bay (inner p.)	19.8	-10	1.70	12	206	150.7	1.6	17.2
	11	Kamyshevaya Bay (middle p.)	20.6	-102	4.50	24	35	5.3	1.9	1.5
	29	Novorossijsk Bay (inner p.)	20.8	-139	3.60	14	215	67.3	1.2	15.4
	15	Balaklava Bay (entrance)	21.4	-39	1.90	4	25	0.9	0.6	6.3
VERY HIGH	30	Novorossijsk Bay (port)	27.1	-174	8.0	5	65	5.3	0.4	13.0
	16	Balaklava Bay (middle p.)	28.6	-104	7.20	14	58	5.1	0.9	4.1
	8	Streletskaya Bay	39.7	-124	16.60	18	82	38.5	1.7	4.6
	17	Balaklava Bay (inner part)	43.8	-111	4.90	3	19	0.5	0.5	6.3
	6	Main Sevastopol Bay (inner p.)	46.2	-101	8.10	10	235	38.8	1.2	23.5
	7	Southern Bay	53.1	-139	33.60	6	70	10.1	1.0	11.7
	5	Main Sevastopol Bay (middle p.)	66.4	-147	34.30	7	130	24.6	0.6	18.6

Table 3.4-3. Comparison of the average values of variables distributed in 4 logical categories in accordance with arrangement of studied regions by gradients of two key parameters: TOC and Eh.

Logical categories (by TOC load)	**Key parameter**	**Range of changes of key pameters**	**Average values of variables**						
			Abiotic			**Biotic**			
			TOC	**Eh**	**CEB**	**Biomass**	**Abund.**	**Sp.Num.**	**Sh.Index**
1	**TOC -->**	1 - 6 mg/g sed	3.4	+175	0.43	97.4	338	27.6	1.94
(Low)	**Eh -->**	+320....+160 mv	6.4	+213	0.49	119.0	468	29.6	2.15
2	**TOC -->**	6 -15 mg/g sed	10.1	+88	0.31	212.0	897	27.3	1.82
(Moderate)	**Eh -->**	+160....+30 mv	10.0	+95	0.86	104.2	380	27.4	1.71
3	**TOC -->**	15 -25 mg/g sed	18.6	-5	2.47	55.0	146	18.3	1.78
(High)	**Eh -->**	+30....-100 mv	14.2	-11	0.94	214.9	754	14.8	1.56
4	**TOC -->**	25 - 65 mg/g sed	43.5	-129	14.33	17.5	94	9.0	0.89
(Very high)	**Eh -->**	-100....-230 mv	38.5	-127	13.42	21.7	101	11.2	1.04

