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IMO/FAO/UNESCO-IOC/WMO/WHO/IAEA/UN/UNEP
Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP)

Biological Indicators and their Use in the Measurement of the Condition of the Marine Environment

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Joint Group of Experts on the Scientific Aspects of Marine Pollution (GESAMP)

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NOTES

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Definition of marine pollution by GESAMP:

Pollution means the introduction by man, directly or indirectly, of substances or energy into the marine environment (including estuaries) resulting in such deleterious effects as harm to living resources, hazards to human health, hindrance to marine activities including fishing, impairment of quality for use of seawater and reduction of amenities.

Members of the GESAMP Working Group on Biological Indicators and their Use in the Measurement of the Condition of the Marine Environment

Professor John S. Gray (Chairman)
Department of Marine Zoology and Marine Chemistry
Biological Institute
P.O. Box 1064
0316 Oslo
Norway

Dr. Lee Shugart
Environmental Sciences Division
Oak Ridge National Laboratory
P.O. Box 2008
Oak Ridge, Tennessee 37831
U.S.A.

Dr. Susan Anderson
Lawrence Berkeley Laboratory (MS70-193A)
1 Cyclotron Road
Berkeley, California 94720
U.S.A.

Professor Antony J. Underwood
Institute of Marine Ecology
Marine Ecology Laboratories A11
University of Sydney
Sydney, NSW 2006
Australia

Dr. Brian L. Bayne
Plymouth Marine Laboratory
Prospect Place
Plymouth PL1 3DH
United Kingdom

Dr. Helen Yap
Marine Science Institute
University of the Philippines
U.P.P.O. Box 1
Diliman
Quezon City 1101
Philippines

Dr. Mikael Hilden
Environmental Impact Assessment Unit
National Board of Waters and the Environment
P.O. Box 250
FIN-00101 Helsinki
Finland

Dr. Manfred Nauke
IMO Technical Secretary of GESAMP
Marine Environment Division
4 Albert Embankment
London SE1 7SR
United Kingdom

Dr. James R. Pratt
School of Forest Resources
7 Ferguson Building
Pennsylvania State University
University Park
Pennsylvania 16802-4302
U.S.A.

Dr. Ivan Zrajevskij
UNEP Technical Secretary of GESAMP
UNEP Regional Office for Europe
Palais des Nations
1211 Geneva 10
Switzerland

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Foreward

This publication is the result of the GESAMP Working Group on "Indicators of Ecosystem Health" which was established by GESAMP following a proposal from the members at its 22nd session.

The working group was jointly spondored by the United Nations Enviornment Programme (UNEP), the International Maritime Organisation of the United Nations (IMO), the Food and Agricultural Organisation of the United Nations (FAO), the United Nations Educational Scientific and Cultural Organisation (UNESCO) the International Atomic Energy Agency (IAEA) and the United Nations Assembly (UN).

This report was adopted by GESAMP at its twenty-fourth session (New York, 21-25 March 1994) for publication in the GESAMP Reports and Studies series.

Executive Summary

The Group considered ideas of a "healthy ecosystem" and concluded that the normal functioning of an ecosystem is only definable in a comparative sense, in which the system under study is compared with an *a priori* condition or with control areas. "Health", even when applied to human health, is a subjective term which can only be loosely defined. What is viewed as a "healthy" system in one region may not be so valued in another. This document therefore deals with the "condition" of marine ecosystems rather than their "health".

The goal of this document is to give a framework of how appropriate techniques can be used to detect and assess the physical, chemical and biological impacts on marine ecosystems.

The WG recommend a phased approach to the use of biological indicators in the measurement of the condition of the marine environment with appropriate statistical and design procedures embedded within each phase. The initial phase is detection of a problem. The second phase of the framework is the assessment, definition and characterisation of the problem and the final phase is managerial activity to solve the problem. In each phase, the aims and uses of indicators may vary.

Typical impacts on the marine environment are discussed, and a specific illustrative example of potential impacts on mangrove forests is used. Indicators of exposure and effect are reviewed, ranging from the molecular to the individual, population and assemblage levels. In addition, examples are given of their use in marine environmental assessments. How such indicators may be selected for use in the three phases of the process are described.

Application of the phased approach is illustrated using four examples. These include chemical contamination, physical destruction, nutrient enrichment, and increased ultraviolet-B (UV-B) radiation on marine systems.

Subsequently, the importance of proper sampling design to detect environmental stresses is discussed considering univariate and multivariate statistical approaches, responses of populations to changes and techniques to estimate the power of a given monitoring programme to detect a given change.

A suggested implementation of such a framework in South East Asia is given and needs for training are briefly discussed.

1 Terms of Reference

The group was given the following terms of reference:

- identify characteristics of components of marine ecosystems that can generally be used to indicate the normal functioning of those systems;

- consider the origin, and value, for these purposes, of terms such as stress, population, community and ecosystem with specific reference to space and time scales and energy flow in the marine environment;

- review the methods used to detect stress on marine populations, communities and ecosystems and assess their value and limitations;

- review the methods used to detect stress on individual organisms, in a field situation and assess their value and limitations;

- identify, on the basis of the above, suites of indicators of the state of marine ecosystems that can be used to assess the impact of anthropogenically induced change of the marine environment

The WG approached these terms of reference by concentrating on indicators of exposure and effects which have been well-tested and which the group is confident can be applied globally.

GESAMP has reviewed a number of topics that are relevant to this issue, such as the Global Strategies for Marine Environmental Protection (Report 45 and Addendum, 1991).

In relation to the first term of reference, the group regards the **normal functioning** of a system in a comparative sense where the condition of a system under study is compared to a previous condition or contemporaneously to conditions in control or reference areas. In order to make such assessments, a phased approach was envisaged, paying particular attention to sample design (see Section 6). The WG believe that "health", even when applied to human health, is a subjective term that can only be loosely defined. In the context of the marine environment, a system that is regarded to be "healthy" in one region may not be so appraised in another. A formal definition will, in our view, only lead to conflicts because different societies value systems differently. Hence, a comparative approach has been used whereby systems or states of a system are ranked using a suite of indicators of condition.

Likewise, in current usage, the term **ecosystem** has no commonly accepted definition. The Baltic Sea is called an ecosystem as are coral reefs and mangrove forests. These are vastly different systems. Indeed a much used textbook in ecology (Begon et al., 1990) states that communities encompass all the properties commonly defined as ecosystems. In this document the WG has chosen to use the term ecosystem in a purely operational sense to include habitats and their assemblages of organisms in addition to any functions of such organisms and processes within the habitats, such as energy and element cycling.

Stress was originally used in physical mechanics - stress meaning applied force per unit area and strain meaning the response e.g. deformation. Widespread use of this term in environmental research has, however, resulted in numerous definitions (see Calow, 1989 and other articles in the same volume). Here, stress is defined in the box below. A response to stress may be positive or negative. Increased growth rates or catches of fish may be responses to stress. Not all responses are therefore negative changes in variables.

Monitoring in the context of this report is viewed in relation to an *a priori* hypothesised or identified stress.

The Group has not provided a detailed review of methods used to detect stress on marine organisms because these topics have been extensively treated elsewhere in the results of the GEEP workshops (Bayne et al., 1988; Addison & Clarke, 1991; Stebbing et al., 1992). Instead, the Group has concentrated on giving a context and framework for the use of the available methods.

Definitions of terms

Contamination	An increase of background concentration of a chemical or radio-nuclide.
Pollution	The introduction by humans, directly or indirectly, of substances or energy into the marine environment (including estuaries) resulting in such deleterious effects as harm to living resources, hazards to human health, hindrance to marine activities including fishing, impairment of quality for use of seawater and reduction of amenities.
Stress	A chemical or physical process that leads to a response within an organism, or at the levels of whole organisms or assemblages.
Response to stress	The response to (or biological effect of) a stress of a system within an organism or a whole organism or assemblage. These include what are widely termed impacts.
Assessment	The orderly process of gathering information about exposure and effect in a possibly stressed system and determining the significance and causes of any observed changes.
Disturbance	A chemical or physical process caused by humans that may or may not lead to a response in a biological system within an organism or at the level of whole organisms or assemblages. Disturbances include stresses.
Monitoring	Observation of a variable over space and or time in order to determine the condition or state of the ecosystem.

The framework provided in this document does not address the following issues:

1) Assessment of the value of an ecosystem. GESAMP believes that different societies will place different values on similar systems and therefore, that there can be no common definition of the value of a system.

2) Management issues are not covered because this was not within the terms of reference. Nevertheless, GESAMP believes that the framework provided will enable managers to incorporate indicators of "condition" or "health" within monitoring programmes and a framework for sample design for such programmes is presented. The WG draw attention to the need for better dialogue between scientists and managers.

3) Suggestions have been made that it is possible to create a rigorous quantification of ecosystem "health" using quantitative variables that can be formalised in ecosystem models (Constanza, 1992). Rather than emphasising this approach, it was decided to attempt to synthesise knowledge of techniques which have been shown to be effective in solving practical environmental problems. So far, quantitative holistic modelling of ecosystems has had limited practical application (see also Suter, 1993, and Calow, 1989, for a critique of the ecosystem model approach).

It is acknowledged that managerial techniques and ecosystem models have important interactions with many levels of the framework described here.

1.1 Scope and Focus

Techniques for assessing the condition of marine ecosystems are numerous and rapidly evolving, yet efforts to develop general principles of appropriate application for such techniques have not kept pace. Similarly, there has been little effort to make these new developments available to scientists and managers in all parts of the world. Thus, there is a need for current and multidisciplinary guidance on the appropriate application of various indicators of the condition of marine ecosystems. Although many of the most recent developments have been achieved in the assessment of chemical impacts on ecosystems, these cannot be considered in isolation. Biological and physical impacts, such as the introduction of exotic species, overexploitation, sedimentation and dredging can also have profound impacts on ecosystems. These may interact with effects of contaminants in marine ecosystems. In addition, within a global context, the level of concern about one type of issue in comparison to another may vary dramatically from country to country and from region to region.

The goal of this document is to provide a general framework for appropriate application of contemporary techniques used to assess chemical, physical and biological impacts on marine ecosystems. Our focus is broad. Consequently, an approach to assessment is described that can be used as a framework for many types of investigation. GESAMP does not consider the detailed validation or use of particular techniques; these have been satisfactorily evaluated and reviewed elsewhere, (the IOC/UNEP/IMO Group of Experts on Effects of Pollution (GEEP) workshops, Bayne et al, 1988; Addison and Clarke 1991; Stebbing et al. 1992). Our approach is illustrated using selected examples of perturbations of marine ecosystems. Also presented are discussions of the types of impact that may be of concern in selected habitats and the range of technique that can best be used for specific perturbations. Principles of experimental design and analysis for these investigations are also described throughout the report.

The approach to environmental assessment has two core components. The first is that of recognizing that there are the different phases in a study which lead to determination of the goals, needs and uses of indicators of the condition of an ecosystem. The second component is that there are tiers of hierarchical sets of indicators that might be examined, depending on the defined goals of the study. This is described more fully in section 4.1.

The WG propose that, in general, there are three main phases in any assessment of environmental well-being. The first phase is identification of an environmental problem. The second phase is definition and characterisation of the problem and the final phase is managerial activity for its solution. The techniques employed in each of these phases may vary. The second phase is frequently the most technically complex because it involves formulating an understanding of the magnitude, duration and scale of the problem.

A tiered structure to investigation may include a range of biological organisation from the molecular level extending to the cellular and organismal levels, populations and assemblages. Investigations at one level of organisation may be appropriate for one type of problem, whereas another type of problem may require investigations at all levels.

A single conceptual model elucidating potential interlinkages among phases of investigation and tiers of study would be an oversimplification of the diversity of threats that currently challenge marine habitats. Consequently, the approach to assessment is developed principally through the use of illustrative examples.

1.2 Background

A prerequisite for assessment of anthropogenic effects is that there is knowledge of the variability in the natural system. A prime example is the occurrence of "unusual" plankton blooms caused by variations in hydrographic conditions, which differ from the "normal" blooms and yet are not due to anthropogenic influences. In order to assess anthropogenic impacts on marine ecosystems, methods for detecting or measuring effects must be well validated for use in the field and the variability in response must be measured. In other words, the effects must be measured precisely and their variability in time and space must have been evaluated. These, and other factors related to validation of specific techniques, have been addressed in recent publications. These include experimental validation, field evaluation and intercomparison of techniques, all discussed in recent practical workshops organised by GEEP and held in Oslo (1986; Bayne et al., 1988), Bermuda (1989; Addison & Clarke, 1991) Bremerhaven (1990; Stebbing et al., 1992) and elsewhere. Such workshops have helped to identify various response indices (or "biomarkers") with real potential for practical application within field programmes. Some of these are discussed in detail elsewhere in this report.

In the past, it was perceived that clear cause and effect relationships had to be developed among the tiers of investigation for a technique to be considered valuable for broad application. It was envisaged that evidence of impacts on cells, for example, must be clearly related to the consequences for the functioning of populations of individuals Subsequently it has been learnt that this is not only an unachievable objective (at least within realistic time-scales) but also that it is an unnecessary one. Rather, measurements of effect on cells, tissues or individuals, say, will all contain different information, expressing different facets of response to a stress and all valid and significant in the evaluation of environmental impact. Molecular biomarkers of exposure help to quantify the links between additional/increased chemical exposure and the first stages of biological response. It is not necessary to require that such measurements also provide information on the performance of the individual organism, or a change in the reproductive potential within the target population. In some applications, however, it is still vital to consider whether a particular effect is more or less useful in establishing connections among tiers of investigation. During the process of designing a study, the potential interpretability on different tiers of the indicators may represent one of the criteria for choosing among them. Indicators with the greatest potential for cross tier use may be more informative than any alternative choice.

Equally, it has been learnt not to expect (nor to require) predictive capability from response indices beyond the boundaries of the operational measurement and its quantitative relationship to the relevant environmental stimulus. To give a specific example, an empirically established dose-response relationship between aromatic hydrocarbon concentrations in the environment and scope for growth of a target organism may provide a powerful predictive capability for assessing the impact of hydrocarbons on the growth of organisms in the field. One should not demand of this relationship a predictive power that extends beyond its empirical base. Equally, a statistically valid interpretation of a change in benthic assemblage caused by sedimentary organic loadings is a valuable aid to an evaluation of stress effects on these assemblages. Nevertheless, one should not require of this evaluation that it support a prediction of the rates of benthic-pelagic exchange of carbon as a measure of "ecosystem level" impact.

