Methods for Estimating Risk of Chemical Injury: Human and Non-human Biota and Ecosystems
Edited by V. B. Vouk, G. C. Butler, D. G. Hoel and D. B. Peakall

Methods for Measuring the Effects of Chemicals on Aquatic Animals as Indicators of Ecological Damage

Michael Waldichuk

ABSTRACT

The effects of chemicals on aquatic organisms can be classified into: (1) lethal or acute, and (2) sublethal or subacute. Mortality is used as the endpoint in bioassays measuring the acute effects. The net result of such tests is an LC_{50} value, usually for an exposure of 96 hours. Control agencies often apply a simple application factor (usually 0.1 for a chemical that is not bioaccumulated and 0.01 for one that is bioaccumulated) to the 96-hour LC_{50} to arrive at the maximum permissible concentration of the chemical in the receiving water.

In case of sublethal effects, the exposed organisms may not be killed but may be debilitated, and some of their functions, including reproduction, may be impaired. In the long run, sublethal effects may be very serious for the survival of populations of aquatic organisms, especially if reproductive success is reduced. Chemicals may affect physiological functions, such as feeding, respiration, blood circulation, excretion and osmoregulation; and various biochemical changes may occur, for example, alterations in enzyme activity, blood chemistry, steroid metabolism, metal-binding proteins and lysosomes; morphological abnormalities and deterioration in resistance to disease can also result from exposure to chemicals. Behavioural responses to chemicals, such as avoidance reactions, chemoreception effects on feeding, mating and homing and alterations in phototaxis are important sublethal effects; and changes in the composition of the gene pool may take place. Ecological effects of chemicals include changes in species diversity, population structure, reproduction and community biomass. Ecological studies are time-consuming and expensive, because a long time series of observations is generally required to yield definitive answers on the effects of a pollutant. Choice of an appropriate technique depends on a number of factors, including complexity of measurement, sensitivity, response rate, range of concentrations of chemicals over which the effect is observable, accuracy and precision with which the effect can be measured, and quantifiability of the

relationship of the effect to the concentration of the chemical and duration of exposure. No single type of measurement can provide information on the effect of a chemical on a population or community of organisms or on an ecosystem over the long term. A carefully selected series of measurements is needed. Predictive testing on single species with the potential for extrapolation of results to higher levels of biological organization that would have universal applicability for the aquatic environment has not yet been developed.

1 INTRODUCTION

There is a wide range of effects of chemicals on aquatic animals from a brief, mild stress to rapid, almost instantaneous death. Measurement of the time to death of a group of organisms exposed to a given concentration of chemical is perhaps the simplest test or biological assay to measure the toxicity of a chemical. This bioassay has been refined to provide a standardized toxicity measurement method (Sprague, 1969; APHA/AWWA/WPCF, 1981), by which one chemical can be compared to another using the same test organism. What is usually obtained is a 96-hour LC_{50} or median lethal concentration, i.e. the concentration causing death of 50% of test organisms within a 96-hour period of exposure.

It has been long recognized, of course, that a substance may also have chronic sublethal effects (Alderdice, 1967; Sprague, 1971) on an aquatic organism, and that this effect may be important in terms of its impact on that organism over its life cycle. Although the organism continues to survive, the performance of various functions may be sufficiently impaired so that the organism fails to complete its life cycle, either through premature death due to various causes, or lack of reproductive success. Obviously an individual may be insignificant in terms of the impact on the community, stock of organisms or population as a whole. Yet, much work has been confined to measurement of effects on groups of animals in the laboratory which may have little application to the situation in the field. There is a need for predictive testing on single species or communities that would allow extrapolation of results to higher levels of biological organization and to aquatic ecosystems. Unfortunately, such predictive testing has not advanced far enough for general applicability. Research on microcosms (Sugiura *et al.*, 1976a,b, 1982; Giesy, 1980; Huckabee, this volume) is promising. Virtually no substitute exists at present, however, for ecological studies in the field, which are painstaking, time-consuming and expensive. Moreover, careful statistical analysis is needed to separate out natural fluctuations and to determine unequivocally ecological damage due to a man-made source of pollution.

