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ORIGINAL ARTICLE

A. Diamant · H. v. Westernhagen

Biological indicators for the detection of natural and man-made changes in coastal waters – an introduction to the MARS project

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Although once regarded as a "bottomless pit", the sea, unfortunately, lacks the desired near-infinite capacity to absorb the vast amounts of sewage, industrial effluents and land runoff which for years have been discharged into it, a process that is still going on. As a result of a faulty approach to the management of waste disposal and the continuous abuse of the coastal marine environment as a dumping ground for all sorts of refuse, indications of possible pollution-induced stress conditions in the European seas became apparent as early as in the 1960s (Kinne and Rosenthal 1967; Caspers 1968; König 1968; Stripp and Gerlach 1969). In an attempt to control and possibly regulate the input of detrimental substances into the sea worldwide, the London Dumping Convention was signed in 1972. In the Oslo Convention (1972) which is valid for the northeast Atlantic and the North Sea, regulations were further specified and inputs restricted. With the signing of the Helsinki Convention (1974) for the Baltic, an even more stringent treaty was agreed upon that did not allow any discharges whatsoever into the Baltic Sea.

In the framework of the Paris Convention (1974), which regulated marine pollution in the northeast Atlantic that originated from land-based sources, a permanent quality control of the marine environment was postulated. This led to the initiation of a "Monitoring Programme" which was meant to closely follow the development of the conditions in the marine environment and the effectiveness of the measures undertaken to improve conditions. It consisted of a chemical and a biological monitoring programme, the Joint Monitoring Programme (JMP). The Joint Monitoring Programme was developed by the Contracting Parties and was established in 1979. In this programme residues of pollutants (i.e. heavy metals, chlorinated hydrocarbons) were determined in water and sediment (chemical monitoring) and in animal and plant tissue (biological monitoring). The programme is divided into four purposes:

1. the assessment of possible hazards to human health;
2. the assessment of harm to living resources and marine life;
3. the assessment of existing levels of marine pollution;
4. the assessment of the effectiveness of measures taken for the reduction of marine pollution in the framework of the conventions.

Of these purposes, only 1, 3 and 4 have been operated regularly, but the assessment of harmful effects on living resources and marine life has been wanting, since at the beginning of the programme the requirements for this purpose had not yet been defined by the Contracting Parties. It was only in 1989 that the North Sea Task Force recommended the monitoring of fish disease ethoxyresorufin O-deethylase (EROD) activity, macrozoobenthos, and the oyster-larva test for a baseline study in their North Sea Task Force Monitoring Master Plan.

Despite a clear rationale and attempts to identify and establish techniques for the assessment of biological effects in environmental monitoring programmes (MacIntyre and Pearce 1980), such techniques have been slow to become adopted. Today, vast resources are still invested each year in order to seek out and develop new biomarkers and biological indicators, but many of these have limited application or are insufficiently sensitive as early warning signals. Moreover, it is not nearly enough to develop a biological marker; it is equally important to assure that usage of the method is as easy and straightforward as possible. Clearly, when a procedure is tedious or requires a high level of technical expertise, laboratories may find it impracticable to adopt, even if it has considerable potential strength for assessment of environmental stress. Criteria for assessing the impact of human activities may be selected on the basis of their

A. Diamant (✉)
National Center for Mariculture,
Israel Oceanographic and Limnological Research Ltd.,
P.O. Box 1212, Eilat, Israel 88112
e-mail: diamant@agri.huji.ac.il

H. v. Westernhagen
Biologische Anstalt Helgoland,
Alfred Wegener Institut für Polar- und Meeresforschung,
Notkestrasse 31, D-22607 Hamburg, Germany

Table 1 Common stressors, which life cycle stages they affect and population parameters altered as a result of those affects (* often; *x* occasionally; *o* rarely). (Modified after Shuter 1990)