GESAMP perceives the application, then, of a suite of indicators, embracing measures at different levels of the hierarchy, but without any necessary causal link among levels. This is coupled with the knowledge that many techniques may be selected to form an appropriate suite, given different constraints that may apply. This statement represents the current scientific view that will be developed further in this report.

An essential aspect of environmental assessment is that statistically-based design criteria are vital in structuring any field programme which sets out to detect or to monitor environmental impact. The WG recognise that many past programmes have been flawed in this respect, leading to wasted effort and lost opportunity. Hence sampling design is given particular emphasis in the report.

The use of rigorous sampling designs also requires clear formulation and testing of hypotheses in environmental impact assessment. Formulation and testing of hypotheses is emphasised in our approach to add power to our recommended framework. Predictions about the potential impacts of planned developments, estimates of the potential consequences of an observed impact, or managerial decisions about environmental problems, all involve statements that are, in effect, testable hypotheses. These predictions are all amenable to test and should be tested (see Green, 1979; Underwood, 1991; for relevant discussion). With the correct combination of experimental design and defined responses, one is able to construct specific hypotheses based on *a priori* expectations. These may then be put to the test in a monitoring programme. This approach introduces a needed element of scientific rigour and also, importantly, provides information that is then expresed in terms more likely to provide a focus for appropriate managerial action.

For example an hypothesis that says that a site (or habitat, or coastal system) is or will be subjected to a particular hazard to a particular degree, which would be measurable in terms of specific indices of known properties, may or may not then be supported by data. If supported, managerial action may be structured on the formal statements of impact and quantitative response. If not supported, the hypothesis may, as a management tool, then be modified to consider a different category of hazard, a different suite of response end-points, or it may be rejected in favour of a decision that no further action is required. In any case, the close interaction between the management concern and the scientific data, effected via the process of hypothesis testing, provides a powerful and clear approach to environmental assessment.

Consideration of this interface between scientific analysis and environmental management raises the question of the role of modelling. Modelling (rather than the scientific data generated) is often the preferred tool for evaluating potential environmental decision-making. Over the last two decades, there have been various approaches to ecological modelling and some of these have been considered in the context of studies of the effects of pollution. It is concluded that the approach of general ecosystems modelling, in which many processes, interactions and forcing functions are represented in a simulation, has little to offer in the context of our immediate concerns. Rather, the approach of more specific process modelling can be extremely valuable. In this approach, a particular feature of stress response is formulated as a model and from this, in turn, a specific hypothesis (or set of hypotheses) is constructed.

The value of this approach is two-fold. First, light that may be thrown by such models on specific aspects of environmental impact. For example, models may suggest rates of spread of toxicants in a habitat. Second, such process modelling can guide managerial action and facilitate "what if?" approaches to particular environmental situations.

There are two primary, but different reasons for environmental monitoring. The first is so-called 'surveillance' monitoring in an attempt to detect unanticipated impacts, particularly ones that may be wide-ranging, subtle or that only slowly become large and obvious. The second type of monitoring is so-called 'compliance' monitoring, done to detect departures from agreed or predicted amounts of disturbance. An example of compliance monitoring is after a licence has been granted to allow discharge of certain amounts of chemical contaminant, which may accumulate in edible bivalves. Agreement has been reached that the contaminant will only affect bivalves over a distance of, say, 100 metres. Monitoring must then be done to determine whether biological effects only occur within this distance.

Compliance monitoring is thus used after a managerial decision is reached - to determine whether subsequent disturbances comply with the regulatory rulings made by managers. It is used in phase three of environmental assessment (see Section 4.1). The hypothesis tested is that impacts are within the scope and scale agreed in making regulations.
Surveillance monitoring tests the hypothesis that some, unspecified, impact is occurring and therefore some chosen biological measure will change in a predicted way through time (for a large-scale impact) or will differ from that in control areas (for some spatially defined, smaller-scale impact). It is used in phase one of environmental study (see Section 4.1).

There is, however, a third sort of monitoring needed in cases where managerial decisions are supposed to solve an environmental problem. Managers have decreed that certain action will remove, or reduce, the impact. Its cause has apparently been identified and action taken to prevent it. Monitoring is now needed (in phase three - Section 4.1) to test the hypothesis that the action taken *does* have the predicted consequences of environmental improvement.

All three types of monitoring (and therefore hypothesis-testing) share the same requirements for understanding the biological system. Without the appropriate scientific understanding of the likely consequences of possible large-scale disturbances, there is no way to determine what to measure. Similarly, where there is inadequate understanding of the mechanisms and processes operating in the system, it is impossible to predict the likely outcome of any regulatory or remedial action. The relationships between measures of environmental stress and the phases and scales of environmental investigations are clarified in this report.

2 Typical impacts in the marine environment

Human influences affect marine habitats by biological, chemical and physical interactions over different temporal and spatial scales. The spatial distribution of an impact may be local, regional or global. The spatial scale of the impact may not necessarily be related to the physical scale of the stress because a range of mechanisms such as hydrodynamic and atmospheric transport processes or the migratory patterns of organisms may extend local effects to regional or global scales. Regional effects may also arise due to the multiplicity of local effects so that they collectively cover a significant regional area. Other relevant aspects of human impacts are their magnitude, frequency (Table 2.1) and reversibility. For impacts that arise as a consequence of accidents or failure of normal operations, the probability of occurrence will also be relevant.

Human activities interact with the characteristics of habitats, making habitats differentially vulnerable to human stresses. The following discussion links typical impacts to vulnerable habitats as a way of illustrating and predicting associations between human activities and environmental degradation. Examples are given to form the basis of scenarios re-examined later in this report.

The marine environment can be classified into shallow and deep-water habitats, generally representing nearshore and offshore environments, respectively. Nearshore habitats can be further distinguished as being estuarine (i.e. subject to freshwater influence from the land) or non-estuarine. Within these broad groupings, habitats may comprise either hard or soft substrata or biologically structured habitats such as sea-grass beds or mangrove forests.

It is understood that the above classification of habitats does not define physical boundaries. Because of the continuity imposed by water, different marine habitats are interconnected and can rarely be treated in isolation. This physical continuity also explains why stresses can cause impacts on different spatial scales. It should be noted that only a few examples have been presented. Other effects and combinations of effects also occur in these habitats.

Table 2.1. Examples of the characterisation of different types of impacts that can affect mangroves

BIOLOGICAL IMPACT

Type	Spatial extent	Magnitude of impact	Duration	Frequency	Reversibility
Invasion and establishment of populations of exotic species	May eventually cover whole region	Absolute or relative abundance	Long term	Continuous new state	Often irreversible

CHEMICAL IMPACT

Type	Spatial extent	Magnitude of impact	Duration	Frequency	Reversibility
Non-point source chronic poisoning	May cover local scales or whole regions	Biological effects (e.g. fish kills)	Long term	Continuous	Slowly reversible
Accidental, acute poisoning	Local	Biological effects effects (e.g. fish kills)	Short term	Low frequency	Reversible for populations and individuals

PHYSICAL IMPACT

Type	Spatial extent	Magnitude of impact	Duration	Frequency	Reversibility
Structural change due to hydrologic change	Local	Magnitude of change in discharge and distribution of discharge over time	Long term	Variable, depending on type of regulation	Often reversible

2.1 Shallow Water Habitats

2.1.1 Estuarine Habitats

Estuarine habitats are heavily influenced by human activity. Throughout the world, there is concern about loss of habitat due to diverse activities such as filling of wetlands, silviculture, the diversion of water, the introduction of exotic species; aquaculture and other stresses which develop in the catchment of rivers.

Nearshore environments under the influence of freshwater from land may be under additional stress caused by contaminants in river run-off. The contaminants may be organic, such as untreated domestic sewage, or chemical, (e.g. industrial wastes or pesticides washed out from agricultural areas). Sedimentation associated with land-based activities such as agriculture, silviculture, and mining and estuarine and marine dredging may also significantly degrade nearshore habitats (Milliman, 1992).

Important ecosystems situated in estuarine areas include wetlands, as exemplified by mangrove forests in the tropics, seagrass beds, and seaweed (macroalgal) assemblages.

Mangrove forests are important ecosystems around the tropical belt. The most significant modern impact on these habitats is physical destruction in the form of extensive clearing for purposes such as construction of fish ponds, creation of areas for human settlement or agriculture. In addition, mangrove trees have traditionally been cut down for their wood.

Seagrass beds occur in areas receiving a significant freshwater input and also in areas characterised by oceanic salinity levels. In the former, they are viewed as estuarine habitats and are subject to impacts that may be associated with riverine outflow, in addition to other perturbations not necessarily associated with the estuarine situation, such as increased sediment load caused by deforestation up river.

Traditionally, seagrass beds have been extensively dredged to improve navigation in shallow coastal waters, so that the predominant impact is again physical habitat destruction. Seagrass beds are often heavily fished for finfish, molluscs, crustaceans and echinoderms. They may therefore be disturbed by over-exploitation, especially where there is a heavy dependence of rapidly-growing human populations on these resources for food.

Plant assemblages aside from seagrass beds include stands of various species of macroalgae. Numerous euryhaline macro-algal species are found in estuarine habitats. These are subject to impacts similar to those on seagrass beds.

In addition to the impacts described above for specific ecosystems, soft-bottom habitats, including seagrass, mangrove and algal assemblages and the expanses of sediment on the seafloor which are devoid of conspicuous epibenthic organisms, are vulnerable to forces affecting sediment movement and alteration of sediment characteristics. Processes of erosion, deposition and alteration of grain size can adversely affect organisms in these soft sediments.

Hard bottom assemblages in estuaries are similarly affected by a range of impacts associated with run-off from land and such factors as excessive exploitation.

2.1.2 Non-Estuarine Habitats

Coral reefs are among the most important non-estuarine ecosystems in shallow water and are hard bottom structures. They line the coastlines of parts of the tropical and sub-tropical belt where environmental conditions generally allow sufficient penetration of light for photosynthesis by their autotrophic symbionts, and fluctuations in temperature and salinity do not exceed the physiological limits of the reef-inhabiting organisms. Over time, these ecosystems have become important sources of food and other products for nearby human populations, in addition to serving other uses such as coastal protection and recreation.

The rapid increase in human population and the heavy dependence on reefs (especially in developing countries) has led to over-exploitation of resources. Certain species of finfish, molluscs, crustaceans and echinoderms are harvested on a selective basis, resulting in changes in the structure of populations and species assemblages.

Coral reefs also suffer from physical destruction in many parts of the developing world due to causes ranging from destructive fishing, to sedimentation from land run-off, to dredging, mining and filling (as in land reclamation). Destructive fishing, which has received considerable attention, refers to the employment of harvesting methods which harm the environment, such as the use of explosives, or trawling which greatly disturbs bottom sediments and biota. It must be remembered that physical damage to reefs may also be caused by natural phenomena such as storms and major events such as El Niño, (Warwick et al. 1990). The majority of reefs have, however, evolved responses over geologic time that allow them to cope with, or recover sufficiently from, the effects of such natural events.

Coral reefs in proximity to large human settlements may be exposed to damage due especially to the effects of particulate material from, amongst other sources, untreated domestic sewage. This leads to shifts in structure of assemblages, the net result of which is a diminished overall quality, defined in terms of human use.

As already indicated, seagrass beds may also occur under full oceanic salinities. The same is true for other plant assemblages such as the macroalgae and many sedimentary ecosystems. They are subject to impacts such as physical destruction and over-exploitation, as mentioned above. Where they occur close to the shore and in the vicinity of human populations, they are also vulnerable to various types of pollution from the land.

In non-estuarine situations, hard-bottom habitats are less susceptible to impacts caused by hydrological modification. Nevertheless, the influence of waste discharges and accidental spills of harmful chemicals remain a concern.

Globally, other diverse impacts occur in nearshore marine environments. These include organic enrichment of kelp forests, disposal of dredge spoil and discharge of thermal effluents into rocky embayments.

2.2 Deep Water Habitats

The open ocean constitutes the largest part of the marine environment. In general because of its greater distance from sites of human activity it is less likely to be subjected to anthropogenic impact than coastal areas. Traditionally, the open ocean has been divided into the benthic realm and the overlying water column (pelagic realm). It is recognised, however, that the water column encompasses thousands of cubic kilometres and is also structured by physical processes. It can therefore be subdivided into a number of different regions which include the ocean surface microlayer, the euphotic zone, intermediate waters and abyssal depths and from oceanic gyres to frontal systems.

Offshore pelagic assemblages, like their shallow water counterparts, are subject to chemical contamination which may lead to effects on biological sysytems. The primary concern has however, been with over-exploitation of their living resources.

Bottom habitats, both hard and soft, are subject to continuing deposition and movement. Deep water assemblages are subject to long-distance influences including low-levels of contaminants, especially those associated with transported sediments.

Additional threats are posed by deep-sea dumping and the prospects of mineral exploitation. Some deep water fish stocks are heavily exploited without adequate knowledge of the stocks or their productivity.

Factors such as long-distance transport of chemical contaminants and nutrients may influence open ocean productivity. In addition, potential effects of UV-B radiation (associated with global ozone depletion) on phytoplankton may eventually alter ecosystem structure and function. Effects of global climate change on ocean circulation and weather patterns, coupled with other stresses, may exert significant changes.

2.3 Impact Scenarios

As indicated above, ecosystems may be influenced by a multitude of human activities. This should be taken into account in the development of impact scenarios for specific systems. Such scenarios should include the sequence of events for single stresses and the interactions and common features of sequences of stresses. This will help to provide a common framework for the application of indicators and methods to assess the state of the system. Thus, the development of impact scenarios should also clarify the connection between management and scientific information. If well-formed, scenarios should simplify the formulation of hypotheses to be tested through monitoring or specific research programs.