Whereas it has been difficult to establish the loss or decline of stocks of fish or other organisms in the sea due to pollution alone, it has been relatively easy to do this in lakes, where discrete stocks of fish could be monitored while exploitation is controlled. This has been dramatically demonstrated with thousands of lakes that have been rendered fishless by acid rain in the Scandinavian countries

(Jensen and Snekvik, 1972; Leivestad and Muniz, 1976), north-eastern USA (Schofield, 1976), and the south-central part of Canada (Beamish, 1974). Acidic precipitation, which lowers pH in fresh waters that are poorly buffered, has had disastrous consequences on fish reproduction.

There have been several significant documents in the last five years, summarizing the biological effects of marine pollution, biological response measurements and the feasibility of biological effects monitoring, particularly at the sublethal level (ICES, 1978; Cole, 1979; GESAMP, 1980; McIntyre and Pearce, 1980; Bayne, 1983). No comparable overviews have been published for the freshwater environment, but many of the principles established for the marine environment apply also in freshwater systems. Sublethal effects of chemicals on aquatic organisms have been equated to stress by some investigators, and several symposia have been convened on stress in fish (Pickering, 1981) and stress effects in natural ecosystems including the response to stress in marine benthic communities (Boesch and Rosenberg, 1981). Some earlier documents on the selection of biological tests to evaluate marine pollution (FAO, 1977), on indices for measuring responses of aquatic ecosystems to human influences (FAO, 1976) and on techniques for biological surveillance in various ecosystems (NERC, 1976) contain useful information and should also be consulted. The PRIMA (Pollutant Responses in Marine Animals) programme, now underway with funding by the US National Science Foundation as part of the International Decade of Ocean Exploration, promises to produce important results in this area (NSF, 1982).

2 DOSE–RESPONSE RELATIONSHIP

The principles involved in biological assays and the uses of various terms have been summarized by Alderdice (1967) and Sprague (1969, 1971, 1973). Quayle (1969) defined a bioassay as 'a test in which the quantity or strength of material is determined by the reaction of a living organism to it'. There are basically three components of a bioassay: (1) stimulus, (2) subject, and (3) response. The stimulus may range from an electrical shock to a chemical used such as an insecticide; the subject may be an animal, a tissue or a cell culture; and the response may be a change in behaviour of the subject, decreased enzyme activity, morphological change in the cell, or death of the organism. The stimulus may rouse an organism to activity and it may impose a stress. The objectives of a bioassay are essentially to:

(1) establish the maximum concentration of a particular substance that a representative organism can withstand without causing unacceptable damage to that organism or to the biological community; and
(2) determine the potency of the substance relative to some standard or reference material.

A host of other questions can be answered when these objectives are met. For example, are treatment methods adequate to sufficiently reduce the toxicity of the substance?; does the toxicity of the substance vary with time? It is obvious that for a meaningful intercomparison of different substances to meet objective (2), conditions of the test must be comparable.

The response of an aquatic organism to a given substance, or the observed effect on the organism with changing concentration of the substance, can usually be represented by a simple equation, $R = f(tcA)$, where R is response, f is a function, t is time, c is concentration of the chemical and A is its activity. A dose–response curve illustrates this relationship for a given period of exposure (Figure 1). Dose is defined as the amount of a substance at the site of effect. Dose can be estimated from measurements of exposure and metabolic information. Indirect estimation of dose can be obtained from metabolic information. The time period over which the response is measured can be varied according to the activity of the particular chemical. The response of an organism to different concentrations of a substance—manifested as the change of a physiological function, accumulation of the substance in the tissues or an alteration of its behaviour—can be demonstrated by a dose–response curve. The curve may be linear, which it seldom is, or it may be curvilinear, as shown in Figure 1. There may be no measurable response at low doses, so that the curve does not necessarily pass through the origin, but intercepts the dose coordinate at some point above zero concentration. Usually there is an effect even at the lowest concentrations, either inhibitory or stimulatory, but our techniques to measure effects at those concentrations are often inadequate. At different points along the dose–response curve, the various descriptors for the effect of a substance can be shown (see Figure 1) ranging from the sublethal threshold, through the LC_{50} to 100% mortality. Dose–response relationships can be established for different effects, such as respiration, blood pressure, enzyme activity, swimming performance, avoidance reaction, food consumption, growth and selected blood characteristics. [Wilson (1980) discusses some aspects of biological effects of pollutants which determine their suitability for monitoring pollution.]

