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- GESAMP -

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No. 12

Monitoring biological variables related to marine pollution

United Nations Educational, Scientific and Cultural Organization

IMCO/FAO/UNESCO/WMO/WHO/IAEA/UN/UNEP Joint Group of Experts
on the Scientific Aspects of Marine Pollution (GESAMP)

MONITORING BIOLOGICAL VARIABLES RELATED TO MARINE POLLUTION

Unesco, 1980

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* For the period 1977-1978.

NOTES

1. GESAMP is an advisory body consisting of specialized experts nominated by the Sponsoring Agencies (IMCO, FAO, UNESCO, WMO, WHO, IAEA, UN, UNEP). Its principal task is to provide scientific advice on marine pollution problems to the Sponsoring Agencies and to the Intergovernmental Oceanographic Commission (IOC).

2. This report is available in English, French, Russian and Spanish from any of the Sponsoring Agencies.

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The training aspect

In most of the plans referred to above there is emphasis on the need for communication and training. The Working Group recognized a similar need in the field of monitoring biological variables. This is obvious in relation to the more sophisticated techniques when instruction in the use of instruments and in the interpretation of results is clearly desirable. But even for the more straightforward methods, when a routine and uniform application is required by different personnel from various laboratories over a wide area, some attempt at intercalibration is essential.

There is considerable value in making available manuals of instruction that provide detailed step-by-step guidance on procedures. It should be possible to collect methods in the form of a "cook book".

The most useful instruction, however, is likely to be in the participants' own laboratories and on board their own research ships, and with this in mind, selected experts might be encouraged to visit regional centres, where programmes are being executed or planned, to give advice and instruction. An extension of this might be a series of lectures, courses or workshops designed to focus on important aspects of biological monitoring, arranged for appropriate groups of scientists and administrators. Finally, the funding of selected scientists to study at suitable centres for limited periods could provide direct supervised experience of some of the more sophisticated techniques.

SUMMARY AND RECOMMENDATIONS

The Working Group studied the problems of measuring biological variables related to marine pollution, and concluded that the case was established for incorporating such measurements in monitoring programmes.

In discussing the scientific requirements for biological monitoring, the Working Group proposed a set of principles for selecting suitable variables. It then evaluated a selection of possible variables in the light of the principles, and listed measurements that could be recommended for immediate inclusion in monitoring programmes. The list included certain biochemical and physiological procedures, as well as several morphological, population and community measurements.

In addition to these procedures and measurements, which are sufficiently well developed for immediate use as monitoring tools, there are other approaches that show promise, but require further study. It is recommended that countries with well developed research organizations take the lead in such development.

The Working Group noted the lack of any general framework for applying biology to monitoring programmes. It therefore developed a three-part strategy for dealing with the three phases: identification, quantification and causation; and it provided guidelines for implementing it. The strategy recognizes that appropriate chemical analysis is always required and that the biological input is most effectively deployed in a suite of procedures carefully tailored to the requirements of individual programmes.

The Working Group studied several current projects and, for the most advanced of these, suggested some additional biological measurements. It is recommended that concerted efforts be made to apply the strategy in existing local and regional programmes and to build it into new programmes at the planning stage. Existing methods should be used since significant advances are more likely to follow at present from an evaluation of practical experience than from new theoretical or laboratory exercises. After the strategy has been pursued for some time, it should be reviewed and modified as necessary.

On the subject of open-ocean monitoring, the Working Group recognized substantial problems at present. It suggested that open-ocean biological monitoring might be most usefully focused on the benthos at a few sites specially selected for their stability and where the benefits of other, relevant, ongoing research would be available.

Finally, the need for training in some of the biological techniques was discussed. It was proposed that this be done by visits from experts, by lectures and courses, and by periods of training at selected centres of expertise. The value of compiling a manual of methods was noted.

INTRODUCTION

The terms of reference of the Working Group were:

(i) "To formulate a rationale for monitoring biological variables taking into account existing and planned regional and global monitoring programmes, paying particular attention to those which include monitoring of biological variables.

(ii) To determine the scientific requirements for monitoring relevant biological variables.

(iii) To assess the feasibility of establishing practical procedures for the monitoring of biological variables related to marine pollution". (GESAMP Reports and Studies No.8).

The membership of the Working Group is given in Annex I. The Group met in December 1977, May 1978 and June 1979, after which a small drafting group assembled the Final Report.

None of these six approaches could be highly recommended, according to our scoring, for general use in routine monitoring programmes at present because: (i) they lack an applied quantitative relationship with pollution; (ii) they have not been tested and evaluated sufficiently in a field programme; and (iii) the two specific biochemical effects have a low signal-to-noise ratio and can only be applied to a limited number of species (e.g., amino-levulinic acid dehydratase is specific to lead pollution and is restricted to organisms with haemoglobin; acetylcholinesterase in the brain is specific to organo-phosphate pollution in fish and crustacea). In spite of such comments, the fact that these approaches may be found useful at specific sites is perhaps a useful reminder that generalizations should always be treated with understanding and caution.

If the working groups were to make any additional proposals for inclusion in the Mediterranean programme they would be as follows:

(i) bioassay for general water-quality assessment;

(ii) assessment of vertebral damage, asymmetry, fin erosion, ulcers, neoplasia/tumours and liver/digestive gland and kidney structure alteration;

(iii) measurement of feeding rate, scope for growth, oxygen-to-nitrogen ratio and body-condition indices;

(iv) measurement of taurine-to-glycine ratio, lysosomal stability and metallothionein.