Figure 2.1 provides an example of an impact scenario for mangrove habitats (but which could be generally applied to other habitats). It starts with human activities that might lead to detectable responses in the system. The stresses and intermediate impacts represent the links between the human activities and biological responses. The choice of human activities and links to the responses will obviously depend on what is a priori considered important for the system in question. In this case, land clearing can, for example, be considered as one aspect of agriculture, but for other systems it may be identified as an explicitly separate activity. Similarly, erosion and deposition of material is implicit in the structural change, but, in a system in which this is the major change, these processes could be expressed explicitly.

To strengthen the base for assessing the biological responses, the characteristics of impacts should also be examined. In Fig 2.1, examples of the characteristics of some impacts that affect mangrove habitats are given. Each of the activities listed may, however, elicit a greater response than that shown.

When dealing with stresses in the marine environment and the effects they elicit, it is useful to note that certain techniques may be applied for a broad range of habitat types and impact scenarios. This is because certain biological responses are the same and unambiguous regardless of the physical setting or location. Examples of techniques that could be widely used to detect environmental impacts are DNA biomarkers, indices of physiological stress at the organismal level (metabolism, mortality) and changes in population structure.

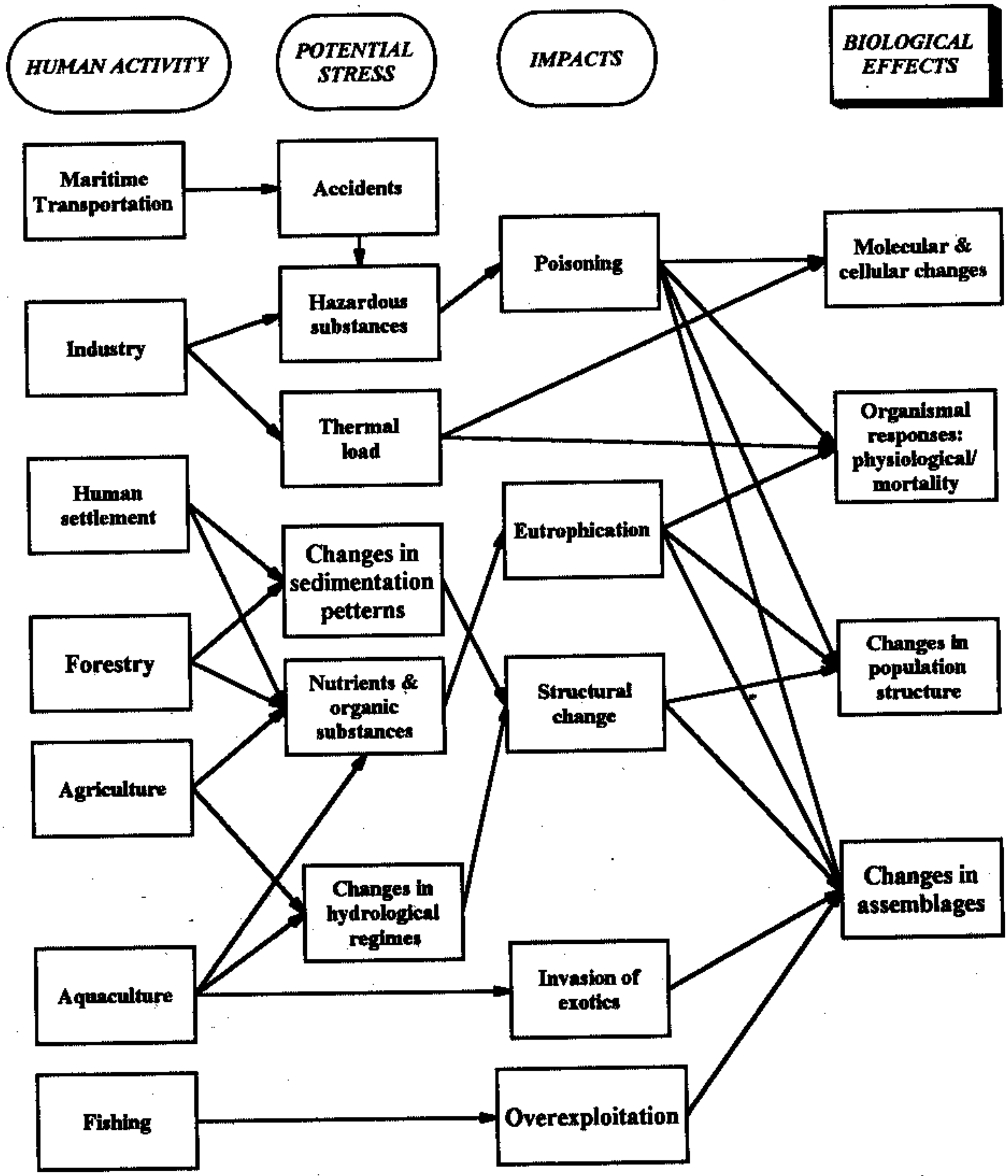

Figure 2.1 Major and general stress scenarios for mangrove forests

A parallel consideration, however, is that alongside their similarities, different habitats and impact scenarios also possess unique characteristics that should influence the choice of biomarkers or stress indicators employed in a particular situation/location. Changes in diversity are difficult to interpret unless the particular ecological context in which they occur is known. Differences in growth rate would also have different meaning in different systems due, for example, to ambient temperature and its variability.

The similarities and differences among impact scenarios require careful formulation of the objectives and hypotheses leading to the use of the chosen indicators or methods in different phases of an investigation. Some of these aspects are elaborated further in Sections 4 and 5.

3 Indicators of Exposure and Effect

This section provides examples of methods used to detect stress effects on individuals, populations and assemblages as discussed in the Terms of Reference. Some key references and techniques have been selected. Such a broad overview has utility in illustrating the potential value of understanding and dealing with problems in distinct phases, in addition to using techniques from several tiers. It is also important to have co mmensurate chemical data on both the environment and from organisms and information on the regimes of physical disturbance since this information will help in understanding what is happening at any phase of a study. Nevertheless, in this report methods for analysis of chemical contamination are not considered. Estimating the extent to which contamination may adversely affect the biota within marine ecosystems is an important goal of a programme that monitors environmental quality.

3.1 Bioassays

Bioassays (also commonly called toxicity tests) are experimental procedures in which organisms are exposed to different substances or combinations of substances to determine concentrations that adversely affect them. The period of exposure may be short (in acute tests, not constituting a substantial portion of their life span) or longer-term (in chronic tests, covering longer periods or a substantial portion of the life cycle) (adapted from ASTM, 1993). Measured effects include mortality and sublethal biological responses such as impaired growth, irregular development, abnormal behaviour, or reduced reproductive output.

Different bioassays should be used for specific purposes, such as: 1) comparing the toxicity of effluents from industry and their treatment plants; 2) comparing the toxicity of chemicals or chemical mixtures; 3) comparing the toxicity of field samples from different locations; 4) comparing the sensitivities of different species to the same substances; or 5) identifying new problems. The choice of bioassays should always match the question being posed.

The advantages of marine bioassays include their standardisation, reliability, ease of use, low cost and sensitivity. The short-term nature of most bioassays, which only consider single species under controlled conditions, rather than many species under natural conditions, limits their predictive value as indicators of complex chemical and biological interactions.

It is well-recognised that precise exposure condition is a very important variable affecting organism-toxicant contact and partitioning within the organism (McCarty and Mackay, 1993; Rand et al., 1994). Subsequent chemical uptake, organ-system chemical transfer, toxic response, metabolism and depuration all depend on exposure concentration and composition. Hence, bioassays on fresh field-collected samples or conducted *in situ* can be more realistic indicators of field exposures and may be able to identify available toxicants.

Numerous single-species marine bioassays are available as standard methods or protocols (e.g. ASTM; OECD; ICES; Envionment Canada, Blaise et al. 1988; US EPA). Many more bioassay procedures are available as experimental laboratory or field procedures from individual investigators (e.g. those tested by the Global Investigation of Pollution of the Marine Environment (GIPME) programme of Unesco/International Oceanographic Commission (IOC)/International Maritime Organisation/United Nations Environment Programme and it's Group of Experts on Effects of Pollution (GEEP)). The standardised bioassays cover the span from acute sublethal and lethal assays to chronic sublethal assays, measuring the bioaccumulation and effects of contaminants. They have recently been described in Persoone et al. (1984), and Rand (1985, 1994 in press), among others, and in detailed methods documents such as ASTM (1993). Commonly-used tests are those that measure mortalities in marine fish, bivalve molluscs, mysids, copepods, microalgae, rotifers, amphipods, bacteria, echinoid gametes, and crustacean larvae. Recently cryopreserved oyster and clam larvae have been used in bioassays (McFadzen, 1992) and such techniques promise further standardisation of tests. Endpoints include mortality, abnormal behaviour, slowed or delayed growth, inhibited photoluminescence, inhibition of fertilisation, abnormal or delayed embryonic development, decreased moulting success, and others. The precision of short-term chronic bioassay tests in multi-laboratory studies has also been documented (Anderson and Norberg, 1991).

Bioassays can be used on sediment samples, for which the most common tests involve measuring survival of oyster larvae to extracted pore water (Thain, 1992; Butler et al., 1992) and survival and burrowing of amphipods (Swartz et al., 1985) into putative contaminated sediments, (see also Chapman et al. 1992). These tests are adequate for predicting the potential for effects in highly contaminated areas. At sites where more subtle effects of contaminants are expected, potential interferences such as sediment grain size may be of concern (DeWitt et al., 1988). The problem with these tests is that increasing effects are frequently not correlated with increasing concentrations of contaminants in bulk sediment. This is because the bioavailibility of contaminants varies markedly and is often difficult to predict in a medium as complex as sediment (Knezovich and Harrison, 1987). Numerous efforts are under way to develop techniques using pore water extracts. There is no currently-accepted standardised way of extracting pore water; thus, use of these techniques is still experimental.

Recent applications of toxicity testing have included assessments of the effects of contaminants in the sea surface microlayer (Hardy and Cleary, 1992; Karbe, 1992). The microlayer was sampled and then thoroughly mixed with clean water and toxicity tests done on this water. Although such tests have indicated significant toxicity there is still an unresolved issue as to what extent organisms in nature are actually exposed to the surface microlayer. Thus there is a need for further studies. As with methods for collecting pore water, new techniques are being developed and should soon be more widely available. (GESAMP has just completed a report on the sea-surface microlayer).

3.2 Biomarkers

Biological responses to chemicals in the environment can be measured at all levels of biological organisation, from molecular to species assemblages. A variety of tissue, cellular and molecular biomarkers have been developed as diagnostic screening indicators, to evaluate the status of an organism and to detect exposure to contaminants (McCarthy and Shugart, 1990).

The term "biomarker" refers to a biological response that can be specified in terms of a molecular or cellular event, measured with precision and confidently yield information on either the degree of exposure to a chemical and/or its effect upon the organism or both.

Biomarkers are influenced by the bioavailability of contaminants, the routes of exposure and the level and time of exposure. The first response of organisms takes place at molecular and cellular levels of target organs and tissues before effects become visible at higher levels of biological organisation (individual, population or assemblage). Because responses at the molecular and biochemical levels are similar across phyletic boundaries they are often not merely species specific..

A variety of biomarkers at the tissue, cellular, and molecular levels are available to evaluate the status of an organism and to detect exposure to contaminants (Huggett et al., 1992). At the molecular level, for example, biomarkers of exposure to contaminants and the potential for subsequent effects include the induction of detoxicative enzyme systems, the alteration in enzyme activities, and perturbations in DNA structure and function. An important consideration for molecular biomarkers is the fact that they are tightly linked to the insult, and therefore, they tend to be sensitive indicators of specific types of exposure.

Molecular biomarkers may require sophisticated technology (access to molecular probes, micro-plate readers, confocal microscopy). Although in theory possessing significant phylogenetic generality, they may need research validation on chosen target species before general application. Even where specific responses to particular classes of chemical compounds have been described (e.g. P-450 enzymes, metallothionein, choline esterase inhibition), responses may bear uncertain dose-response relationships to the causal agents; be specific to certain types of organisms; and for their expression may depend upon seasonal metabolic cycles (Huggett, et al., 1992, Peakall & Shugart, 1993).

Biomarkers may fall into several categories depending upon their diagnostic value, (Table 3.1). Such categories are:

(A) biomarkers of exposure only;

(B) biomarkers of exposure with uncertain eventual consequence;

(C) biomarkers of known deleterious consequence based on mechanistic understanding.

Table 3.1 Categorisation of biomarkers based on their diagnostic value

Biomarker	Category	Remark
I. DNA Integrity		
a) Strand breakage	A	Potential genotoxic insult
b) Adducts	B	Exposure to specific class of chemicals (mutagens, carcinogens)
c) Photoproducts	C	Exposure to UV-B radiation
d) Chromosomal aberrations	B	Detected by flow cytometry and cytogenetic analysis
II. Protein		
a) P-450 IA	B	Exposure to classes of chemicals (PAHs)
b) Alad and porphyrins	B/C	Exposure to classes of chemicals (lead; PCBs)
c) Choline esterase	C	Exposure to classes of chemicals (organophosphates)
d) GTH metabolism	A	Chemically-induced oxidative stress
e) Metallothioneins	C	Exposure to metals

Biomarkers of exposure are biological responses that can be linked directly (and preferably mechanistically) to selected chemicals, or groups of chemicals. The direct toxic consequence of a response may not have been demonstrated. In such cases the biological responses can, however, indicate the bioavailibility of the toxic agent and the magnitude of response may relate directly to the degree of exposure.

Biomarkers of exposure represent either general or specific responses. General biomarkers of exposure include those responses that are not compound- or class-specific but indicate merely that exposure to some exogenous agent might have occurred (e.g., DNA strand breaks or chemically-induced oxidative stress). Changes in some general biomarkers can be caused by environmental variables unrelated to toxic chemical exposure. For example, temperature increase has been shown to stimulate selective protein synthesis or modulate the frequency of occurrence of chromosomal aberrations. Specific biomarkers of exposure are used to indicate or confirm the class of chemicals or toxic agent involved. Examples include organo-phosphate/carbamate inhibition of serum cholinesterase activity, polyaromatic hydrocarbon (PAH) adducts with DNA and protein, lead inhibition of aminolevulinic acid dehydratase (ALAD), PAH and planar halogenated aromatic hydrocarbon induction of $P_{450}1A$, and DNA photoproducts caused by ultraviolet radiation. Many of these biomarkers are considered in much greater detail elsewhere (Huggett et al., 1992).