Stressor	Life cycle stages affected			Population parameter	
	Egg to juvenile	Juvenile to mature	Mature adult	Reproductive reserve	Environment carrying capacity
Contaminants	*	x	*	*	*
Fishing	o	*	*	*	o
Species introduction	*	*	*	*	*
Habitat destruction	*	*	*	*	*

merit and relevance at the population or individual level (e.g. extinction and mortality, respectively). There is, however, little consensus on appropriate endpoints for assessing impacts on ecosystems or communities. In the absence of a priori specification of ecosystem effects, a wide variety of potentially relevant ecological variables are typically measured in environmental monitoring programmes (Akkerman et al. 1990). Ecosystem response to disturbances is then reflected as a multivariate quantity, by changes in this set of variables, whose number is expected to provide a comprehensive description of the state of the ecosystem at large. This approach introduces difficulties from the standpoint of ecosystem protection or management, since routine monitoring of a large suit of ecological state variables can be a formidable task and a considerable strain on available resources. Practical constraints on the expenditure of time, effort, and money often dictate a sampling scheme, which allows routine monitoring of only part of the desired and relevant parameters. Ideally, a small subset of easily measured variables, which would provide early, adequate warning of ecosystem and/or population damage, is desired.

A population that is under stress due to some anthropogenic changes in its environment is frequently characterised by a limited distribution, low abundance of its individuals and/or reduced reproductive potential. Thus, the ultimate expression of stress at the population level is a decrease in the absolute number of adults. The environmental changes of particular concern are, of course, those produced by human activities that are likely to exert a significant impact on the biota. Respective activities may be fishing, contaminant loading, introduction of exotic species, including disease agents, physical destruction of the habitat, and others. Such activities constitute "stressors" whose effects on the target population may lead to a decline in adult abundance through the direct promotion of higher mortality or reduced fecundity, the net result being a reduction in the reproductive reserve of the population. Other stressors may act individually by reducing the environment's carrying capacity for the population through habitat destruction, prey or host elimination, introduction or enhancement of competition or by other means. How individual stressors may affect a population is determined by the parts of the life cycle that are affected (Table 1).

The ultimate effect of a specific stressor on adult organism abundance can vary considerably, depending on when in the course of its life history the animal is impacted and whether or not population abundance is determined by a bottleneck which is larval or adult. When a simple linear relation links increasing stressor effect and equilibrium adult abundance, the ultimate consequences of observed parameter changes can be predicted with some confidence. In populations regulated by a larval bottleneck, the ultimate consequences of changes in egg production or larval survival are not immediately obvious. Here, compensatory processes mask low-to-moderate stressor impacts; the linear dose-response relations are transformed to threshold relations, and the threshold is very difficult to determine accurately (Goodyear 1980).

In principle, exposure to contaminants or any other stressor can change any of the distinct processes that govern the dynamics of a population (e.g. reproductive capacity or growth, mortality) and the effects of these on adult abundance will depend on the stage of development in which a bottleneck exists (see Table 1). A useful indicator will flag the occurrence of stress-induced changes in a specific population and help in predicting whether such changes will lead to serious damage. In this context we define serious damage as an irreversible decrease in adult abundance. The greater the indicator reliability and the earlier the warning provided by it, the more useful it is.

Following this philosophy, the ICES Working Group on Biological Effects of Contaminants (WGBEC) (Anonymous 1996) developed an implementation strategy to establish where contaminants cause deleterious biological effects. This programme identifies two generic objectives:

1. to monitor the general quality status of an area so that environmental impacts of contaminants can be identified;
2. to identify biological effects in areas with known or suspected elevated levels of contaminants, e.g. areas receiving major point-source inputs, such as estuaries receiving significant contaminant effluents.

Within the framework of this programme it was also agreed that a combination of techniques should be used (see also Stebbing and Dethlefsen 1992). It is apparent,

Table 2 Summary of recommended methods for general monitoring of the biological effects of contaminants in the (Oslo-Paris Convention for the Protection of the North Atlantic Marine Environment – 1992) area (*A* suitable for immediate application; *B* suitable for application as soon as quality assurance is in place). (Modified after Anonymous 1996)

	Status	Specificity of contaminant response
Bioassays		
Whole sediment	B	General toxicity
Pore water	B	General toxicity
Water-column	B	General toxicity
Biomarkers		
P4501A (EROD)	B	Planar molecules, PAHs, PCBs
Lysosomal stability	B	Organic contaminants
Liver pathology	B	General
Liver nodules	A	Cancer-forming chemicals
Population/community responses		
External fish diseases	A	Not specific to contaminants
Fish reproductive success	B	Not specific to contaminants
Macrobenthic fauna	A	Not specific to contaminants
Parasite/host index	B	Not specific to contaminants

that in such a programme multiple measures of effects are essential, and that measurements should include those indicative of both exposure and pathology. The combined set of measurements should integrate responses across organisational levels, and, wherever possible, be readily interpreted in terms of cause and effect. Whenever possible, both invertebrate animals and fish should be used as environmental sentinels.