Furthermore, it would be suggested that whenever possible the majority of these biological effects should be measured on the same indicator species or sentinel organism used for chemical minitoring; e.g., the mussel, Mytilus galloprovincialis, the deep-water pink shrimp, Parapenaeus longirostris, the striped mullet, mullus barbatus, and the bluefin tuna, Thunnus thynnus. An integrated programme that examines chemical effects combined with general and contaminant-specific biological effects should provide information on which to base environmental management decisions.

The Working Group then reviewed other United Nations activities, noting the following initiatives:

1. UNEP/Kuwait Action Plan.

2. IOC Programme for the Western Pacific (WESTPAC).

3. UNEP/ECLA Caribbean Environment Programme.

4. UNEP Action Plan for the Gulf of Guinea and adjacent areas.

5. CPPS Action Plan for the Southeast Pacific.

6. IOC Co-operative Investigation in the North and Central Western Indian Ocean (CINCWIO).

7. WHO/UNEP Pilot Project on Coastal Water Quality Control (MED-VII) in the Mediterranean.

The Working Group's Terms of Reference would clearly have permitted it to make a detailed examination of those plans in an effort to produce explicit biological monitoring programmes for each. Unfortunately, time was less permissive. Our hope, however, is that the guidelines set out in this report will be of use to those involved in further elaborating and carrying out the plans.

In setting up the strategy and the method for implementing it, the Working Group attempted to maintain a flexible approach that could be readily adopted in programmes already under way or at an advanced stage of planning. There are many such programmes, and they are run by local authorities, national governments, international pollution commissions, scientific bodies (e.g., ICES) or by the United Nations agencies.

These programmes are for the most part focused on local situations as already described (outfalls, dumping grounds) where the most obvious biological monitoring component may have been readily identified and used. But there is an increasing number of programmes concerned with environmental contamination on larger scales (regional or global) that attempt to encompass all contaminants from whatever source that are likely to affect environmental quality.

The "Ocean Pulse" programme off the east coast of the United States of America is an example of a comprehensive project. It is designed to provide, among other things, monitoring data on the continental shelf waters between Cape Hatteras and the Canadian border. The very diverse programme includes the monitoring of several biological variables such as mutagenetic analysis of fish eggs and larvae, structural analysis of benthic communities, and biochemical/physiological measurements on blood and tissues of various invertebrates and fish.

In general, however, regional monitoring programmes in the past have, quite properly in view of the earlier state of the art, focused on chemistry, and the biological aspects are either lacking or are at an experimental level. The Working Group paid considerable attention to the United Nations agency programmes, and examined the reports of several workshops and projects published up to the beginning of 1979. The most advanced of these was the UNEP Co-ordinated Mediterranean Pollution Monitoring and Research Programme (MEDPOL). Two Pilot Projects, one on the effects of pollutants on marine organisms (MED IV), and another on the effects of pollutants on populations and communities (MED-V) were of particular interest.

In these two projects, most of the biological effects measured fell into the general categories listed in Table 1, and several were in our "highly recommended" groups; e.g., bioassays and community approaches. There were a few effects we had not listed, however, and we scored these against our criteria and arrived at the following evaluations:

		Evaluation
Biochemical effects		
	Amino-levulinic acid dehydratase	3
	Protein synthesis (regenerating sponges)	3
	Glycolytic and citric acid cycle enzymes	3
	Acetylcholinesterase in brain	3
Behavioural effects		
	Righting behaviour in *Paracentrotus lividus*	2
Ecological effects		
	Colonization by fouling communities	3

In most monitoring programmes, contamination is measured in terms of chemistry, and chemical analysis of material from the various compartments of the environment can provide a sensitive indication of the concentrations of substances that have been selected for study. But pollution, as defined by GESAMP, implies deleterious effects and these are usually assessed in relation to a biological system. In pollution monitoring, therefore, biological information is required at some stage, but there is considerable scope for discussion as to what sort of biology is most appropriate, and at what stage in a monitoring programme it may be most effectively applied. Those who manage and control resources have tended to avoid the wide use of biological parameters because of the high degree of variability in natural systems, and the complexity of an organism's reaction to stress from many physical and chemical factors. Rather, they have preferred the use of chemical analyses as a routine procedure, and biological criteria have been introduced only at the evaluation stage.

However, reliance on chemical analyses alone for studies of biological effects has serious shortcomings. Thus slight and, in analytical chemical terms, insignificant changes in the concentrations of certain chemicals can markedly affect water quality from the biological standpoint. Also, chemicals which by themselves would have been innocuous, may cause effects by interacting in the general milieu of contaminated waters. Further, chemicals whose identity is unknown or whose presence is not even suspected may produce effects. Finally, without observations linking levels in the water or sediment with tissue concentrations and then with effects on organisms and populations, and ultimately, with the well-being of the ecosystem as a whole, an adequate assessment of pollution is impossible.

Clearly, then, there is a case for using biological variables to monitor the effects of pollution. However, it should be emphasized that the optimum monitoring programme will include physical, chemical and biological observations in the field, and, moreover, that these observations should be backed up by appropriate experimental work.