Biomarkers of effect detect a wide variety of responses to pollutants. Many response indicators have been proposed which measure the effects of contaminants on the whole organism. These include physiological, behavioural and ecological variables, and are discussed in the reports of three practical workshops in which various indicators were compared for application within field-based programmes of pollution assessment (Bayne et al., 1988; Addison & Clarke, 1990; Stebbing et al., 1993). In the present report no further assessment of these various procedures is given. Rather, here some general features, with examples, in order to illustrate the circumstances where different types of response indicator may, or may not, be useful are discussed.

3.3 Histopathology

Using the multi-tiered approach, molecular and cellular biomarkers (section 4.1) may usefully be linked with other measures of cellular and tissue damage in an assessment of the histopathology associated with exposure to chemical insult. This strategy not only has the potential for detection of early warning of effects of stress that will be diagnostic for both exposure and injury, but will also provide prognostic capability for predicting the likely consequences for the condition of individuals in a population if the stress is sustained (Moore et al. 1994).

Some tests use cells in body fluids, for example blood, which can be used non-destructively, such as pathological changes in intracellular membranes of lysosomes. In fact, lysosomal membrane damage appears to be a universal marker for effects of stresses in most if not all nucleated cells (Moore, 1990; Moore *et al.*, 1994). Furthermore, many toxic organic chemicals accumulate in lysosomes and here molecular modelling and quantitative structure-activity relationships, (QSARs) are revealing how such xenobiotics enter cells, specifically target the lysosomal compartment and exert their toxic action (Moore *et al.*, in preparation).

The use of molecular and cellular biomarkers coupled with cellular pathology (or histopathology) has the potential to reveal significant differences between impacted and non-impacted (or reference) organisms (Hinton and Lauren, 1990; Moore and Simpson, 1992). Furthermore, the compilation of tests used will indicate whether some differences result from exposure to xenobiotics, metals or other causative agents. It is important to note that by relating biomarkers of cell injury to significant pathological consequences for the individual, their diagnostic value and predictive capability for further damage at higher organisational levels will be strengthened (Moore and Simpson, 1992).

Many antibody-based recognition tests for specific proteins (e.g., cytochromes P-450, stress proteins, oncoproteins, etc.) can now be applied directly to histological samples. This can provide useful information on the spatial distributions of such proteins in relation to stress-induced structural and organisational alterations in cells and tissues (Moore and Simpson, 1992).

Histopathological changes can be easily and accurately quantified using microstereological procedures applied to tissue sections, and these data can be correlated with both cell injury processes and abnormal physiology. Such techniques are relatively simple, low cost, and rapid, yet are capable of providing information of a very high level of biological/pathological sophistication. Histopathological samples can be archived indefinitely and yet can be made readily available for subsequent application of advanced molecular recognition tests for specific proteins and other products.

It has often been suggested that gross pathological conditions in fish, such as the frequency of diseases, increases along pollution gradients and is therefore, a useful indicator of stress. Yet detailed studies done in the Bremerhaven workshop, (Vethaak et al. 1992) on dabs showed no correlation between gross and histologically identified liver lesions and chemical contamination. Epidermal hyperplasia/papilloma, however, showed some promise but the need was expressed for more data on migration patterns, natural background levels of disease, and on disease induction before these can be used as reliable stress indicators.

3.4 Physiology

Physiological responses of marine organisms to pollutant exposure depend on the biological availability, uptake and distribution of the chemicals within the body. By their nature, physiological responses integrate sub-cellular and cellular processes and can be chosen to be representative of the fitness of the whole organism. As Capuzzo (1988) has pointed out, "the most important physiological changes associated with contaminant exposure are those that may adversely affect the organism's growth and survival and, thus, its ability to contribute to the population gene pool. Physiological indices linked to the survival and growth potential of the individual (such as the bioenergetic variables, feeding, digestion and respiration), or to the reproductive potential of the population (such as reproductive effort and larval viability), are therefore potentially most effective in assessing the effects of contamination gradients" (p. 111).

Various techniques attempt to measure the effects of disturbance on the whole organism (i.e. the physiological level of the biological hierarchy). This includes, for example, measures of metabolic rate, growth and reproductive behaviour. One such approach which is being increasingly applied in field programmes is the determination of Scope for Growth (SFG). This is based on a simple analysis of the main components of energy balance within an individual animal, i.e. measures of energy input (ingestion, assimilation) and output (respiration, excretion) which, when integrated, provide a snapshot of the potential for growth by the organism at that point in time. This approach has proved most useful when applied to sessile marine invertebrates such as bivalve molluscs (Widdows and Johnston, 1988), in which the various components of the energy budget are readily measured and are representative of the physiological status of the individual. The procedures are described in detail by Widdows and Salkeld (1992).

Extensive laboratory and field studies (see Bayne et al.,1988) have demonstrated the effects of various pollutants on the SFG of mussels and the utility of the approach in field monitoring. Further, by equating the physico-chemical characteristics of classes of organic compounds to their toxicity, as measured by the SFG (the QSAR approach), Widdows and Donkin (1991) have demonstrated the feasibility of providing a quantitative assessment of the cause of observed impacts on physiological condition in the field, and also of making predictions of effect given knowledge of the local concentrations of contaminants.

Physiological responses, such as the SFG, although also generally applicable in theory (all organisms must balance energy intake against expenditure in order to acquire surplus energy for growth), may be difficult to measure in some types of organism. Respiratory energy losses, for example, is an essential component in the SFG determination, that are dependent on rates of maintenance metabolism, growth and locomotory activity. Only when these are controlled experimentally can a reproducible estimate of SFG be made and this constraint may limit the technique in practice to certain sessile organisms such as bivalve molluscs (Koehn & Bayne 1989).

Such whole organism studies provide an extremely powerful and apt assessment of impact in natural assemblages of organisms when properly linked to the use of molecular biomarkers, and to related studies on assemblage structure (see below). Such approaches are potentially available for wide application in marine pollution studies. For example, biological monitoring of SFG, coupled with good laboratory data on dose-response for various classes of contaminants, can be used to diagnose the chemicals responsible for observed field impacts over a wide geographical area. Since such measurements are relatively inexpensive, they can be used prior to any chemical monitoring to optimise use of expensive chemical analyses on which to base remedial action.

3.5 Ecology

There are several indices of population structure or species assemblages that measure ecological responses. In broad terms, some of these are intrinsically univariate measures - i.e. a single measurable variable responds to an environmental stress. Others are contrived univariate measures of the collective properties of more than one variable. For example, indices of diversity reduce all the information on abundances of a subset of different species into a single measure of diversity of the entire set. In contrast, multivariate measures retain the information about more than one variable (e.g. the numbers of all species present in an area). As such, the latter more completely represent the range of properties of an ecosystem.. The methods used to sample or measure ecological (and other) univariate and multivariate variables are considered in Section 6.4. Here, a number of indices that are useful for detecting environmental change are briefly introduced.

Many univariate indices are used to assess changes in populations and assemblages, but five types of indices are most commonly considered. Loss of species is the first type of index. When species data have been collected periodically over time it may be possible to detect loss of species. For major biologically-determined habitats (sea-grasses, corals, mangroves, kelp forests), loss of the primary species will entail loss of dependent species. In most marine assemblages, many species are rare with small abundances whatever the scale sampled. Thus, it is not a trivial task to assess species loss at levels other than the easily observable.

The second type of index is diversity. Diversity indices integrate the number of species and the number of individuals into a single value, in an attempt to simplify the expression of biological complexity. Many diversity indices have been proposed, but calculations of many different indices from the same data sets show high correlations and therefore high redundancy (Warwick and Clarke, 1991). There are many examples of reduced diversity in response to a variety of stresses (organic enrichment, Pearson & Rosenberg, 1978; oil pollution, Gray et al., 1990; mining waste, Olsgard, 1993). Recent data, however (Gray et al., 1990, Warwick and Clarke, 1991), show clearly that diversity decreases significantly only under severe stress. Thus, reductions in diversity can only be used to indicate severe effects and are not specific to the type of stress.

Changes in the type or abundance of dominant species is another index of change. Under severe effects of stress there are many examples to demonstrate that dominance by a few species increases and that these are small-sized, rapidly growing, so-called opportunist species. Examples are dominance by capitellid and spionid polychaetes under extreme organic enrichment (Pearson and Rosenberg, 1978 give many examples) and the influence of mine waste discharge (Olsgard, 1993). On coral reefs, macroalgal dominance increases in response to nutrients (Smith et al. 1981). Warwick (1986) has clearly shown that biomass dominance patterns change with increased stress and has proposed comparing dominance patterns of abundance and biomass changes. In unpolluted areas, the biomass dominance curve always lies above the abundance dominance curve, whereas under polluted conditions the reverse is true. Yet, natural physical changes can also produce similar effects, (Beukema, 1988). Thus, the method is not always indicative of pollution.

Reductions in the size of harvested species are indicators of population change due to overharvesting. Over-exploitation of commercial species leads to reductions in mean size. The fisheries literature shows countless examples. In the Philippines, the exploitation of spiny lobsters has led to an overall reduction in larger size classes (Juinio, 1987). Reductions in the mean sizes of sea urchins, due to intense fishing, was observed in both Barbados (Scheibling and Mladenov, 1987) and British Columbia, Canada (Sloan et al., 1987). In Chile, heavy harvesting of limpets has reduced the mean size of the affected populations (Oliva and Castilla, 1986). With properly designed sampling programmes, any significant reductions in size of harvested species should be easy to detect. The effects may however, be confounded by variation in the year class strength which in most cases is unrelated to the level of exploitation. Correct interpretation of changes in size structure often requires information on year class strength.

In addition to changes in mean size, other changes in population structure may indicate significant impacts on a population. Mean abundance (the average numbers of animals in an area of habitat) is the most widely used variable for detecting changes in populations. As indicated in Section 5.3, the spatial distribution of individuals in a population (i.e. how they are dispersed throughout the habitat) and their temporal variability (the rates and patterns of change) are also important indicators of well-being of populations.

All of the usually investigated ecological characteristics of populations (e.g. fecundity, age- or size-specific patterns of mortality, rates of birth or recruitment and measures of activity or behaviour) are useful for determining whether populations are stressed.

What is needed, however, is a much better understanding of how different types of stress are likely to affect characteristics of populations. For example, successful predictions of effects of stress or valid interpretation of patterns in stressed populations, requires knowledge of how the disturbance acts. If chemical pollutants, for example, kill juveniles faster than adults, predictable changes in size will occur. On the other hand, disturbances removing existing populations (e.g. as a result of episodic floods releasing excess sediments into an estuary) will lead to quite different size-frequencies.

In general, ecologists feel comfortable about interpreting changes in abundance, coupled with knowledge of sizes of members of a population, so these two variables tend to be those most applied to the measurement of the condition of the environment.

Multivariate statistical analyses are valuable techniques for assessing change due to stress in complex assemblages of many species. Analyses (see Section 6.4 for a more detailed discussion of methods) based on matrices of species abundance in several sites have been shown to detect subtle effects of stress on natural systems (e.g. on soft sediment benthos, Gray et al., 1988; Gray et al., 1990, Warwick, 1988a; Warwick & Clarke, 1991, 1993 and on coral Warwick et al., 1990). Such methods incorporate changes in abundance patterns and species differences among sites, thus integrating many of the properties discussed. Analyses suggest that data from families and even phyla are still able to detect environmental gradients (Heip, 1988; Warwick, 1988b).

Recently, Clarke and Ainsworth (1993) have developed methods to relate the patterns found in a set of sites to possible causative agents. These greatly aid determination of cause-effect relationships.

4 Using Indicators to Measure the Condition of Marine Ecosystems

Sampling to detect changes in components and functions of ecosystems over time will need to include an appropriate mixture of indicators, because of the range and scale of potential stresses acting at any time in a given area. Wherever possible, the assessment of the well-being of a system needs to include indicators of chemically-induced effects (e.g. biomarkers) in the biota. There also needs to be a range of measures to detect responses to disturbances such as organic or nutrient enrichment, changes in sedimentation, erosion or hydrography, exploitation and other processes that influence whole organisms, populations, assemblages and the processes and interactions operating at these levels of biological organisation. Finally, there must be indicators of the risk of, or the actual ,change of habitat (due to such processes as dredging, clearing, land-fill and development of the shore-line) that will lead to alterations or losses of populations or sub-systems in an area.

Depending on the nature and the spatial and temporal characteristics of the prevailing stresses, different weight will be given to different biological effects in determining the well-being of a system. For example, dredging or clearing a mangrove forest will obviously affect local populations and assemblages but may also have wider implications for conservation of marine ecosystems in an entire region. The steady erosion of well-being in a patch of habitat, due, for example, to the effects of chronic pollution, may eventually lead to loss of the entire habitat, through the indirect effects of local destruction of patches of habitat. Thus, the sorts of measures that indicate the nature of local, very destructive impacts will be very different from the measures needed to investigate regional consequences of local problems.

To reduce the potential confusion, it is helpful to consider investigations as having three general phases. First is the regular measurement or monitoring of the condition of a system to detect changes, or early warnings of change, that are indicative of stress. Once a response has been identified, in the second phase the emphasis changes to a determination of the nature and cause of the response measured. This usually involves measures to detect changes through time, or better, differences in places subject to known disturbances, relative to undisturbed sites. The sorts of questions to be answered include the following. What are the consequences for this, or other nearby and other connected parts of the ecosystem? How widespread is the response and is it increasing?

Once these issues are determined, the third phase is to implement managerial solutions to the problem, to remove or ameliorate the stresses, to regulate the rate or magnitude of stresses, to rehabilitate the species, assemblages or habitat and to evaluate the effectiveness of each and all of these. In some circumstances, the three phases are not really separated in time. Responses to the detection of an impact may already have been planned, so that the second phase is not necessary. For example, contingency planning for an accidental spill of chemicals, or an oil-spill obviates the need for phases one or two. The disturbance leads to phase three because the biological effects and the appropriate managerial responses can be predicted before the event occurs. Nevertheless, it is instructive to consider each of these three phases even when drawing up precautionary measures or response plans in the event of some stress being detected.