WGBEC developed an implementation strategy which incorporates all of the above-mentioned points and places them within the context of an environmental management plan that integrates scientific interpretation and management decision-making. A key aspect of this programme, which is illustrated in Table 2, is the use of a variety of low-cost techniques which have a high signal-to-analytical-noise or natural level ratio and which, when used in combination, are indicative of both exposure and pathology. This strategy incorporates biological effects measurements at all organisational levels and is sufficiently flexible to accommodate new diagnostic tests for both pathology and exposure as they become available (see also Sindermann 1993).

Many of the existing bioassay methods for evaluating environmental quality, carcinogenicity, toxicity and risk may be grouped for convenience into four categories based on procedures and applications. These are: population dynamics, ecosystem processes, descriptive toxicology and molecular activity.

Bioassays for molecular activity are helpful in the study of the effects of minute doses of chemicals upon critical cellular macromolecules and provide a means for new, more efficient epidemiology aimed at identifying substances that are health hazards. Many of these bioassays are classed as methods for detecting the activity of biotransformation, quantification or cellular disturbances, immunological responses to pollutants as well as DNA-damaging agents, the action of which cause neurotoxicity or genotoxic interactions between chemicals and DNA or proteins. Bioassays for effects at the population level are elucidating changes in interspecific interactions, such as competition, predation and parasitism. The morbidity of parasitism and disease in dynamic populations may increase when normal physiological and nutritional states are compromised. Morbidity may increase when environmental pollution reduces a host's resistance to parasite infection. Consequently, bioassays have detected increased parasitism in fish exposed to metals or chlorinated hydrocarbons but also a decrease in parasite diversity due to, for example, loss of intermediate hosts. Descriptive toxicology bioassays are those that directly determine the toxicity of materials on an organism. The bioassays used in descriptive toxicology are designed to characterise what toxic effects a specific chemical can induce. These are usually conducted in the laboratory or under controlled conditions. Dose-response curves are derived from these assays and portray the effective and toxic doses. These bioassays provide perspicacious descriptions of the pharmacokinetics of specific chemicals under controlled conditions.

MARS 1 was designed to combine a variety of approaches and develop a set of methods for employment of biological indicators in pollution monitoring and environmental quality assessment. The activities of the individual subprojects of the project focused on the evaluation of effects of chemicals on molecular activity (MARS 1/1, 1/2, 1/3), population dynamics (MARS 1/2) and descriptive toxicology (MARS 1/3) (Fig. 1). The work encompassed field sampling in the North Sea (collection during research vessel cruises), Mediterranean and Red Sea (self-collection or purchase of samples from fishermen). Tissue samples of the same fish were shared by the different groups, whenever feasible, to obtain as much information as possible per sample. Brief summaries of the three subprojects and the adjoining chemistry workplan and the results obtained within are as follows.

MARS 1/1 dealt with the interrelationship between level of pollution-mediated anti-xenobiotic cellular defense responses. The project included in vivo and in vitro study of organisms from all three study areas in the