4. Some Preliminary Interpretations and Recommendations

- In general, the responses of key benthic variables (abundance, biomass, numbers of species, and H′ diversity) to increasing organic-carbon (TOC) concentration in sediment followed patterns predicted by the conceptual model. In most cases, benthic variables showed predicted maximums at intermediate TOC levels and minimums at both low and high levels. Such patterns seemed to be the most pronounced for abundance and biomass. In some cases, numbers of species and H′ showed moderate to highest values within the lowest TOC range and then progressively declined with increasing TOC concentration (characteristic of a negative exponential decay).
- Best-fit regression curves for plots of benthic variables versus TOC concentration have fairly low R^2 values. This is due to the wide distribution of values of benthic variables at specific TOC concentrations, which reflect several co-occurring processes affecting benthic abundance, biomass, and diversity. In spite of this variation, benthic variables show some distinct patterns in relation to TOC concentration that are consistent with ecological theory.
- Comparison of benthic and other abiotic environmental variables (e.g. contaminant levels, Eh) across different ranges in TOC concentration suggest that it is possible to identify TOC thresholds that could serve as indicators of high vs. low risks of benthic impacts and related sources of stress. Exact values of thresholds vary among regions and choice of variable (Table 4-1). However, there are some basic similarities in their patterns that appear to support the following general classification scheme:
- A low TOC range (<about 0.5 or 1 mg/g) in which organic levels may be too low to support high macrobenthic biomass and abundance. When undisturbed, macrofaunal assemblages in this type of environment are composed typically of a high diversity of K-selection equilibrium species at low densities. When low TOC is related to physical disturbance (e.g. strong tidal currents, storms, or anthropogenic physical stress), the assemblages may be dominated by opportunistic species and show relatively low levels of biomass, low diversity, and unpredictable abundance;
- A high TOC range (> about 30 mg/g) in which associated environmental stressors (low oxygen, high sediment contaminants, high porewater ammonia and sulfide) exceed the physiological tolerance limits of a large percentage of species. As a result, benthic variables decline sharply within this range. Typically, benthic assemblages under such conditions are dominated by a few pollution-tolerant and r-selection opportunistic species. However, it is possible that "healthy" assemblages may be encountered in high TOC environments when a very large proportion of the carbon is refractory; and
- An intermediate range (about 1-30 mg/g) in which benthic variables will typically show distribution peaks due to the co-existence of species with varying life-history strategies (e.g., presence of both equilibrium and opportunistic species) and levels of tolerance to stress (both sensitive and hearty forms). Within the upper end of this range (“moderately high” TOC values in Table 4-1), most benthic variables will tend to decline with increasing TOC due to the onset of adverse environmental conditions for some of the more sensitive species. However, abundance may remain high in some cases due to the presence of opportunistic/pollution-tolerant species at high densities. Biomass may also show a secondary peak at these moderately high levels of TOC, though such a pattern was not detected in the present data.
- Thresholds for the Seto Inland Sea, Japan seem to depart the most from these general patterns. For example, the lower TOC threshold for subtidal samples is < 16 mg/g, which is considerably higher than the corresponding lower thresholds for other regions. TOC concentrations below 16 mg/g (down to about 1 to 0.5 mg/g) are within the optimum range for other regions. This variation may be attributable to the limited number and localized nature of sampling points for the Seto Inland Sea study.
- As a next analysis step, data from all regions should be merged together and used to help define the general patterns and resulting thresholds from common plots of the combined data. Data sets

from additional regions (e.g., North Sea, coastal areas of Great Britain and Scandinavia, Chilean coast) should be included in the analysis as well.

Table 4-1. Comparison of proposed ranges in TOC concentration (mg/g) indicative of possible shifts in the integrity of benthic assemblages across four coastal regions of the world.

Mean, median, minimum, and maximum TOC concentrations for each region are included.

	Southeastern U.S.A	Seto Inland Sea, Japan:		Eastern Mediterranean	Northern Black Sea
		Intertidal	Subtidal		
TOC Indicator Range:					
Low[1]	< 0.5	< 6.0	< 16	<1.0	< 6.0
Low-Moderate[2]	0.5-10	6-25	16-22	1-10	6-15
Moderate-High[3]	10-30	> 25	> 22	10-25	15-25
High[4]	> 30	-	-	> 25	> 25
TOC Distribution Properties:					
Mean	16.6	8.5	19.2	8.0	17.8
Median	6.3	8.5	20.1	3.9	13.7
Minimum	0.05	2.2	11.6	0.03	1.1
Maximum	175	44	28.2	190	66.4

[1] TOC range in which organic levels may be too low to support high benthic abundance and biomass.

[2] TOC range in which environmental conditions are favorable for survival of most species. Typically, benthic variables have distribution peaks within this range.

[3] TOC range in which some benthic variables begin to decline due to the onset of adverse environmental conditions for some of the most sensitive species. Abundance and biomass may show peaks within this range, due possibly to the presence of opportunistic species.

[4] TOC range in which related environmental stresses (low dissolved oxygen, high sediment contaminants, high porewater ammonia and sulfide) may be too high for most species to tolerate. Typically, benthic variables decline sharply within this range.

5. References

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