Using the dose–response technique, the activity of a substance can be described by the median lethal concentration, LC_{50}, sometimes referred to as the median tolerance limit, TL_m. Test organisms are exposed to the substance at different concentrations for a given period of time, say 96 hours. A sigmoid-type curve often depicts response *vs* concentration. The concentration of pollutant associated with a 50% response mortality gives an estimate of the 96-hour LC_{50}. Another useful measure that can be acquired from such a test is the median lethal time, LT_{50}, the time to death of one-half of test organisms. An estimate of the response threshold and the associated concentration can be obtained from the asymptote of the distribution of LT_{50} estimates at which exposure becomes infinitely long.

In recognition of the fact that sublethal effects occur at much lower exposure

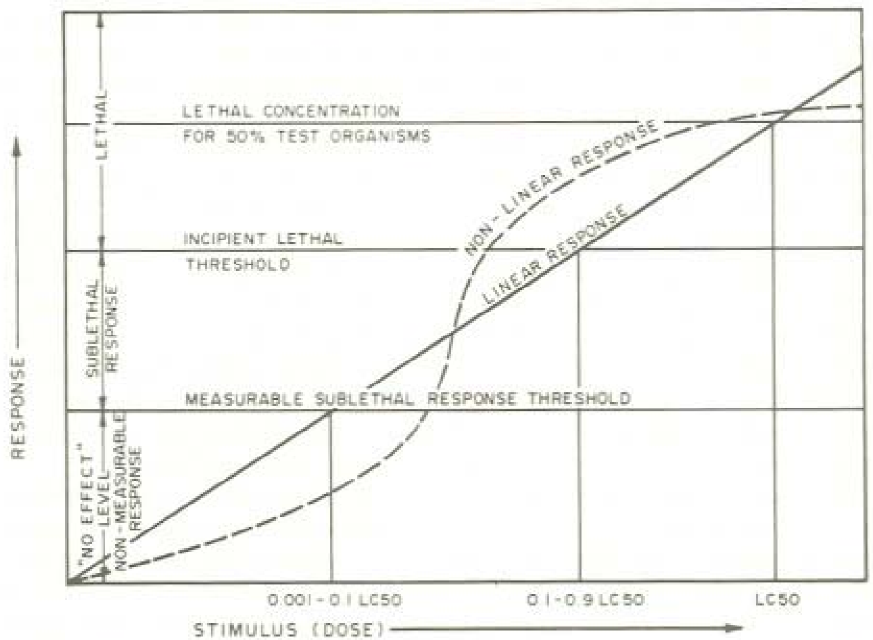

Figure 1 Hypothetical relationship of the response of an aquatic organism to concentration of a chemical, showing some significant points and regions on the curve. (Modified from Waldichuk, 1979)

than that causing death, regulatory agencies often apply a rather arbitrary safety factor to the LC_{50} for a particular substance, in order to set a limit for its concentration in a given water body. Depending on the toxicity, bioaccumulation and persistence of the substance, the application factor may range from 0.1 to 0.001, but it is most often 0.1. More recently, the application factor has been derived experimentally from a 96-hour acute bioassay and tests to determine the threshold of sublethal toxicity. Depending on the type of tests used, the factor may vary from one laboratory to another. For example, for kraft pulpmill effluent, a factor of 0.05 was reported by one investigator (Walden, 1976) and 0.02 by another (Davis, 1976).

Environmental variables, such as water temperature, hardness, pH, salinity, dissolved oxygen concentration and the presence of metals, must be taken into account when determining the toxicity for aquatic organisms. Each organism has an optimum combination of environmental variables under which it performs best. When it is exposed to a toxic substance outside this optimum, there is a stress imposed on the animal owing to environmental effects in addition to that of the toxicant. This effect can be shown by response surfaces in a three-dimensional system (Alderdice, 1972).