THE USE OF BIOLOGICAL VARIABLES

Previous work

The use of biological variables in the context of monitoring for marine pollution has already been the topic of considerable discussion in GESAMP. At the sixth session of GESAMP, a panel, chaired by Dr. Stjepan Keckes, was set up to specify parameters relating to effects monitoring, and its report (IMCO et al., 1974) recognized the problem of defining a clear cause-effect relationship between changes observed in marine communities and specific contaminants. It emphasized the value of experimental studies, in the laboratory and in situ, for establishing links between the body burden of contaminants in organisms and levels of these contaminants in the sea water. Regarding laboratory tests, it discussed the use of indicator organisms and experimental ecosystems, and noted the importance of determining the point at which effects on individual organisms manifest themselves as harmful at the population level. Regarding field studies, it pointed out that bio-accumulating organisms, such as mussels and barnacles, or communities on fouling plates, could be transported to ocean study areas where they are not indigenous. It concluded by recommending a number of monitoring exercises, including observations on phytoplankton communities, fish populations, and open-ocean island systems.

Three FAO fisheries technical papers are particularly relevant. One (FAO, 1976 a) reviews the use of marine organisms as accumulators of pollutants, evaluates their role, and provides guidance on their selection and utilization in relation to the main pollutants.

The second (FAO, 1976 b) is concerned with devising a system of indices to measure effects of stress, due to human activities, on aquatic resources. It reviews appropriate indices and their advantages, disadvantages and constraints. While it deals with population, community and ecosystem levels, it concentrates on community indices and specifically excludes bioassay and toxicity studies. It recommends the establishment of an international network of scientists to produce, in about five years, a synthesis in the field of utilization of indices. Specifically, it suggests setting up two multidisciplinary panels whose deliberations would lead to an international symposium. It notes the need for long-term, baseline studies.

The third (FAO, 1977) deals with the bio-evaluation of marine pollutants and concentrates on toxic chemicals rather than organic wastes causing oxygen depletion. It refers to the types of laboratory procedures and the types of organisms most likely to be useful in reinforcing ecological studies of marine pollution effects.

Two recent reports focus on field situations. One (NERC, 1976) is particularly concerned with biological change, and for the marine environment It lists and evaluates some time-series records available for plankton, fish and benthos, and discusses the organization of surveillance programmes.

The other (Unesco, 1973) concentrates on the continuous measurement of biological variables and is not directed particularly towards pollution, but most of the variables it discusses are relevant to pollution monitoring. The report deals with monitoring strategy and statistical design problems, and its major section, on the technical feasibility of individual methods, constitutes a main part of the recommendations. It stresses the importance of monitoring natural variability and calls for work on assembling, processing, indexing and assessing time-series data from the ocean. The setting up of several working groups and review bodies is recommended.

We paid particular attention to this, and Dr. Longhurst, who had been Chairman of the Working Group, reviewed its conclusions in the light of current conditions. He suggested that substantial progress had been made in the development of biological survey equipment since the report's publication, and that the means needed were now to hand or could be readily developed.

A research programme in the United States of America sponsored by the National Science Foundation (the Manganese Nodule Project) has been developing instrument platforms to perform long-term (up to 1 year) observations on the deep sea-floor. The instrumentation is primarily designed to carry out measurements of geochemical fluxes at the water-sediment boundary, but also includes sediment traps, current meters, light-scattering meters and cameras for time-lapse photography of the sea-floor. This programme and others designed to examine the problems of deep-ocean dumping suggest that the techniques required for examining the deep benthos mentioned above may soon be available.

Geographical scales

Application of the strategy will vary, depending on the geographical scale, and in general three ranges may usefully be recognized. First, at the local level, one can think in terms of point-source pollution from individual discharges or of localized hot-spots on dumping grounds or around industrial terminals, where the details of the input may be known and the strategy may therefore be more finely focused. The Working Group discussed this aspect, but in view of its terms of reference, concentrated on the second level: the regional, which will encompass the sum of the effects of the local-scale pollution and include diffuse inputs from land and river run-off and from the atmosphere.

At the third level, the global scale, the Working Group concluded that there was sufficient reason to modify its strategy with respect to open oceans.

There is increasing evidence that the division between estuarine and shelf waters, on the one hand, and the open oceans, on the other, is surprisingly firm in that "leakage" of pollutants between the two is slight. Since most of the inputs of contaminants are to shelf waters (by main rivers, land run-off, direct discharge), it is to be expected that biological effects will occur in these regions more quickly than in the open oceans. Accordingly, the greater proportion of monitoring effort should be concentrated on these shelf waters.

In the open oceans the main input of pollutants is from the atmosphere, but the relatively short residence time of pollutants in the surface waters (≤ 5 years) is much less than can be accounted for by the average mixing times (<20 years) of the surface waters with the deeper water. It appears that pollutants, on entering the aquatic phase, rapidly become associated, actively and passively, with detrital particulate material of relatively large size (≥ 50 um). These particles, almost entirely derived from the biological cycle in the surface waters, are continuously leaving the surface water reservoir and may reach great depths almost intact within days or weeks. There they accumulate as a superficial detrital layer on the sediment.

It is this same recent allochthonous detrital material that contributes 15-30 per cent of the required organic carbon input to the deep benthic community as a whole, and for some species, which scavenge selectively as epibenthos, it constitutes their primary energy supply. These species, in particular, are likely to be highly susceptible to the quality and quantity of the settling material from the surface water.