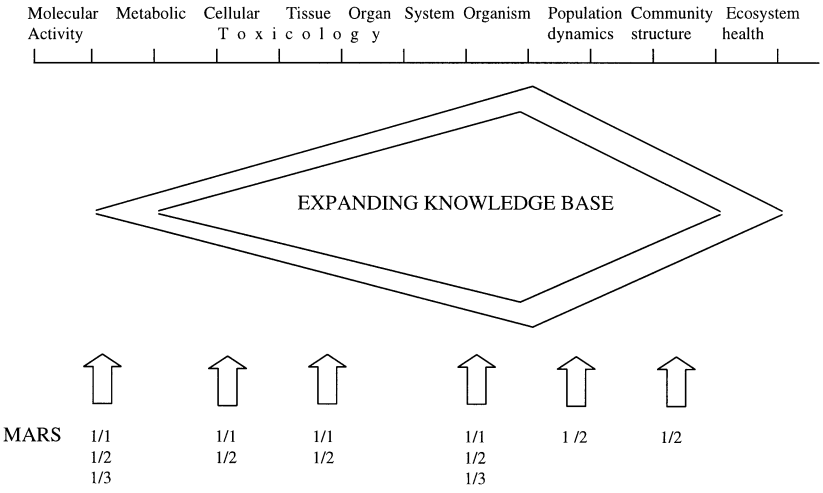

Fig. 1 Illustration of levels of biological organisation investigated in MARS 1/1, 1/2 and 1/3 subprojects in relation to the expanding knowledge base on the marine environment. (Modified after Couch 1993)

North Sea (bivalve and fish), Mediterranean and Red Seas (bivalves and gastropods). The study, using vital and supravital fluorescent contact microscopy and microfluorometry, analysed the early manifestations of genotoxicity, clastogenicity and environmental pathology. The objective was to select several reliable biophysical, cytochemical, cytophysiological, cytogenetic, immunological and cytopathological responses and assess their suitability as early warning tools.

MARS 1/2 was aimed at developing a quantitative, operational model for the utilisation of fish parasite communities as a tool for the detection of environmental stress. The basic hypothesis was that species diversity and abundance of heteroxenic (H) and monoxenic (M) fish parasites, and their ratio (H/M), are indicative of the condition of the environment. The underlying concept was based on the assumption that the H/M ratio will reflect the presence or absence of taxa of organisms in the ecosystem on which complex life cycles of heteroxenic parasites are dependent. In addition, biomarkers at the molecular level of biological organisation (the activity of CYP1A-dependent monooxygenase EROD), the subcellular level (lysosomal membrane stability) and the cellular level (activity of non-specific immune response) were applied to each individual fish. The project included study of fish from all three study areas in the North, Mediterranean and Red Seas.

MARS 1/3 studied hydrogen peroxide as a possible early tracer of pollution. Hydrogen peroxide production in seawater is positively correlated with concentration of dissolved organic matter and intensity of ultraviolet radiation. Hydrogen peroxide is highly toxic to living cells, which counteract its activity with enzymes such as catalase and peroxidases. By measuring peroxide levels in coastal polluted vs. offshore, supposedly “clean” waters, and by measuring peroxide-scavenging enzymes, the group attempted to assess environmental stress of the substance on the environment. The research activity focused on three parts: the influence of various factors on H_2O_2 production and decay in coastal seawater National Institute of Oceanography (NIO, Haifa); the role of catalase in peroxidase decomposition in seawater and factors that affect the enzyme activity National Center for Mariculture (NCM, Eilat); and the biological impact of H_2O_2 by measurement of physiological responses of bivalves Institut für Meeresforschung (IfM, Kiel).

The Chemical and Environmental Research Supplement to MARS 1 was directed towards providing supplementary environmental chemistry data linked with the work of the three research groups involved in the joint project. The workplan was designed to assist in the interpretation of the biological measurements and furnish the background conditions of the marine environment at the sampling sites. Part of the environmental data collected (water and sediment quality) was aimed at corroborating the a priori knowledge on the environmental status of the different sampling sites while the trace element composition and organic screening of the different species were designed to add additional chemical information to aid the interpretation of the biological results. Analysis were performed on fish and mollusc specimens that were collected in conjunction with the MARS 1 groups.

Since the intention of the project was to develop an approach that could be applied on a supra-regional scale, investigations regarding the expression of biological effects were conducted in three different seas, i.e. the North Sea, the Mediterranean Sea and the Red Sea. Figure 2 illustrates the particular sampling sites in the respective areas that were subjected to common investigations by the individual groups.