In each phase (detection of the response, definition of the cause and a solution to the problems), there are different needs and requirements for measurements of the condition of an ecosystem. Different methods and different sets of indicators are therefore likely to be appropriate. Thus, considerable thought must go into defining the problems and choosing the best set of indicators for each phase. These will probably be different from phase to phase.

4.1 A Tiered Approach in Each Phase of an Environmental Study

In field studies, it is recommended using indicators in a tiered approach, specifically designed to meet the needs of the three phases of an investigation. The composition of each phase and the number of tiers appropriate for a given situation would be tied to the specific objectives of the research and the characteristics of the field site. A general framework can, however, be identified, as in Fig. 4.1.

The first phase is to identify a response. This may happen because of some observed biological effect (a fishery fails or a series of algal blooms occur). Normally, however, phase one will involve sampling to detect predictable and other, generally unpredictable responses to stress. Measurements could include a suite of relatively inexpensive, rapid, and general chemical, biomarker and whole organismal studies. In the case of chemical contamination or other chemical stresses, variables should be selected that are sensitive indicators of major physiological or molecular responses to a wide variety of chemicals. For other types of disturbance, there should also be general measures of well-being of whole organisms, populations and assemblages. Often, there will need to be mapping of habitat and identification of the general structural features of the habitat and documentation of their changes through time.

If an environmental response is detected, the second phase will be to characterise and understand the cause. The probable cause of the response may be inferred from the nature of the biological effects seen in phase one. Otherwise, multi-tiered assessment is needed to identify the causative contaminant(s) or disturbance(s). This "downward" or explanatory investigation must be coupled with an "outward" or predictive study. What other species, processes and habitats are going to be affected? What are the potential consequences of this type of stress to local, regional or global marine ecosystems? How will the consequences be translated or transformed into other problems, including socio-economic, cultural and political ones?

Tests in phase two should be more specific, so that the effects of specific chemicals or groups of chemicals, can be ruled in or out or so that particular types of disturbances and stresses can be identified as causing the problems.

The third phase could involve long-term monitoring using indicators to document recovery, with confirmatory chemical and ecosystem studies, once some solution to the problem has been implemented by managers and regulators.

As an example, consider the analysis of a problem in a mangrove forest as in Section 5. Tables 5.1, 5.2 and 5.3. In a (phase 1) routine assessment of estuarine areas and their catchments, changes in land-use may be detected through synthesis of information in a Geographical Information System (GIS) or by using other systematic techniques for mapping. As a result, a decrease in area of mangrove forest might be detected.

Phase two requires identification of the potential causes of the response - from relatively straightforward detection of the planned destruction of the forests or by seeking sources of pollutants, nutrient enrichment, increased transport of sediment or whatever other agent/activity/contaminant/process might be responsible. This requires a quite different suite of techniques from those used in phase one. The techniques would be chosen to evaluate the likelihood that some agent/activity/contaminant/-process has, in fact, caused the observed decline in mangrove forest.

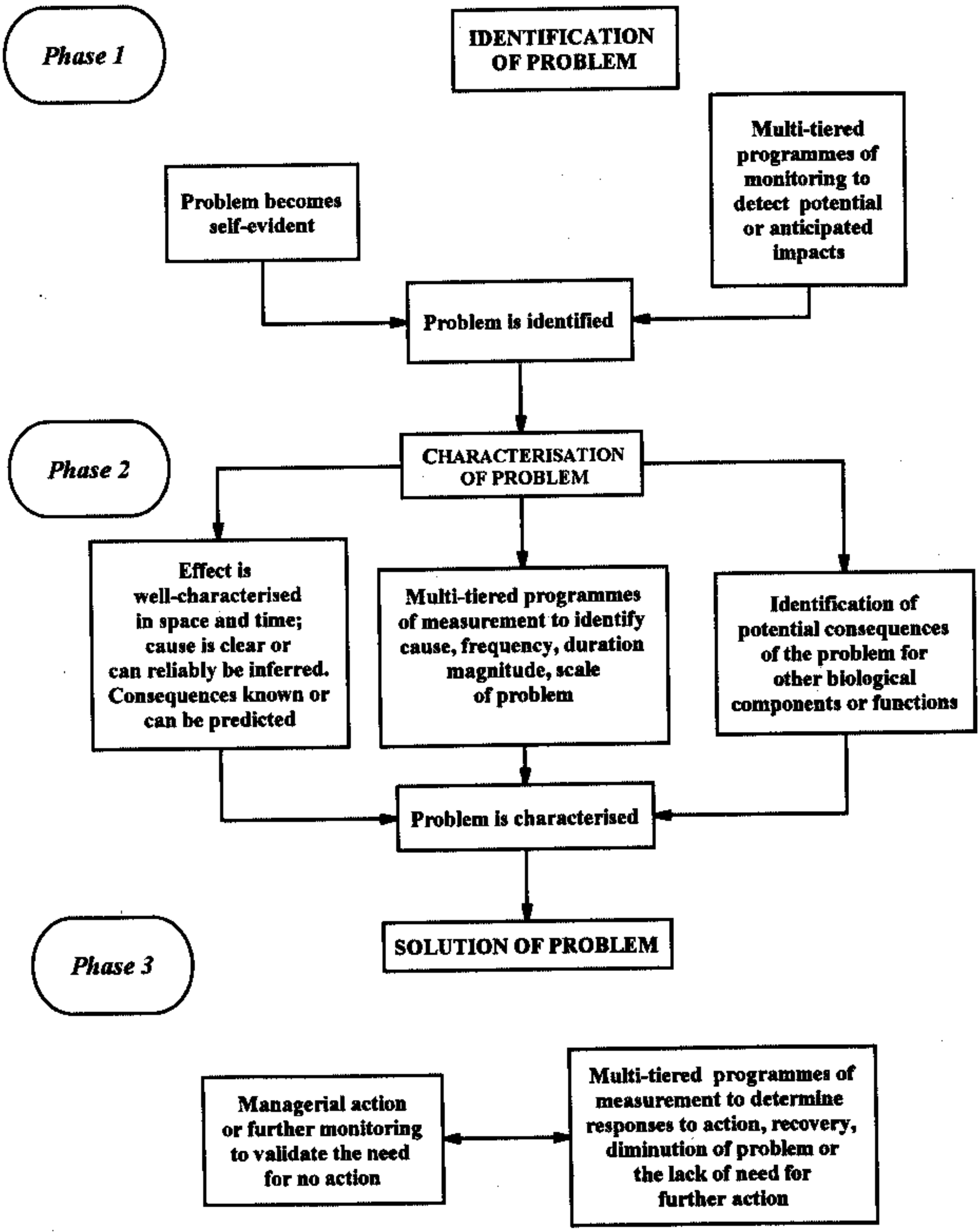

Figure 4.1. A summary of the three phases of an environmental study to identify, to characterise and to manage an environmental problem

Phase two also requires an assessment of the potential impact of loss of mangrove forest on other habitats and on such activities as commercial fisheries (as in matrix 1A).

Finally, once the cause, scope and consequences of the response have been identified, it is possible to suggest options for management to reduce the response or its ultimate consequences. The options should be analysed carefully and then implemented with due regard to what they are designed to achieve. The expected outcome of a managerial action, in terms of the supposed cause and in terms of the outcome of the chosen action should be identified clearly and phrased as testable hypotheses. The final phase is the assessment of the effectiveness of the implemented managerial action (see Fig. 4.1). The nature and predicted consequences of management will determine which biological indicators are most appropriate to measure to test the effectiveness of the action. Outcomes might perhaps be measured by biomarkers, physiological functions or, ultimately, restoration of mangrove forests. In a broader context GESAMP has suggested more general strategies for marine environmental protection, (GESAMP, 1991).

Some indicators can be used with relative ease and speed, providing information rapidly and at low cost. Thus, they may be suitable for long-term monitoring of the marine environment and initial identification of areas requiring additional investigation. General measures of stress can integrate the effects of the stresses to which the organism may be exposed, making these measures useful in understanding the susceptibility and condition of an organism. For example, more specific measures of che mical exposure can provide information on the classes of chemicals to which the organism has been exposed. In addition, they provide information on the mechanism inducing a response and its progression. The use of a suite of indicators in a well-conceived and executed research programme can enhance understanding of the interactions between organisms and the stresses in their environment.

As alluded to above, hierarchies or tiers of indicators might be created that may have different utility depending on the specific questions addressed in any given study or monitoring situation. For example, among the various indicators, some would give direct indications of the condition or Darwinian fitness of the organisms. Others, while responding in some specific or general manner to toxicant exposure, may not be predictive of toxicity. The latter, however, might be useful in providing information regarding exposure to contaminants without providing information on condition or fitness. Obviously, some indicators may reflect both exposure and impaired condition.

The fact that different indicators respond to different stresses points to the advantages of employing a suite of indicators to assess the condition of different components of the ecosystem. Obviously, the success of such a programme would depend on the intelligent choice of this set of indicators. Ideally, this set should be modified to fit the need presented in terms of the type, range, magnitude, frequency, etc., of potential disturbance to a particular habitat.

It is important to carefully consider which tier(s) to examine in each of the phases and when to move from one tier to another. The nature of the problem being investigated and the constraints imposed by costs and resources will largely determine what can and cannot be sampled. Nevertheless, it is crucial to make the decisions using a systematic framework so that the proposed suite of measurements can always be justified in relation to the objectives and purposes of that phase of the study. This is particularly important under the fairly common circumstances where a stress is being examined against a background of other, interactive stresses.

4.2 Establishing and Integrating Field Programmes

Prior to beginning a programme of measurement to determine the state or well-being of a marine ecosystem, it is necessary to understand the constraints imposed by the available monitoring protocols. Ideally, indicators would be included during the planning stages of the monitoring programme, allowing adjustments to be made in the sampling protocols to accommodate the particular needs of the indicator selected. Frequently, however, prior constraints exist because of fiscal limitations with the result that indicators are added to an existing programme and not in a systematic phased approach. In these cases, choices of indicator may be limited. In addition, the goals of the monitoring programme should be well thought out. This can be done by asking questions: For example, is the programme designed to detect change due to any environmental stress or to chemical contamination only? Does the programme intend to diagnose cause or only monitor for effect? and so on.

Following selection of stress responses to be examined and possible indicators, the appropriate ways of getting the measurements should be identified. The specificity or generality of the selected indicators may dictate which species should be utilised. The biomonitoring programme design may also constrain which species can be sampled, forcing some assays to be modified or dropped altogether. In either case, selection of appropriate species depends upon a multiplicity of factors, including geographic distribution, home range, size, trophic level, abundance, and ease of capture.

It is clear that environmental stresses of several kinds may need to be studied simultaneously in any locality or region. In what follows, possible approaches to the measurement of biological effects in the three phases discussed above are illustrated. The reader can first establish which techniques are appropriate to apply and then determine the advantages and limitations of the suggested methods.

4.3 Scales of Effects

In each phase of a study and for every possible measurement of biological effect, great thought will have to be given to the temporal and spatial scales (and other characteristics of an effect; see Table 2.1) that are relevant to that measurement. Otherwise, it will not be possible to design a relevant sampling programme to allow the measurements to be taken with some confidence of detecting the changes or differences that would indicate a stress. For example, the loss of an area of habitat may have limited importance in a regional context. Nevertheless, care must be taken to ascertain whether the rate of loss of that habitat in many local areas is sufficient to cause a potential regional impact. Thus, despite the biological effect being local, there is a need to examine potential larger-scale consequences.

Chronic effects of contaminants or of eutrophication or altered sedimentation require measurement of the levels of variables after the onset of the stress, or the steady rate of change of the variable as the effects of the stress accumulate. Acute scenarios are much more likely to be measurable in the context of "pulse" responses, so attention should be focused on reliable measures of rates of change in the various indices.

The easiest stresses to detect are those that are clearly localised and that have impacts that are quite distinctive from other stresses. These are probably rare. Most stresses act in concert and a large proportion of stresses are diffuse and of uncertain timing and frequency.

Weak, diffuse impacts do not affect populations or assemblages quickly. Sub-organismal measures are the only possibilities of detecting them. For these chronic problems, emphasis must therefore be placed on precise estimation of the magnitude of the chosen indicator(s) relative to undisturbed, background or standard measures.

In contrast, many acute events are a result of accidents (e.g. oil-spills) for which it is difficult to design an advance programme of monitoring and detection. This type of environmental disturbance requires monitoring of very general indicators so that a range of disturbances may be detected. It also requires widespread and frequent sampling. This makes the whole process of sampling difficult and expensive.

Unpredictable episodic events pose particular problems for environmental assessment. Monitoring programmes will not detect sudden events unless they happen to coincide with predetermined times of sampling. Subsequent effects of a disturbance should, however, be detected provided they persist until the next time of sampling, but peaks of concentration or exposure may be missed. Unpredicatable events will usually only be detected in surveillance monitoring if they cause long-term changes.

Probably the only way to determine what happens as a result of episodic disturbances, such as oil-spills, destruction of an area of mangrove forest, accidental discharges of chemicals and so forth, is to use "event-driven" sampling. Thus, sampling for these episodic events must be organised in advance and then implementedas soon as the event occurs. In this way, the effects can be detected and interpreted to develop appropriate managerial responses should future events occur. Such "event-driven" monitoring is often done after floods, fires, earthquakes and oil-spills. The point being made here is that sampling programmes should be designed before the event and modified in the light of specific details of any particular case. This would be a great improvement over *ad hoc* designs, hastily organised in response to a crisis.

It should be noted that many natural episodic events that may lead to large environmental consequences. Flash floods leading to transport of sediment and associated contaminants to coastal areas and storms which cause increased sediment load are two examples. Recent data suggest that 90% of the sediment flux in the North Sea occurs over a few days in response to large storms. In order to assess the effects of such storms, event recorders have been developed which only switch on when the waves are over a specified size. Such advance information from research greatly aids the design of useful monitoring studies.

Spatial scales of environmental disturbances are no simpler (Green, 1979; Underwood, 1992). The crucial factor is to have sufficient understanding of the disturbance and the processes that disseminate or constrain its influence. For example, dredging for the development of a marina may be claimed to be a localised effect and any influence claimed to be of small scale. Yet, dredging may release contaminants bound to fine particles that might wash far and wide, causing large-scale disturbances.