It appears that this deep benthic fauna may be the most suitable component for monitoring the open oceans. Monitoring phytoplankton and zooplankton in the surface waters is technically feasible, and remote sensing devices will play an increasingly important role, but at present the factors controlling spatial variability, especially the influence of rings and fronts, are so poorly understood as to argue against the use of the plankters. The technical problems of examining the deep benthos demand complex and sophisticated sampling methods. Further, the spatial heterogeneity of the deep benthic communities is largely unknown, and together these factors argue against widespread benthic surveys. Indeed, such a strategy would make sense, given the relative exposure of deep and shelf benthos, only if the deep benthos were to be much more susceptible to pollutants.

The Working Group therefore recommends that if open-ocean monitoring is to be included, it should at present be focused on the examination of deep benthos and be confined to a few selected sites preferably covering each major ocean, and located where physical conditions and the ocean floor are relatively stable - so-called "quiet" areas - and where synoptic observations on inputs to the detritus are being made. At these sites, sensitive physiological indices such as scope for growth may be apposite.

Finally, the feasibility of using biological effects in monitoring exercises was examined by a study group set up by the International Council for the Exploration of the Sea. Its report (ICES, 1978) briefly reviews recent knowledge of the effects of pollutants on marine organisms and how such effects can be demonstrated and measured experimentally and detected and evaluated in the field. It focuses intentionally on shelf regions of industrialized countries, and explicitly omits consideration of open seas because of the low levels of contaminants and the apparent uniformity of these areas. Further, while recognizing that what matters in the long-term are effects on the population and the community, the ICES report concentrates on the individual organism and on lower levels of biological organization as a means of approaching higher levels and of obtaining earlier recognition of effects. It makes general suggestions about how such work could be fitted into certain types of ongoing programmes, but it does not attempt to advise on specific input to selected programmes in named geographical areas. Finally, it recommended the convening of a small international workshop of specialists to expand its conclusions. This Workshop on Pollution Effects Monitoring met in the United States of America in early spring 1979. It divided into seven panels, dealing with biochemistry, physiology, pathobiology, behaviour, genetics, ecology and bioassay. Each panel recommended a number of procedures considered suitable for inclusion in programmes of biological monitoring. The published proceedings of the Workshop (McIntyre and Whittle, 1980) provide extensive references to the literature on biological effects monitoring.

Discussion

To ensure that any GESAMP initiative did not repeat or significantly overlap earlier work, the Working Group carefully reviewed the publications referred to above, which, taken together, provide a substantial commentary on biological variability and on the problems this causes in monitoring.

The group concluded from these documents and from other material at its disposal that much of the earlier argument against adding a biological component to monitoring programmes was no longer tenable and that in general it is no more difficult to mount a biological programme than a chemical one. However, a consistent strategy for monitoring biological effects has not been developed, and the procedures for implementing such a strategy have not so far been clearly set out together in a unified account. Further, there has been little integration of papers dealing with such aspects as principles, methods and strategies. In spite of these deficiencies, the fundamental principles for biological monitoring have been fully explored before, and it is unlikely that any major new insight will be forthcoming in the immediate future. On the other hand, recent advances in science particularly concerned with biochemical and physiological responses within organisms to sublethal stress, have introduced significant new techniques which are in some cases now ready to be applied in monitoring programmes. The application of these will help to avoid problems inherent in the more classical population approach to monitoring caused by natural variability in population abundance in space and time.

It is noteworthy that several of the reports referred to above call for specific initiatives and it is not clear why some of these have not been followed up. The need would therefore appear to be not for further basic discussion but rather for specific recommendations followed by immediate action.

The Working Group felt, therefore, that it could contribute most usefully by

(i) establishing a set of criteria for evaluating the usefulness of particular biological variables in pollution monitoring;

(ii) evaluating a wide range of biological variables according to this set of criteria;

(iii) developing a strategy for biological monitoring based on, and incorporating, appropriate techniques identified by the evaluation process.

THE SCIENTIFIC REQUIREMENTS FOR BIOLOGICAL MONITORING

Having established that there is a need for including biological variables in marine pollution monitoring programmes, it is necessary to consider the selection of suitable variables and the context in which measurements of them may be applied.

Principles for selecting variables

Not all biological variables will be equally appropriate for inclusion in pollution monitoring programmes, and their suitability for inclusion may be assessed in terms of a number of criteria. Some of these criteria (listed as category A below) refer to the fundamental scientific aspects of assessing the biological impact of an environmental change. Other criteria (category B) refer to the efficiency of the biological measurements and to their practical value as indices of impact. A third group of criteria (category C) is related to administrative questions which may be important in selecting indices for inclusion in monitoring programmes. In the following list the categories are not ranked in order of priority, and no attempt is made to provide a quantitative evaluation of the criteria.

A

1. Ecological significance: can the effect be shown, or convincingly argued, to be related to an adverse or damaging effect on the growth, reproduction or survival of the individual or the population, and ultimately on the well-being of the community/ecosystem?
2. Relevance to other effects: can the effect be related to other effects at higher or lower levels or organization?
3. Specificity: how specific is the effect in relation to the causative agent?
4. Reversibility: to what degree can the variable return to its original level when the causative agent is removed?
5. Range of taxa: is the effect specific to particular taxa?

This criterion may be relevant also to categories B and C.