The MARS 1 subproject results presented in the following pages add to the huge amount of data that accumulates each year in the diverse fields of marine toxicology, pathobiology and parasitological ecology. The question arises as to how the scientific community can effectively utilise this varied and exponentially growing body of knowledge. How can it help enhance our predictive capacity concerning anthropogenic impacts on the marine environment? Clearly, the usefulness of any contribution, including that of the present project, is measured by our capacity to comprehend, integrate and generalise details, with the objective of developing

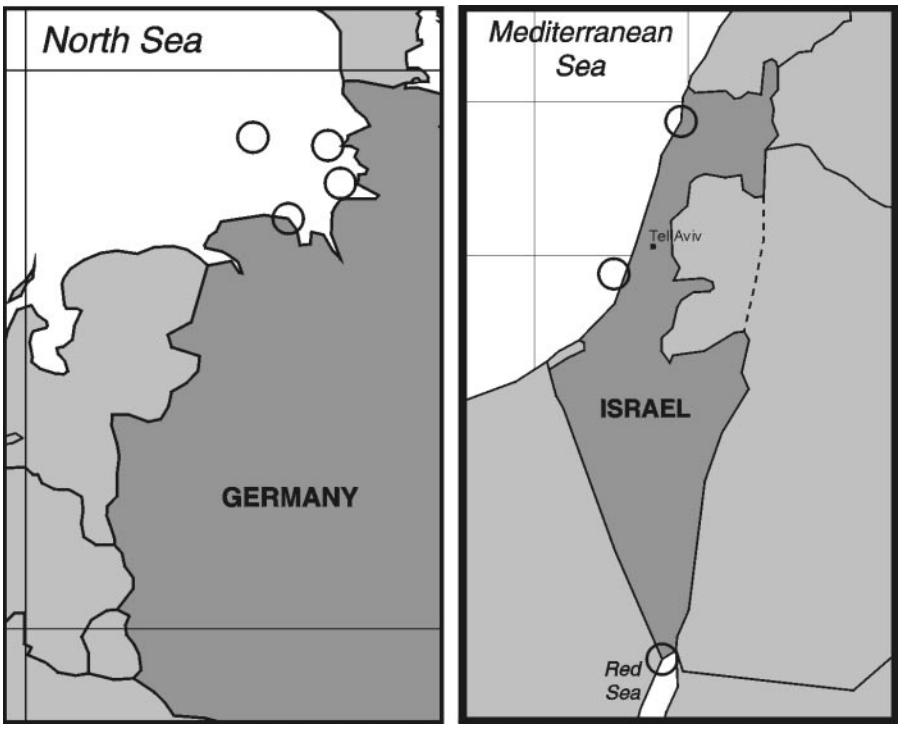

Fig. 2 Sampling areas in different geographical locations of the North Sea, Mediterranean Sea and Red Sea. Approximate sampling areas marked with *circles*

means for predicting consistent and valid endpoints. Thus, the accumulation of a large body of information, such as generated here, is the prerequisite for an in-depth comprehension of the phenomena under study (Couch 1993).

During the preparatory phase of the project, the issue of why people and institutions should collaborate on a supranational basis was discussed very strongly. Consultation and voluntary coordination between partners with converging interests were explicitly seen as advantageous, with the project providing a suitable platform. In many areas, generating solutions to identified problems exceeds the scientific capacity of any single group or even country. Therefore it is wise to join forces, expertise and resources of several countries to address the various components of often complex problems by forming multidisciplinary teams. The design of experiments by a supraregional collaboration can be much more comprehensive than what could have been achieved were the scientists to operate individually or exclusively at national or institutional levels. Incremental benefits arising from shared experience and consultation are therefore a key driving force to the project. Furthermore, consultation is building the trust required for effective collaboration of all actors, i.e. not only the scientists but also the "end users" such as administrative decision-makers and regulatory authorities. This is a consequence of the recognition that all stakeholders need to participate in addressing not only pure science but also social problems, such as development of management tools for pollution monitoring through application of knowledge gained through biological effects monitoring exercises.

Thus, following this line of reasoning, and following the encouragement of the MARS Joint Advisory Committe, which closely followed the work from its onset, the partners invested considerable effort in integrating results and correlating data from different levels of biological organisation. This was facilitated by background chemical and environmental data provided by the Chemical and Environmental Research group, and, in the final phase, also the addition of two groups which supplied the statistical expertise needed to perform multivariate analyses of the collected data. We believe that the outcome of MARS 1, as well as that of the continuing second phase, MARS 2, will not only deepen our understanding of basic marine biological processes but also, in the applied sense, provide new and improved biological indicators of natural and man-made changes in marine and coastal waters.

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