5*

So, unless the spatial scale has been properly defined, monitoring will miss the outcome.

Except for the most widespread disturbances that affect the whole world, or whole ocean basins, all scales of disturbance can be investigated by appropriate definition and measurement. Large-scale stresses will, presumably, cause some stress responses at any hierarchically chosen smaller scale. However, at a small scale the detection of large patterns may be difficult due to the multitude of small scale events that may mask the large scale signal. Detecting large scale stresses may, therefore, require examination of processes at a sufficient scale to filter out local stress responses. A problem is that very large-scale disturbances cannot be detected by any comparisons of disturbed and control or reference locations. The problem of scale and scaling will, therefore, have to be addressed in each case. There may not be a universally correct scale for a particular stress, but there may be scaling laws (Ricklefs, 1990; Levin, 1993). These can be taken into account in developing programs for the detection of stress responses and stresses.

For example, the presence of one dead seal on a shore may be due to a wide range of causes, which cannot easily be ascertained. In contrast, dead seals drifting ashore on a large, regional, scale provide strong evidence that a large-scale stress response has occurred.

As a result, there are only two possible methods for detecting deterioration of the well-being of ecosystems due to very large-scale disturbances. First, is the comparison of temporal trends which, if sufficient data are available from before the disturbance began, will be reliable. The data however, will never provide any directly interpretable indication of the cause of any change, so the existence of a particular disturbance has to be known. Major forcing functions for marine ecosystems are physical structures and processes related to the hydrology and hydrodynamics of the system. Any major changes in these are likely to bring about changes in the ecosystem.

It is sometimes useful to measure the conditions prevailing before, or in the absence of, an impact. This provides a yardstick, or objective for actions taken to rehabilitate the habitat after an impact. The variables investigated must be ones that change as the processes in or structure of the habitat are altered by rehabilitatory action. Only by measuring such variables will it be possible to track the progress towards recovery (i.e. the objective of management in these circumstances) or to determine that rehabilitation is not occurring.

Managerial decisions must, however, also be reached and be reached quickly in poorly investigated systems for which there may be only crude qualitative historical data. The interpretation of variables may differ from system to system. For example, year-class variability in commercially exploited populations close to the distributional limits of a species may be very large. A sequence of poor year-classes provides little information on the state of the system. In contrast, in areas of more regular recruitment, a sequence of poor year-classes may be an important sign of initial degradation.

Practically all systems influenced by human activities will experience local changes. The crucial question is when the local changes should be regarded as symptoms of a general decline of the state of the system. Simply assessing the intensity of the change is not sufficient.

The second method for examining widespread or ecosystem-wide changes is to compare measures of stress against some absolute standard. For example, measures of body-burdens of contaminants are, in undisturbed habitats, supposed to be below some defined concentration. If measured concentrations in samples from widespread parts of a habitat are above this standard, clearly there is a widespread problem.

As with most aspects of environmental measurements, clear thinking about the nature and scales of disturbances and biological responses to them is absolutely crucial. This will apply at each of the three phases of an environmental study, regardless of the choice or variety of indicator used to measure the condition of an environment.

5 Examples Illustrating Procedures for Selecting Indicators

In this Section, four scenarios are taken representing different examples of pollution, (i.e. adverse effects) and/or disturbance, to illustrate the kinds of approaches that may be taken to assess impacts on some marine ecosystems. In each case a specific "concern" is linked with the techniques that may be appropriate. This is followed by a brief discussion of relevant criteria and constraints. For each scenario, a different type of programme of research and measurement is required, with different foci, different techniques, deployed on different spatial and temporal scales and ultimately requiring different responses in terms of managerial action. These illustrations are based on a basic appreciation of the "concern". In reality, simple linearity between each phase is unlikely; "phase 3" activities in one situation may represent "phase 1" under different circumstances, and feedback between monitoring, research and evaluation of the research data is essential. Nevertheless, it is hoped that the following discussion will help focus on the types of action demanded of different situations and illustrate something of the complex interplay necessary between the phases of a problem, the application of the tiered approach to its definition and resolution, and the over-riding need to formulate these actions within the context of hypothesis testing and objective assessment of results.

5.1 Scenarios

Scenario 1.

In situations of "point source" chemical contamination, *phase 1* will comprise the identification of impact on the biota. This may be identified using data from bioassays and about known chemical factors which, by virtue of their bioaccumulation potential, environmental persistence and known biological activity (established by appropriate bioassays) are expected *a priori* to be the cause of the primary concern. Molecular and cellular biomarkers of exposure may be used at this stage to confirm that target species are indeed being exposed, both within the mixing zone *per se* and further afield.

Table 5.1. Matrix 1A. An example of issues and techniques for detecting biological effects of chemical contamination

Concern	Technique
Phase 1: After authorisation of conditions for a point-source discharge, concerns may arise: - are the set conditions being met? - do the expected effects cover a wider area than expected? - are unexpected effects occurring?	- Use of bioassays for toxicity, assays for persistence of the contaminants, etc.
Phase 2: Detailed characterisation of problem: - examination of toxicological data; - analysis of spatial distribution of identified effects; - assessment of other potential effects	- Specific molecular markers of exposure and effect - Scope for growth for key 'target' species - Assemblage analysis
Phase 3: Managerial action: - additional control of effluent and other conditions for continuing the discharge; - new programmes of monitoring	Monitoring using an optimal response index from the phase 2 tiered approach, linked to chemical ("body burden") analysis of a target species, and couched as a null hypothesis of "no effect" within a prescribed region around the discharge.

In *phase 2*, it is the geographical extent of this exposure, beyond the mixing zone, and its biological effects that are the concern. Here, a combination of exposure and effects indices, at the molecular/cell level and above, become appropriate. A tiered approach would be used to explore the effects, if any, at all biological levels, including cellular effects, impacts on individuals (e.g. as impairment of growth and/or reproductive potential) and on structure of assemblages in the area affected. This phase will represent an intensive period of research and application, requiring careful attention to sampling design. In *phase 3,* monitoring to ascertain compliance with legal requirements, combined with monitoring to detect any improvements following remedial action, may be achieved by a combination of chemical analysis linked to sensitive and easily deployed biomarkers of effect. Ideally, at this stage of the study, the legal constraints on environmental damage will have been drawn up with reference to particular biomarkers, which have been proven to be effective during phase 2 and which then comprise the variables to be monitored.

In this scenario careful choice of indices which are capable of providing evidence of chemical effect (i.e. biomarkers of exposure) and measures of effect based on a tiered approach. When these indicators have been sampled at appropriate spatial and temporal scales, they may give direct input to managerial action such as reduction or remediation of the effects of this contamination. Appropriately designed monitoring may also provide the means for "feedback" evaluation of the efficacy of the actions taken.

Scenario 2

Loss of habitat on local and regional scales is undoubtedly the most extensive form of human impact on the marine environment (see Section 2). The primary concern will be to detect the early stages of loss of habitat (as in Table 5.2). Mapping the extent of such losses by ground and aerial surveys and where possible by satellite imagery provides essential data followed by quantitative comparisons with data collected over time.

Table 5.2. Matrix 1B. Example of the concerns and some relevant techniques for examining biological effects associated with the destruction of habitat in a mangrove forest

<u>Concern</u>	<u>Technique</u>
Phase 1: Risk of habitat alteration Areal extent of habitat loss	- Geographical survey of land use change within catchment - Analysis of hydrological regime - Geographical survey of habitat types
Phase 2: Impact on local (non- mangrove) habitats Impact on fisheries	- Structural analysis of benthic assemblages - Stock/yield assessments including survey of spawning and nursery areas
Phase 3: Potential for rehabilitation	- Analysis of nutrient status, trophic structures, carbon flows; modelling

In the case of destruction of mangrove habitat because of forestry, agriculture or aquaculture (i.e. culture ponds) phase 1 will comprise surveys to establish the extent of loss of habitat and changes in patterns of land-use. This would be by airborne remote sensing, with the information compiled in the form of a Geographical Information Systems (GIS). The problem posed in phase 2 may then be to determine the extent to which this loss of habitat is affecting the ecological integrity within the rest of the mangrove and the wider environment. This phase should employ a widely-based benthic assemblage analysis employing uni- and multi-variate statistical analyses techniques, coupled with analysis of fish yields in the various sectors of the fishery (which would include further geographical survey of spawning and nursery areas for the important fish species). The use of biomarkers may be inappropriate in this phase; physiological response indices in key target species, however, may be of use in assessing impacts (e.g. increased sediment run-off, disturbed patterns of primary production) within the wider environment. Finally, phase 3 of this scenario would necessarily draw on knowledge of ecological dynamics within undisturbed mangrove systems to establish parameters for nutrient flux (both within the mangrove system proper and exchange with neighbouring systems), trophic web structure and energy flow, and use this analysis to construct *a priori* expectations on the capacity for recovery. In this situation, a model which formalises an hypothesis concerning the response of the system to modified nutrient and energy flux, might usefully be employed. Once the potential for rehabilitation has been quantified, a tiered approach may be adopted to identify biological effects likely to serve as useful indicators of recovery and a monitoring programme then constructed around these indices.

Scenario 3.

A third example of disturbance is eutrophication caused by excessive nutrients or organic material. The general features of eutrophication are shown in Fig. 5.1 (from Gray, 1992). Eutrophication in the past has often referred solely to the extreme effects of mass growth of opportunistic algae such as in the Lagoon of Venice (Sfriso et al., 1987) or mass moralities such as in Laholm's Bay in Sweden (Baden et al., 1990).

Now however, there are, formal definitions of eutrophication. The European Union definition, (Council of European Communities, 1991) is, " the enrichment of water by nutrients, especially compounds of nitrogen and phosphorus, causing accelerated growth of algae and higher forms of plant life to produce an undesirable disturbance to the balance of organisms present in the water concerned". The primary interest should be in the initial effects of nutrient enrichment. Fig. 5.1 shows the effects that occur during eutrophication. Increases in growth rates of individuals and populations are among the first signs of enrichment by nutrients. Often the total biomass increases such as, for example, the total amount of cholorophyll in the plankton. Groweth rate changes lead to changes in species compositions in both planktonic and benthic assemblages. In particular, benthic macroalgal assemblages often show clear indications of change that can be related to nutrient enrichment. Species compositional changes are best measured by using multivariate statistical analyses (see Section 6.3). Long-term effects such as reductions in distributional depth of macroalgal species have been recorded by comparing data over time (e.g. Kautsky et al., 1986 in the Baltic Sea). Likewise, low oxygen conditions (below 4 mg l^{-1}) which accompany severe eutrophication often lead to behavioural changes, such as fish leaving the area and, should oxygen fall below 2 mg l^{-1}, then sediment-dwelling species leave their burrows (e.g. burrowing decapods) and bivalves gape open. Analyses of assemblages would be expected to record some of these changes as local species extinctions.

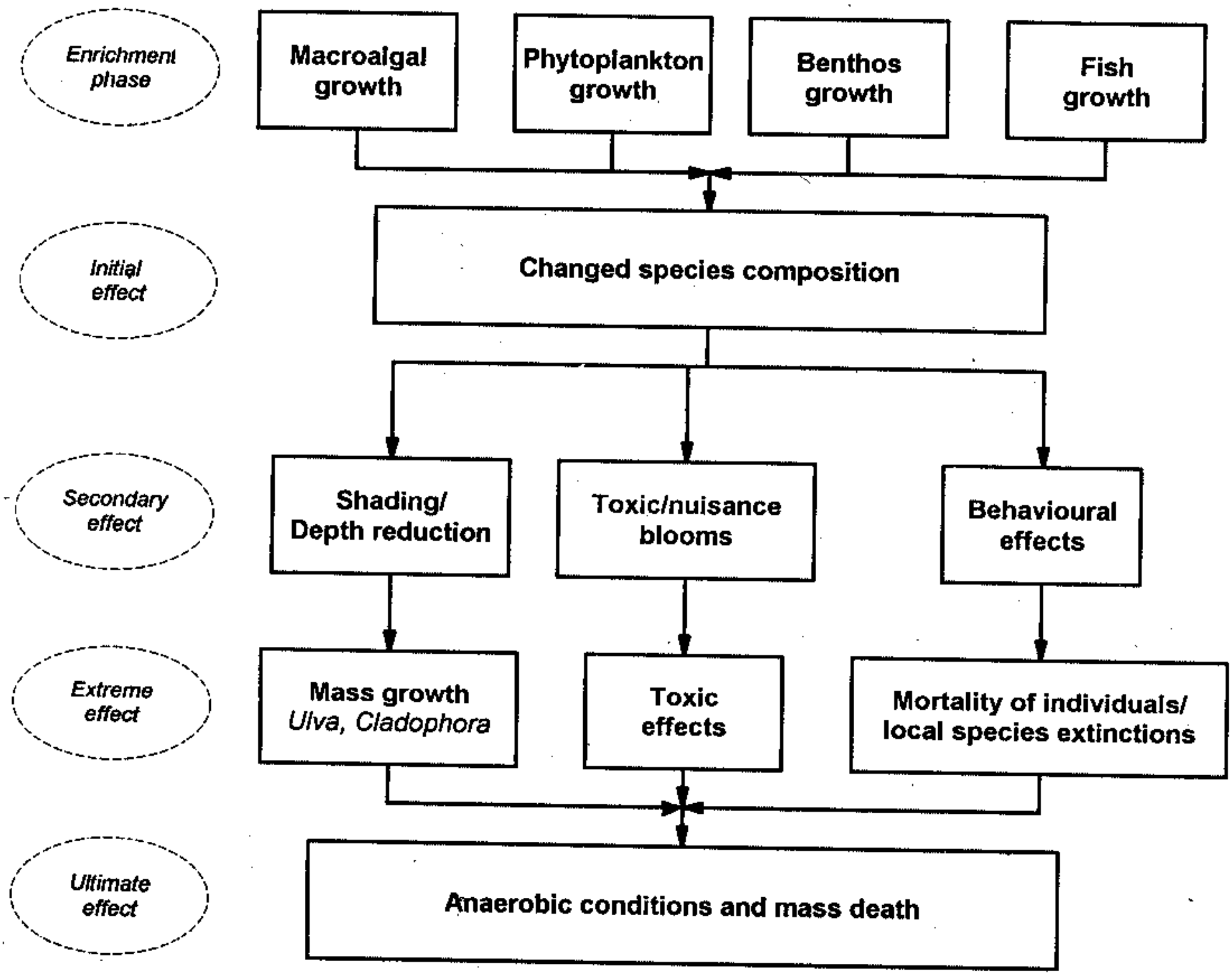

Fig 5.1. General model of effects of eutrophication on marine sysytems (from Gray, 1992)

Table 5.3 shows the approach recommended.