B

1. Quantitative aspects: does the effect bear a quantitative or predictable relationship to the cause (i.e., pollution)?
2. Sensitivity: what intensity of stressor is required to elicit the effect?
3. Scope: over what range of intensity of stressor is the effect observable?
4. Response rate: how quickly is there an observable effect? (hours, days, years)?
5. Signal noise ratio: can the effect (signal) be easily detected above the natural variability (noise)?
6. Precision: can the effect be measured accurately and precisely?

C

1. Cost: how expensive is the measurement of the variable in terms of capital equipment, running costs, training costs and manpower?
2. Application: to what extent has the effect been used in a field monitoring programme and shown to be related to pollution?

Effects measurements at any one level of biological organization will not score high by all the above-mentioned criteria because some, such as "ecological significance" and "sensitivity"/"specificity" may be inversely related over the range of

TABLE 2

ELEMENTS IN STUDYING THE DISTRIBUTION OF BIOLOGICAL EFFECTS

Phase I	Identification:	Distribution of known inputs: - chemical analyses of water, sediment and biota; - selected bioassay of organisms (e.g., estimates of lysosomal fragility); - elevated incidence of morphological/pathological abnormalities in fish populations; - bioassay of surface/deep water with oyster/echinoderm larvae, hydroids.
Phase II	Quantification:	To be implemented on detection of spatial variability of any phase I determinations. In order: - survey of benthic community structure; - survey of benthic population parameters; - physiological indices (e.g., scope for growth) in selected widespread species (e.g., *Crassostrea*, *Mytilus*); - biochemical indices in above-mentioned species.
Phase III	Causation:	To be implemented on confirmation of significant effect assessed by the above-mentioned procedures: - by specific chemical analyses of water, sediment, biota for suspected contaminants; - bioassay, with specific chemical modifications to water samples. - biochemical techniques specific to chemicals or chemical classes.

Temporal aspects. Study of changes in intensity with time is concerned with establishing trends. This can be done: (i) in conjunction with a spatial mapping exercise to demonstrate changes in geographical distribution, one spatial survey identifying the distribution being repeated on an appropriate time sequence; or (ii) by repeated observations at the same site to demonstrate station-specific trends. The approach can clearly be applied over various time scales, depending on the nature of the contaminant inputs.

The strategy for determining temporal changes is similar to that for spatial changes as set out in Table 2, except that the objectives become the identification of changing inputs, changing levels of chemicals in the environment, changing incidences of morphological modifications in fish, and changing size and frequency of hot-spots. The use of bioassays provides a good basis for measuring changes in water quality, as long as a suitable baseline such as synthetic sea water can be established. In such work, variations in water quality due to seasonal influence, blooms, changes in coastal run-off, etc., must be taken into account. An adequate trend-monitoring study will require a suite of biological procedures covering, in particular, sensitivity, causation and ecological relevance, drawn from the possibilities listed under phases II and III of the spatial study.

Biological variables in phase I must be sensitive and precise. That is, they should be responsive to very slight changes in the chemical composition of their environment but, at the same time, they should be capable of precise measurement and discrimination against normal variability - they should have a high "signal to noise" ratio. Also, to be useful on a wide scale, they should be cheap and generally applicable. The incidence of abnormal morphological and pathobiological conditions in fish, as defined earlier, appears to be highly suitable for broad-scale discrimination and over long time periods. Bioassays of sea water samples, although probably subject to greater temporal variability, fulfil all other requirements, and resolution can be increased merely by increasing the intensity of the sampling grid. Observations on lysosomal stability of the bioassay organisms can also be a useful additional technique.

Phase II. The demonstration of a "hot-spot" by the above-mentioned procedures does not of itself indicate biological damage. Confirmation and quantification is then required, and this constitutes phase II, which relies on the examination of more ecologically significant variables. Since one purpose of biological monitoring is to ascertain the health of the ecosystem, gross measurements at community level are important. This approach would have been advocated in phase I but for its relatively high cost, for its insensitivity, and for the problems of interpretation. The Working Group endorsed the conclusion reached by the Ecological Panel of the ICES Workshop on Pollution Effects Monitoring, that analysis of the benthic and epibenthic communities, including littoral communities, is likely to be more cost-effective than plankton work. As indicated earlier, however, the case for detailed analysis of benthic communities in a monitoring role is not yet proven, and the Working Group recommends that only gross measures such as total abundance, total biomass, etc., be done initially. To obtain increased sensitivity, it is recommended that general physiological measurements, such as scope for growth and biochemical measurements, be made.

Phase III. At this stage it may be possible to ascribe the causes (where these are not already known) of any effects using circumstantial evidence, and this can be enhanced by increased chemical sampling, with specific analyses using advanced techniques. It may be noted that phase III requires a rather different type of chemistry from that likely to be used in phase I. The later phases are concerned with quantification and with understanding dose/response relationships so that knowledge of the speciation of chemicals and of their partition in different biological compartments is needed. Bioassays on seawater samples modified in specific ways to alter their chemical quality is a relevant biological approach, and it is at this stage that the extensive use of laboratory experiments on the effects of substances (i.e., retrospective testing) becomes particularly useful.

For a limited number of substances, or types of substances, specific biological effects can be sought, but it should be noted that effects will often be produced by the interactions of numerous contaminants rather than by single substances acting alone.

The essential aspects of the strategy are set out in Table 2.

organizational levels. For example, effects at higher levels of organization (community, population) are generally more significant ecologically but relatively insensitive and non-specific, in contrast to changes at lower levels of organization (cellular and molecular) which are usually more sensitive and specific but less significant ecologically. In any biological effects monitoring programme there may be a requirement for highly specific or for non-specific biological effects measurements, or both. Each biological variable selected will then be either: (i) a general (non-specific) stress response to the total environmental stimulus - because pollution is often not due to a specific causative agent but rather to a complex combination of factors acting in concert with natural environmental variables, or (ii) a specific (selective) response to a particular class of contaminants chosen in an attempt to establish a cause-effect relationship.

Clearly, to meet both basic requirements (i.e., generality and specificity), it is necessary to include biological effects at several levels of organization.

Evaluation of selected biological variables

There are many variables that could be evaluated by the criteria set out above, but the Working Group agreed to focus on those that had already been subjected to some systematic examination. Among these were the variables referred to by the seven panels at the ICES Workshop on Pollution Effects Monitoring. We listed 37 variables (Table 1) and assessed them according to the 13 criteria. Each variable was discussed at length, then allocated a score, on a three-point scale, based on its assessment according to the criteria. After consideration of the scores, each variable was given a general evaluation (to some extent on a subjective basis since it proved difficult to apply weightings to the different scores and criteria) and categorized 1, 2 or 3 as follows:

1. Highly recommended for immediate use in monitoring programmes in all regions; or

2. Recommended only for selective use because it was more costly or required further field testing before it could be used routinely; or

3. Potentially useful but not recommended at present because the approach and techniques required further development.

A biological effect highly recommended for immediate use may not be entirely free of problems in its routine monitoring. An investigator must be aware of the limitations and the necessary scientific considerations (e.g., statistical requirements, importance of endogenous and exogenous factors) associated with each effect measurement before planning and implementing biological monitoring programmes. These detailed scientific considerations were discussed at the ICES Workshop on Pollution Effects Monitoring (McIntyre and Whittle 1980) and are dealt with in a practical manual in preparation at the Institute of Marine Environmental Research (Plymouth, United Kingdom).

The evaluations of 37 biological variables are summarized in Tables 1a and 1b. The major advantages and disadvantages of each are briefly outlined below. A more detailed review is given by the reports of the seven panels of the ICES Workshop on Pollution Effects Monitoring (McIntyre and Whittle, 1980).

TABLE 1a

VARIABLES DISCUSSED AND THEIR EVALUATION (SEE TEXT FOR EXPLANATION)

Measurement	Evaluation
1. BIOCHEMICAL EFFECTS	
Mixed function oxidase	2
Metallothionein	2
Lysosomal stability	1
Steroids	3
Energy charge	2
Blood chemistry	2
Taurine/Glycine	1
Primary production	2
2. GENETIC EFFECTS	
Chromosomal abnormalities	3
Mutagenicity assay	3
3. PHYSIOLOGICAL EFFECTS	
Respiration	3
Feeding rate	1
Body condition index	1
Scope for growth (+ growth efficiency)	1
Oxygen/Nitrogen	1
4. MORPHOLOGICAL AND PATHOLOGICAL EFFECTS	
Gill deformity	3
Liver structure	2
Gametogenic cycle	2
Liver as per cent body weight	1
Ulcers	1
Fin erosion	1
Neoplasia/Tumours	2
Asymmetry	1
Early developmental stage	2
5. BEHAVIOURAL EFFECTS	
Torque test	2
6. ECOLOGICAL EFFECTS	
Community biomass	1
Abundance	1
Diversity	1
Alterations in distribution	1
Species density	1
Growth rate	1
Reproduction (gonad as % body wt.)	1
Population structure	1
7. BIOASSAY	
Bivalve/Echinoderm larvae	1
Microalgae bioassay	1
Hydroid bioassay	1

THE FEASIBILITY OF ESTABLISHING PRACTICAL PROCEDURES

Strategy for biological monitoring

There are several possible monitoring objectives: for input control; the protection of human health; the determination of spatial and temporal trends in contamination and its effects on the ecosystem; the provision of environmental management data. Constraints of time and resources often make it necessary that a single monitoring exercise subserve several requirements, but the programme, whether single or multi-purpose, may usefully be built up according to a consistent strategy that will enable the requirements of different objectives to be identified and appropriate techniques used.

In developing such a strategy, the Working Group recognized three phases:

Phase I identification: detecting a change in time and/or space;

Phase II quantification: establishing the degree or extent of the change;

Phase III causation: determining the cause of the observed change.

The recognition of these phases is important because techniques appropriate to one phase will usually be less appropriate to another. For example, it has been pointed out that many of the readily observable biological changes can be produced by a wide variety of causes, not all related to pollution, but by selecting a suite of non-specific biological effects measurements, changes from the normal state, however caused, can be quickly identified and quantified. On the other hand, the measurement of biological effects specific to one particular pollutant will not identify effects produced by other pollutants.

These phases may be regarded as a temporal sequence of discrete investigations for monitoring biological effects of pollution. It should be noted that in many cases (for example, when hot-spots are already known) phase I, which concentrates on chemistry and involves biology largely to detect a signal, may have been accomplished before the start of the investigation. It is then possible to begin with phase II. At each phase, techniques could be used to increase the precision or sensitivity before the next phase was entered into. Furthermore, at any point within this strategy, an evaluation of the situation based on scientific or economic considerations could indicate that further investigation is unwarranted.