Table 5.3. Matrix 1C. Example of phases, issues and techniques relevant to the study of stresses due to increased nutrient inputs

Concern	Technique
Phase 1: Nutrient enrichment suspected or known	- Measure changes in biomass e.g. chlorophyll (phytoplankton, macroalgae); - Physiological analyses of growth - Assemblage analyses (changed species composition)
Phase 2: Eutrophication	- Measure oxygen concentration - Screen for toxic/nuisance blooms - Physiological analyses of growth - Assemblage analyses (for local species extinctions)
Phase 3: Remediation	- Assemblage analyses (for recolonisation in benthos and changes in plankton assemblage structure) - Physiological analyses (reductions in growth rates)

Scenario 4.

Another example of potential impacts on marine ecosystems on large spatial scales are the effects of UV-B radiation as a consequence of depletion of stratospheric ozone. Estimates of ozone depletion up to 50% during the Antarctic spring and of 3-8% in selected seasons in temperate environments are now widely supported (Solomon, 1990; Stamnes et al., 1992). In the marine environment, the amount of radiation reaching any given depth will depend upon the total amount reaching the surface (largely a function of latitude, season, time of day and cloudiness), the degree of surface roughness (which determines the amount reflected back into space) and the scattering and absorption within the water column. Increased UV-B radiation has been shown to have a variety of deleterious effects on individual marine organisms (Worrest, 1986; Hardy and Gucinski, 1989). In order to assess impacts, new monitoring programs have been proposed to include a wide geographic and species coverage. However, this approach can be both time consuming and expensive. Since the primary UV-B damage induced in biological systems is to DNA, the use of DNA dosimeters (DNA molecules in quartz tubes) offers a sensitive, convenient and relatively inexpensive system (Regan, et al., 1992) for measuring the effects within selected target species. The opportunity exists to establish real cause and effect relationships for this scenario, a situation somewhat unique in global ecotoxicology.

Table 5.4. Matrix 1D. Techniques relevant to studies of the biological effects of increased UV-B radiation

Concern	Technique
Phase 1: Early warning of increased UV-B radiation	DNA dosimetry coupled with photo-optical measurements.
Phase 2: Impacts	Phytoplankton productivity; DNA damage indicators (cytogenetic effects and DNA strand breakage).
Phase 3: Intervention and monitoring	Provide feedback for UV-B modelling and information to strength compliance with the Montreal Convention; Characterise potential interactions with other human perturbations.

5.2 Constraints

All of the techniques mentioned above (see also Section 3), and indeed all techniques currently in use or proposed for evaluating the effects of contamination, have disadvantages as well as advantages. As an example Table 5.5 indicates some technical constraints that may be encountered with techniques that detect change at various levels of biological organisation (molecular: EROD and metallothionein; organismal: Scope for Growth; population: Stocks/Yield analysis; assemblages: structure of benthic assemblages and geographical surveys).

It should be noted that the type of constraint changes with techniques. At the lower tiers of the hierarchy, uncertainties related to the dose-response relationships often dominate. In the higher tiers, the dose-response relationships are multi-factorial and the complex interplay of density-dependent and density-independent controls on the dynamics of populations. The uncertainty of quantitative relationships linking adult fecundity with life-time reproductive success, and the existence of feedback mechanisms regulating population growth, render it difficult to derive precise information, for example on fishery stock yield estimates and the stock/recruitment relationship. Furthermore, analysis of assemblages may be very labour intensive, since it depends on taxonomic identification of species' abundance, often in species-rich assemblages. Recent research, however (Heip, 1988, Warwick, 1988b, 1993), has suggested that resolution of assemblage structure to higher than species levels of taxonomic aggregation (e.g. families, phyla) may ease this problem, but certainly a degree of taxonomic knowledge is required which may render these techniques difficult to deploy in practice. Advanced assemblage analysis requires the use of computers with appropriate software to run the relevant statistical procedures.

Finally, reference is made to the obvious expenses of remote sensing and geographical survey techniques such as GIS and the Continuous Plankton Recorder (CPR), as limiting the application of these techniques in some circumstances.

Table 5.5. Technical constraints

Method	Constraints
EROD	Specific to certain species of fin fish; difficult to establish dose/response.
Metallothionein	Uncertain quantitative relationship to metal exposure; complex interactions between metal species in thionein induction.
Scope for growth	Limited for practical reasons to certain species (sessile benthic invertebrates).
Stock/yield analysis	"Blunt" instrument, lacking precision, due to uncertain relationships for recruitment.
Benthic assemblage analysis	Labour-intensive (sampling, taxonomic analysis); dependent on availability of Personal Computers.
Geographical Surveys	Expensive, requiring relevant computer hardware and GIS software. Best for mapping, but less effective for dynamic representations of change.

Some of these difficulties may be eased by computer modelling. Is has been argued elsewhere that large, all-encompassing models (e.g. ecosystem "simulations") have limited application because of the uncertainties in parameterisation and in interaction terms within the simulations. More focused and "analytical" models, which use few parameters and which are set up to pose specific hypotheses of cause and effect, can help to integrate experimental data and to suggest what measurements are required in each assessment. For example, linked chemical/physiological models may both direct attention towards those compounds most likely to be causing biological damage, and help to simplify the needs of the biological measures necessary to make an objective impact analysis. Modelling applied to cellular biomarkers shows promise for clarifying inter-relationships amongst different processes and target cellular organelles, thereby simplifying measurement needs. Modelling for the purposes of generalising and simplifying the application of response indicators in environmental assessment is currently a topic for research, rather than application, but promise for practical use in the future.

Finally, the point is made here that all appropriate techniques are the subject of on-going research. There is never a time when a procedure can be "frozen" for application. Rather, the power of particular techniques for detecting change will be a function of the extent and efficacy of continuing research. Any application of these procedures is itself a research event. This does not detract from the value of the data to the particular management need, but rather is to be viewed as a contribution to reducing the uncertainty inherent in applying the information. This further reinforces the need for careful design of the sampling/experimental project and for appropriate statistical assessment of the results.

6 Sampling Designs and Hypothesis Testing to Detect Responses to Environmental Stresses

There are two major areas where attention is needed in the design of sampling programmes. First, is the issue of measuring the biological effects against set standards. For example, sampling is done to determine whether the concentration of a heavy metal in fish is above some preset legislated value. Sampling must be designed to be precise enough to allow determination of whether or not the standard has been exceeded. Second is sampling to compare indicators in disturbed or contaminated sites with those in control or reference sites so that differences (or effects) can be detected. Both need careful thought.

Data and therefore statistical analyses that can be used to detect environmental disturbances of anthropogenic origin are either univariate or multivariate. Each type has particular uses and, often, a combination of approaches is appropriate. Univariate analyses are essential when the problem relates to a single measure (e.g. the scope for growth or the relative number of strand breaks in DNA of a particular species) or to some characteristic of only a single species (e.g. the abundance of a particular harvested species). Multivariate analyses are essential when the problem relates to numerous measures (e.g. patterns in the body-burden of several potentially toxic compounds) or to collective properties of assemblages of species (e.g. the similarity of abundances of numerous species in samples from soft-sediments). Sometimes, multivariate analyses become necessary, even though not the first choice, because there is correlation or interdependence among univariate measures, making analyses of single variables less efficient.

In this report, the importance and relevance of formally structured hypotheses is also noted and tests of these hypotheses as important components of environmental work.

6.1 Design of Sampling

Whatever variable (biological indicator) is chosen for measurement, there are important general issues about sampling. The minimal requirement for an ideal sampling and detecting programme is the so-called BACI (Before/After, Control/Impact) design (Green, 1979). Routinely, this includes some sort of repeated measures, hopefully at independently sampled times (Bernstein and Zalinski, 1983, Stewart-Oaten et al., 1986). It is, however, not at all usual to include replicated locations (the 'replicates' are usually within a location at each time). The reason for this is a combination of two things. First, there is usually only a single putatively impacted location, so it is often assumed that only a single, unreplicated control is appropriate. Second is the misplaced belief that temporal trends of mean values of any biological variable should be similar at any two undisturbed locations. That is, it is assumed that processes causing change are invariant in their magnitude and effects among locations, despite abundant evidence that they are not. As a result, it is assumed, illogically, that any difference between the time-courses of biological variables from one control to one putatively impacted location must be due to the disturbance in that location.

Newer designs ('beyond BACI'; Underwood, 1992, 1994) involve an asymmetrical contrast of a single supposed impacted location with a set of control locations. These are potentially much more useful because they can be modified to investigate disturbances that affect temporal and spatial variations and affect mean abundance in various ways (Section 4.3). Effects can be detected at a number of spatial or temporal scales which is very useful when the potential scale of disturbance is unclear. The same methods can easily be adapted to investigate changes and differences in indicators at any tier (in individuals, organs, cells, etc.).

Where there are no 'before' data and the study is designed to investigate potential changes in the condition of a variable, the same principles can be applied (Green, 1993). Investigations based on sampling designs with several spatial and temporal components will be more useful than sets of data from a single location or with inadequate temporal structure. Hierarchical sampling designs are well-suited to investigating a number of scales simultaneously, particularly where there is insufficient information to determine in advance the relevant pattern or scales.

6.2 Univariate Approaches

Univariate measures are those of a single variable, for example, the proportion of animals showing histopathological abnormalities. In what follows, abundance of a population is considered the appropriate variable. This is not restrictive. Most univariate measures (e.g. diversity indices, species number, scope for growth, fecundity, size, metallothionein concentration, mean number of adducts per unit measure of DNA) will have similar problems of measurement of natural, background variation in space and time. Against this background of interactive spatial and temporal variation, human disturbances must be detected, measured and controlled. Sampling designs to detect environmental disturbances must therefore be well-constructed in relation to the life-histories of target organisms and the appropriate spatial and temporal scales over which variations occur.

6.2.1 Relevant Characteristics of Variables that Affect Responses to Disturbances

Mean abundance (and many other characteristics of natural environments), on its own, rarely indicates much, because there is no theoretical, nor empirical, basis from which to estimate what the mean abundance "should" be in any area. Thus, mean abundance (size, etc.) is only useful in a comparative sense and logically, must be used in conjunction with data on temporal changes. Therefore, the first characteristic is that a minimal estimate of natural changes through time must be made before a change in mean abundance could be identifiable that might indicate disturbances.

From this, a second most important characteristic that must be understood and estimated is the temporal variance in mean abundance. This is not usually well-measured (see below), particularly because of apparent obsessions with replication within a sampled area, without proper consideration of temporal replication, relevant time-scales, temporal independence, etc. Note also that some environmental disturbances may affect temporal variability directly, without altering the long-run mean abundance (Underwood, 1991).

Third, because the inverse of temporal variability is some sort of apparent equilibrium, concepts of processes maintaining equilibria (if they exist) have been widely cited. These can be considered as functions, or results of combinations of several properties of populations. Such functions include the inertia of a population, which is its capacity to resist change. Inertia is measured in terms of the size of an external disturbance that elicits a response in terms of changed abundance. Another function is the population's resilience, its capacity to recover from disturbances. Resilience is measured in terms of the greatest change in abundance in response to a disturbance from which the population can still recover. Finally, there is the population's stability, or rate of recovery, if it occurs, measured in some relevant time-scale. The relevance and uses of these terms have been discussed, among others, by Connell and Sousa (1983), Gray and Christie (1983), Holling (1973) and Sutherland (1990). Knowledge of these three characteristics is essential to development of mechanistic understanding of temporal patterns in natural populations and, therefore, for predictions of responses and capacity to recover from disturbances (reviewed by Underwood, 1989).

Fourth, there are characteristics of spatial variability that may be indicative of disturbances or stresses. For example, some schooling fish may re-aggregate when populations are sparse, changing the patterns of spatial variability while mean abundance declines. Without careful examination of spatial variability, at appropriate spatial scales (which may themselves be difficult to determine), some potential signals of stress may be unrecognised (Underwood, 1993a).

The linkages between inertia, resilience and stability are complex, but their value lies in providing a formal focus for planned experimental disturbances and for refining hypotheses to examine when rehabilitation or protection of marine areas is done. An experimental approach to estimating these parameters and for understanding their interactions is necessary (Underwood, 1989). Much of the experimental work may, in a sense, already exist because of previous, multiple disturbances. These could be usefully analysed against appropriate undisturbed 'control' areas. This retrospective type of experiment is vastly under-explored in environmental assessment (Hilborn and Walters, 1981).

Table 6.1. Definitions of terms related to population changes

Resistance	The process operating in an organism, a population or an assemblage so that, when disturbed, no serious stress results. For example, many marine populations are resistant to excessive sedimentation because of increased immigration of dispersive larvae from elsewhere, so that abundances change very little.
Resilience	The property of a population (assemblage or individual) that determines how much stress can be tolerated as a result of a disturbance or perturbation so that the population recovers from the impact. Resilience of any function is measured as the magnitude of deviation of that function from undisturbed levels which still allows recovery to control, undisturbed values.
Inertia	The property of a population (assemblage or individual) that determines whether it shows any response to a disturbance. It is measured as the largest magnitude of disturbance that elicits no response in some specified variable (either because the population really is unaffected by that disturbance or because other processes allow the disturbance to be resisted, so that the chosen variable does not change as a result of disturbance).
Stability	The rate at which a population (assemblage or individual) recovers from the stress caused by some disturbance that is greater than its inertia (so that there is a stress), but within its resilience (so that it can recover). More stable populations recover more quickly.