Implementing the strategy

We may now examine how the variables evaluated in the earlier sections can be drawn into this framework to produce practical guidelines for a monitoring programme. It is useful for descriptive purposes to consider spatial and temporal aspects separately, although the components of both are essentially the same.

Phase I. In the context of spatial variability, the identification phase is concerned with mapping potential or actual effects and thus pinpointing "hot-spots" of pollution. This allows the effort in the later phases to be concentrated at those sites where biological effects are most likely to occur.

Potential "hot-spots" of effects can be inferred from the distribution of contaminating inputs and from areas with elevated levels of contaminants in water, sediment and biota. Increased resolution may be achieved using chemical analysis of sessile suspension feeders of wide geographical distribution (such as *Mytilus*); mobile species (such as fish) may also be used but they provide poorer resolution. Spatial discrimination by these methods can be very high. General requirements for biological accumulators are discussed in a report already referred to (FAO, 1976 a).

Alterations in lysosomal stability is a biochemical effect that can be readily incorporated into a pollution monitoring programme because it is quantitative, non-specific, sensitive, has a high response rate and signal/noise ratio, it can be applied to a variety of organisms and has been tested in field studies. The main disadvantages are the relatively high equipment costs (a cryostat is needed) and the low ecological significance, although it has been shown to be correlated with effects measured at higher levels of organization.

Two biochemical effects measurements with some degree of specificity are mixed-function oxidase (MFO) and metallothionein; the former is concerned with the metabolism of xenobiotics (including petroleum hydrocarbons) and the latter is involved in metal detoxification by protein binding. Both biochemical effects are sensitive, probably with a low scope, are responsive, applicable to a wide range of organisms and have a high signal/noise ratio. However, their measurement involves high cost in terms of equipment and training, and more extensive field evaluation is required. Consequently, these two specific biochemical effects can be recommended only for selective use at present.

Although the Group restricted its discussion and evaluation to the 37 variables listed in Table 1, the list is not exhaustive. Additional effects could be proposed, assessed in terms of the 13 criteria, given an appropriate evaluation, and compared with the existing list of variables.

TABLE 1b

HIGHLY RECOMMENDED MEASUREMENTS

Lysosomal stability	Asymmetry
Taurine/Glycine	Community biomass
Feeding rate	Abundance
Body condition index	Diversity
Scope for growth	Alterations in distribution
Oxygen/Nitrogen	Species density
Liver as % body wt.	Growth rate
Ulcers	Gonad as % body wt.
Fin erosion	Population structure
Vertebral damage	Bioassays

Bioassay. Bioassay techniques are used primarily for biological assessment of water quality. Three types of bioassays (on bivalve/echinoderm larvae, microalgae and colonial hydroids) have been applied successfully, albeit on a small scale, and are considered suitable for the identification of pollution "hot-spots" in a routine monitoring programme. They all have the advantage of being highly quantitative, sensitive and precise, with a high signal/noise ratio and response rate (e.g., within 48 hours for oyster and echinoderm larvae and microalgae), combined with a low cost. However, the use of bioassays should be limited to identifying the presence of "hot-spots" because the response measured in isolation has little ecological significance.

Ecological effects. Measurements of all the ecological effects listed in Table 1a are considered suitable for application in a monitoring programme but the effects generally have a low signal/noise ratio and a very slow response rate. An initial assessment of ecological conditions can be achieved by means of community biomass, abundance, diversity indices, and alterations in the distribution of selected species. Measures of community biomass and abundance are included because they are relatively easy to obtain and require little time and expertise in species identification.

Further detailed analysis of community and population structure, species density, and estimates of growth rate and fecundity are considered important ecological effects measurements but involve extra cost, combined with a need for a higher level of training in sampling and statistical procedures. The sensitivity and scope of altered species distribution and density are usually low but are largely a function of the species selected for detailed study.

Growth rate, is an important biological effect measurement but one that is often difficult to determine accurately in invertebrates. Estimates of growth rate, measured in terms of shell length, body length or weight changes, often ignore gamete production which can form a large proportion of the total production in many adult stages and represent a component of growth that is most likely to be affected by stress and pollution.

As well as invertebrates and fish to which the above remarks largely refer, top predators such as birds and mammals are appropriate for ecological studies and may be more amenable to direct observation and counting.

Behavioural effects. The behavioural effects panel at the ICES Workshop did not recommend any specific tests for use in a monitoring programme, partly because behavioural effects are often different to measure in the field. One behavioural response, the "torque test", has been demonstrated as a useful measurement of pollution effects in fish and is therefore considered in Table 1. It is moderately sensitive and has been applied to a limited extent in a field monitoring programme but has the disadvantage of being applicable to only a few fish species, and requiring expensive equipment. In general, the effects of abnormal behaviour may be more readily observed and used than the immediate behavioural response. Thus avoidance in fish may be reflected in altered distribution while egg breaking by birds and abnormal suckling by seals result in changes in population numbers.

Morphological and pathobiological effects. Several morphological and pathobiological effects (e.g., vertebral damage, asymmetry, fin erosion and ulcers) can be examined in fish populations at low cost and without involving highly trained personnel or sophisticated scientific equipment. They are, therefore, considered suitable for the initial identification of hot-spots. However, the collection of data from commercial fisheries may not be feasible if deformed and damaged fish are disposed of before they are landed. It is noted that vertebral damage can be assessed without the use of x-ray techniques but there is a reduction in sensitivity. The assessment of ulcers in terms of sensitivity and scope was not possible owing to lack of experimentation.

Alterations in liver/digestive gland structure and gametogenesis, neoplasia/tumours, granulocytomas and abnormalities in early developmental stages are all measurements suitable for inclusion in a monitoring programme but require scientists trained and experienced in microscopic studies. In many cases these histopathological effects measurements can be performed on the same sections taken from the same individuals used for physiological and/or chemical analyses, and together these measurements can form a major part of an integrated monitoring programme. Gill deformity in fish, crustacea and molluscs is not recommended for use at present because the precision required to detect change cannot be achieved without expensive equipment and highly trained scientists, and it has not been adequately tested in field programmes.

Physiological effects. The physiological responses of organisms are more quantitative, sensitive and responsive to pollution than ecological effects measurements, and have been applied and shown to be suitable for assessing the degree and extent of pollution effects. However, the costs are generally higher in terms of equipment and training.

Respiration is not considered suitable as an isolated response because it lacks a clear quantitative relationship with stress and pollution, and is relatively insensitive, but it should be measured as a component of the balanced energy equation from which "scope for growth" is derived.

Feeding rate is quantitative and sensitive, and is of ecological significance and therefore should be measured whenever possible as an individual rate function or preferably as part of the balanced energy equation.

Scope for growth or the growth potential of an organism is derived from measurements of rates of feeding, respiration, excretion and food absorption efficiency, followed by integration in terms of the balanced energy equation. This physiological integration is recommended since it is quantitative, ecologically significant, relatable, has a wide scope, and has been evaluated in field monitoring programmes. Two additional physiological stress indices, the ratio of oxygen consumed to nitrogen excreted, and growth efficiency, can be obtained from the same set of basic physiological measurements used to compute scope for growth without incurring any additional costs. They provide information on the balance between catabolic processes and the efficiency with which an organism functions, satisfy many of the criteria, and are therefore recommended for inclusion in monitoring programmes.

The body condition and body component indices are recommended for use in monitoring programmes because body weights are usually required for other effects measurements (e.g. physiological, and population structure). They are easily determined at low costs, and are ecologically significant (i.e., reflect changes in nutritional status). However, they have the disadvantage of being insensitive, variable and slow in responding to environmental change.

Genetic effects. None of the genetic effects measurements discussed at the ICES Workshop on Pollution Effects Monitoring can be recommended for use at present but are regarded as having long-term potential following further development and evaluation in pollution studies.

The assessment of chromosomal damage in relation to pollution is considered a useful effects measurement, but at present it has a high cost in terms of trained personnel and high quality microscopes, and may be relatively insensitive. Although the mutagenicity assay has been included as a biological effect of pollution, it is generally regarded as an assay for mutagenic chemicals and therefore difficult to assess in terms of the 13 criteria. It is clearly an important assay that should be developed further, but owing to its cost and insufficient testing in a field programme, it is not suggested as suitable for inclusion in a monitoring programme at present.

Biochemical effects. Several biochemical effects are considered suitable for use in a monitoring programme but only in conjunction with other effects measurements at higher levels of organization. Biochemical effects can be divided into two categories; those that are general (non-specific) and those that are indicative of the specific toxic action of particular pollutants. Most biochemical indices considered at the ICES Workshop were non-specific, and only two specific effects were evaluated (mixed-function oxidase and metallothionein).

Primary production, measured in terms of ^{14}C fixation, is included in the biochemical effects, although it can be regarded as an ecological effects measurement (i.e., total production of microalgae and other micro-organisms in a body of water). It may be useful as a low cost, general indicator of environmental condition, if scintillation counters are available, but has the disadvantage of not showing a clear quantitative relationship with pollution, high variability (low signal/noise) and insufficient field evaluation.

The ratio of two free amino-acids, taurine and glycine, in the tissues of bivalves is recommended for use in monitoring programmes since it has been shown to be quantitative and moderately sensitive, with a wide scope, and has been applied and evaluated in field programmes. However, its use is restricted to marine bivalves and to laboratories with an amino-acid analyser.

Blood chemistry is a general term that includes a wide range of measurements. The conclusions of the biochemistry and physiology panels at the ICES Workshop was that considerable research still remains to be carried out with fish and invertebrates before blood chemistry is developed into a proven monitoring technique. The main disadvantages are that many of the blood components show no predictable and quantitative relationship with stress and pollution, and there has been little field application of the more promising techniques.

Adenylate energy change is a measure of the metabolic energy available to the organism. This general biochemical effect is applicable to all organisms, is responsive and has a high signal/noise ratio; but evidence suggests that it is relatively insensitive with a low scope and a high cost. Although energy change has been proposed as a general biochemical effect, it still requires field application and evaluation.

Changes in steroid hormone metabolism may prove to be sensitive and ecologically significant indicators of pollution effects, but only a few species of fish have been studied in a very limited number of laboratories. Before steroid-metabolism measurements could be applied routinely in monitoring, it would be necessary to carry out basic steroid hormone research on a wide range of vertebrates and invertebrates in order to establish the background information. Consequently, steroids are not suitable for use in monitoring programmes at present.