6.3 Multivariate Approaches

Multivariate sets of data are those with numerous variables measured simultaneously. For example, data on several indicators collected simultaneously at a range of sites. Alternatively, data may be the abundances of all species in an assemblage. Multivariate procedures analyse all of the data together. The general usefulness of multivariate procedures in detection of environmental disturbances was illustrated in an introductory manner by Green (1979). The aim is to detect patterns of difference or similarity among samples containing a variety of organisms, for each of which the density or biomass is estimated. The principles are straightforward, (Field et al. 1982). The relationship between measures of abundance or biomass of species in any two samples is measured by some index of similarity or dissimilarity. All possible pairs of samples therefore produce a matrix of such measures, which can be used to classify samples into groups according to how similar (or dissimilar) they are. There is a large variety of alternative procedures for achieving this (Green, 1980, Mardia et al., 1979, Pielou, 1984, Ter Braak, 1986).

Of the various coefficients of similarity, the Bray and Curtis (1957) similarity index has been found to be very reliable (Faith et al., 1987). It has also been argued that rank ordered similarity is more easily interpreted than any absolute measures (Clarke and Green, 1988, Warwick and Clarke, 1991). This has led to the development of procedures to identify and map relationships among samples using non-metric multi-dimensional scaling (Kruskal and Wish, 1978). This procedure produces a map of the relative closeness or similarity of samples allowing a number of useful procedures to determine whether an environmental disturbance has occurred and, under appropriate conditions, what environmental variables are associated with any patterns in the fauna (Gray et al., 1990).

From these analyses it is possible to determine which species contribute to differences among samples (Field, 1969, Clarke, 1993) and to do statistical tests on formal hypotheses about the differences from control to putatively impacted locations (Clarke and Green, 1988, Gray et al., 1988, reviewed by Clarke, 1993). These tests are, however, limited to relatively simple nested designs of sampling or to the least complex factorial designs (Clarke, 1993).

Recent advances in these procedures have the potential to become very important tools in future assessments of environmental disturbances. There is the possibility of being able to compare the degree of disturbance (or the environmental condition) of various sites, as has recently been done by Warwick and Clarke (1993). The protocol uses the biological reality that different taxa in an assemblage respond to disturbances with differing sensitivities, making multivariate comparisons of assemblages capable of contrasting the resulting disturbances and impacts (Warwick and Clarke, 1991). Taxonomic similarity of geographically distant assemblages is greater at higher taxonomic levels. Thus, Warwick and Clarke (1993) grouped species into large taxa to compare macrobenthic assemblages in several disturbed and undisturbed locations. This produced a gradient of severity of disturbance. Future samples could be contrasted with this gradient to determine how badly disturbed some site is (Warwick, 1993).

There has also been the development of procedures to correlate physico-chemical environmental variables to the patterns in structure of assemblages of organisms. In this, multi-dimensional scaling of physical and chemical data can be compared graphically with that for the fauna. The similarity matrices of the biotic and abiotic data from a set of samples can also be compared to determine which combination of the latter provides the best rank order correlation to the former (Clarke and Ainsworth, 1993). This has been used by Clarke (1993) to examine relationships between physical and chemical variables in sediments and the patterns of disturbance shown by benthic macrofauna around an oil-rig. The potential for such methods is increasing with these new developments.

A final comment is that recent work using a suite of multivariate procedures strongly suggests that marine environmental disturbances can readily be detected without great taxonomic resolution (Ferraro and Cole, 1990; Gray et al., 1990; Herman and Heip, 1988; Warwick, 1988 a,b). This is important for example, for saving time and money on redundant specific identifications. It is also a major breakthrough in solving the dilemma of how to deal with the twin problems of high diversity and the uncertain taxonomic status of species in many contaminated environments. Perhaps some of the emphasis will now be switched away from unnecessary taxonomic rigour to increased numbers of replicated samples? This would vastly increase the power of studies to detect environmental disturbances.

6.4 General Considerations

Three general issues, relevant to the advantages and disadvantages of various analytical procedures, that have to be considered when producing a sampling design are:

(i) How important are estimates and understanding of spatial and temporal variability in the measurements? Where this is important and spatial and temporal variability is complex and/or large, univariate procedures are crucial. They alone are amenable to the complex sampling designs and analytical frameworks appropriate for estimating or testing for significance of complex structured data (e.g. Underwood, 1991).

(ii) How important is it to examine complex sets of variables and co-variables? For example, attempts to choose single (or a few species) in a diverse assemblage to act as indicators or sentinels of environmental change are difficult and controversial (e.g. Underwood and Peterson, 1988). Therefore, univariate approaches are less satisfactory.

(iii) The precision with which correlations must be determined between the data on organisms and on physical and chemical environmental variables. With complex and interactive sets of data, clearer signals will probably be obtained in multivariate analyses (Clarke, 1993).

These considerations must be borne in mind when designing sampling programmes to detect putative environmental disturbances.

6.5 Hypothesis-Testing and Determination of Power of Monitoring and Estimation

One of the key features of environmental assessment, when coupled with determination of the potential disturbances and some estimation of the likely risks, is the capacity to design the sampling programme with sufficient power to detect the anticipated changes if they occur (Fairweather, 1991, Peterman, 1990). Of course, this cannot be done where the types, magnitudes and rates of potential impacts are not known in advance, but even in such a case some general principles will help.

For example, one of the risks to coastal fisheries is the organic enrichment due to constructing fish farms on coastal wetlands. From the literature, it will be possible to estimate (however crudely) the scale of such effects and the concentration or gradient of nutrient-enrichment or contamination from a planned farm. This will determine the boundaries and scale of the potential impact on, say, abundance of some species of amphipod or polychaete that might be used to indicate deleterious effects (i.e. that may predict future changes in abundance of the fished species). Prior knowledge of the biology of the chosen species, or simple guess-work, may provide a frame of reference for determining in advance the appropriate amount of change in the abundance that should be detected. In other words, if a 10 % change in mean abundance of the amphipod would be considered serious and would provide suitable early warning for future changes (perhaps irreversible ones), the study should be designed to be sufficiently powerful to detect a 10 % change. If such a change happens, the farm should be closed down, made to alter its operation, etc., to manage and thereby reduce the stress.

Alternatively, it might be considered, in the light of the total area likely to be affected, that a 50 % change in the numbers of the target species could be accepted recourse to remedial action. Then the study would have to be powerful to detect a change of this magnitude, but would not be concerned about smaller changes.

Armed with this *a priori* estimate of magnitude of change that should be detected, an appropriate sampling design can easily be constructed to compare the potentially affected area with a number of spatially replicated control areas. Or, if there are several farms, to compare several replicated areas with farms with several controls where there are no farms. The intensity of sampling needed to achieve adequate power against the alternative hypothesis (there has been a 10 or 50 % change) can then be determined (Cohen, 1977, Peterman, 1990).

This would save resources. It would also focus attention on the need to try, wherever possible, to contemplate how much change might be tolerated by a natural system being utilised or exploited by man.

It would also lead to considerably improved discussion about the relationship between Type I and Type II error. The former (the probability of detecting apparent change when none has occurred) is usually the basis for planning environmental surveys (the nearly ubiquitous use of $P = 0.05$ demonstrates that). Type II error is the probability of failing to detect a change in abundance when one has occurred. This is far more serious in terms of environmental management and protection. The "precautionary principle" for environmental protection suggests that, wherever possible, errors should be in favour of relatively undisturbed environments (i.e. Type I errors are acceptable, Type II errors are not). It must be remembered that there is a trade-off between Type I and Type II errors, reducing the probability of making a Type I error will increase the risk of making a Type II error and vice-versa.

The power of an experiment or sampling programme is the probability of detecting a real effect of previously determined magnitude, if it occurs (and is therefore 1 - Probability of Type II error). There are several components to the design of a study to ensure that it is sufficiently powerful. The number of replicate samples, the intrinsic variability of the variable being sampled and the choice of probability of Type I error (i.e. $p = 0.05$ or some other value) all determine the power of the experiment. The final ingredient is the hypothesised magnitude of the 'effect' (Winer, 1971, Underwood, 1981, Andrew and Mapstone, 1987). So, in the case of the above example of fish-farms, if the alternative to no change is a 50 % change, the power of the sampling programme will be much larger than if the same study were used in an attempt to detect a 10 % change.

Sometimes, attempts to design a sampling programme powerful enough to detect a possible impact result in a large expense in time and money. This can be very usefully offset by increasing the probability of Type I error, which always increases power. The effect is to be more likely to imagine the existence of an environmental disturbance when none has occurred. This is the right mistake to make in terms of environmental protection and management (Fairweather, 1991). So, more complex designs, if coupled with more thought about the potential risks, the possible sizes of induced changes and the appropriate biology of the system, do not necessarily have to cost more money or take more time.

Finally, "beyond BACI" designs do not necessarily require more effort or resources for sampling. They can probably be efficiently designed by redistributing the sort of sampling intensity done in more traditional frameworks.

7 Managerial Action

It is vital that any programme of monitoring or research be firmly embedded in frameworks of appropriate interpretation and in practices of management, amelioration and rehabilitation. These require several operational considerations.

First, wherever possible, the risks and the nature of likely exposures must be considered in a context of proposing specific hypotheses. Thus, the threat of biological effects due to chemical contamination by disposal of sewage on the coast leads to specific hypotheses about the degree to which certain chosen indicators should be different from those in control or reference sites. The spatial and temporal scales of effects should be assessed before any field programme is implemented. Effort can therefore be directed to specific indices to optimise sampling programmes for the specific purpose of determining whether the hypothesised effect has occurred.

Thus, formal advance consideration of quantitative aspects of the biological effects will greatly simplify the design of sampling, including the power appropriate for the magnitude of potential effect (see Section 6). This "prior work" also allows consideration of possible management if the hypothesised effect is detected. Sensible prior consideration of alternative strategies for management allows the field programme to incorporate assessment of data needed for implementation of appropriate management.

Managerial decisions are not, however, based only on information that is quantifiable in classical statistical terms mentioned above. This means that managerial decisions are subject to a wider set of uncertainties than those normally taken into account in designs for sampling indicators or measuring stress responses. At a managerial level the connections and relationships among biological, technical, socio-economic and cultural information including poorly defined or conflicting societal objectives will have to be evaluated. Thus, a wider range of uncertainties and the need to reduce major uncertainties may affect the choice of indicators and stress responses to be investigated in a programme which is designed to give information on the condition of the marine environment. For example, on their own body burdens of specific contaminants do not provide much information on the state of a system, but can be of concern for the management of risks to human health. Interaction between managerial bodies and scientists is therefore important in phase 1 and phase 2 (the problem identification and characterisation).

The interface between science and management is rapidly evolving and effective communication is essential to improve the use of scientific information in management, to direct the development of scientific methods in areas of primary managerial concern and to make efficient use of new developments in key areas of research.

In practice, this means that there is a need to explicitly formulate the mechanisms underlying the choice of action and the predicted response of the system to managerial action. For example, when decisions are made to introduce more stringent control the decision is based on the hypothesis that this will reduce levels of contamination and some biological responses in the system. This prediction can be tested by directed (experimental hypothesis-testing) research, which is research to evaluate managerial decisions.

More complex cases will require more complex subsequent research. Such scientific evaluation of environmental decision-making is crucial for verification that appropriate responses have been made once an environmental disturbance has led to action. Clearly, research into management must be done when a managerial decision does NOT lead to the predicted response by the biological system. Here, research is urgently needed to determine what went wrong.

Thus, research into the process of management itself is needed (How were decisions made? Were data misinterpreted? How did the underlying models get chosen?) (see also Walters, 1986). This has not been a traditional area of research because many managerial decisions about environmental disturbances have not been evaluated (Buckley, 1991). Gathering good data and using well-tested and appropriate methods will not help to improve the condition of the marine environment if they cannot be used in making effective decisions.

8 Implementation

As presented in this document, the proposed strategy for the evaluation of marine ecosystem condition, with its suites of biomarkers and other indicators of stress, is ready for implementation. An ideal candidate site for this purpose is Southeast Asia. There are many compelling reasons for such a choice. The region is believed to lie at the centre of global biodiversity for species inhabiting coral reefs, mangroves, sea grass and sedimentary habitats. At the same time, such an immense concentration of living resources is being threatened by the tremendous growth of human populations, with its accompanying impacts on the marine environment from socio-economic activities. It is a rapidly industrialising region, and a strong impetus for this is the prospect of the existence of large oil reserves in the South China Sea area. The region's waters are also important shipping lanes for the transport of goods between Europe, the Middle East and Japan.

The South China Sea is a focal point for research on large marine ecosystems (LME's, Sherman, 1993). The study of the condition of marine ecosystems can, therefore, be conducted on a number of scales, from the very localised (individual habitats within countries) to the context of the entire sea.

Among the Association of South East Asian Nations (ASEAN), (Brunei Darussalam, Indonesia, Malaysia, the Philippines, Singapore and Thailand), a relatively well established scientific infrastructure already exists. The potentials of other countries in the region such as Vietnam and Cambodia remain to be tapped. Thus, a major goal of a program on assessing the condition of marine ecosystems would be capacity building where needed. In addition to a number of other international and regional organisations the ASEAN region is a focus of interest and activity by the International Geosphere-Biosphere Programme (and in particular the Land Ocean Interactions in the Coastal Zone or LOICZ programme), the Regional Seas Programme of UNEP and the World Bank's Global Environmental Facility (GEF).

Regions in other parts of the world should be considered for purposes of implementing the proposed strategy for evaluating the condition of marine ecosystems, provided that such regions possess and characteristics of global significance and the results of the monitoring programmes, when applied, have universal application.

9 Capacity-building and Training Needs

There will be many needs for increased availability of training and technology transfer about environmental methods. Many of the techniques used are novel and still being developed. Some of them are complex. The inherent variability of many biological measures requires more attention to the complexities of appropriate sampling and experimental designs than has usually been the case.

It is therefore recommended that training programmes be organised through the relevant UN organisations, such as the IOC/UNEP/IMO GIPME programme, in the areas of specific techniques, general aspects of experimental design and statistical analysis of data, general procedures for decision making about appropriate choices of indicators of environmental stress and about adaptive management of research programmes in environmental investigation.

There will need to be a variety of approaches, including "specific-theme" workshops, consultancies by relevant experts and establishment of centres for formal training and less formal consultative networks of expertise. Unless these initiatives are properly established and funded, the prospects will continue to be bleak for better future detection, prevention and remediation of marine environmental disturbances.

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