For a given chemical, LC_{50} values may vary by two or three orders of magnitude depending on the species tested. There can also be a large range in LC_{50} values according to the life-cycle stage of the species tested and the sensitivity of that stage. An animal that has a life cycle synchronized with the seasons can exhibit different sensitivities to a toxic chemical, if bioassays are conducted at different times of the year. The toxicity of a chemical may be different in freshwaters than it is in the marine environment. Although substances are generally more toxic in freshwater, this is not always so, especially with organic compounds such as phenol, the anionic detergent sodium lauryl sulphate and organophosphorus pesticides. The fitness of the test animal is important, if results are to have meaning with respect to the animal in the natural environment. Some knowledge should also be available on the normal variability of responses in animals used in bioassays. All these factors must be taken into account when designing bioassays. Above all, it must be realized that laboratory experiments should bear some relationship to the field situation.

Bioassays have a recognized role in aquatic pollution monitoring (Stebbing *et al.*, 1980) and in establishing maximum permissible concentrations of pollutants. Aquatic animals have often been selected for bioassays on the basis of their availability, adaptability to laboratory experiments and commercial value of the species. Bioassays are often conducted with such species as rainbow trout, *Salmo gairdneri*, or coho salmon, *Oncorhynchus kisutch*. In some cases, species with a short lifetime are chosen, for example, *Mysidopsis bahia*, in order that life-cycle toxicity tests can be conducted (Nimmo *et al.*, 1977). There are arguments for using invertebrates in bioassays (Åkesson, 1980; Maciorowski and Clarke, 1980), and particularly eggs of some species, such as sea urchins (Kobayashi, 1971, 1974) or larvae of other species (Woelke, 1965, 1967, 1972). Efforts have been made (Davis and Hoos, 1975) to standardize bioassays with interlaboratory calibration, using reference chemicals.

3 EFFECTS OF CHEMICALS ON AQUATIC ANIMALS

Chemicals can exert an impact on aquatic organisms that may be expressed in a variety of ways through behaviour, physiology, biochemistry, pathobiology and morphology of the organisms, genetic changes in cell structure and ecological alterations at the community level. There may be an effect on all the foregoing functions simultaneously, but the response in one of the life processes may be greater than in others. It is this feature that is important in selecting an appropriate response for measuring adverse effects of a chemical. If it impacts directly or indirectly on the reproductive process, then it can have significance at the population level. The biological effects of a chemical on aquatic animals will have a bearing on the choice of appropriate methods for measuring the impact the chemical may have on a community, on a stock or population of organisms. Ecological fitness is the criterion that really counts, and when ecological fitness declines, then a pollution effect has really occurred. An animal that is alive but moribund can be considered as ecologically dead.

3.1 Physiology

The degree to which physiological functions deviate from the 'norm' can be considered a measure of stress imposed by a chemical pollutant. Such deviations reflect the fitness of the organism. Physiological functions may range from feeding behaviour through swimming performance and respiration to excretion. The question that must be answered, of course, is what is the 'norm' for a particular physiological function and what is the normal range of fluctuations in that function under natural conditions. Only after the natural variability has been taken into account, is it possible to ascribe an aberration in a physiological function to the effect of a pollutant.

There are many techniques for evaluating the impact of pollutants on physiological functions. Some are relatively simple, such as measuring the rate of growth of byssal threads in mussels and recording a cough response in fish, while others are more complex, as exemplified by the analysis of the scope for growth and osmoregulation in fish and invertebrates exposed to a pollutant. In some cases, the response is comparatively rapid with a high degree of sensitivity, and specificity; in others, the response, sensitivity and specificity are low. Bayne (1980) discussed the physiological measurement of stress, some of which may be due to pollutants. An evaluation of the physiological techniques available for measuring the biological effects of pollutants in the sea was prepared by a Physiology Panel of a Workshop on Biological Effects of Marine Pollution and the Problems of Monitoring, arranged by the International Council for the Exploration of the Sea (ICES) at Duke University in Beaufort, North Carolina (Bayne *et al.*, 1980). Some views expressed by this Panel are summarized here.

3.1.1 Feeding

The process of feeding involves several activities such as searching for and locating food, selecting and capturing prey, filtering food out of water if the organism is a filter feeder, and burrowing through the sediment to find the food, if the organism is a deposit feeder. The rate of food intake will depend on the ability of the animal to find food and on its appetite. Most research on the effects of pollutants on feeding is confined to laboratory experiments on the rate of feeding.

Blaxter (1977) exposed in the laboratory yolk-sac plaice, *Pleuronectes platessa* L., to different concentrations of copper in seawater and found that feeding was totally inhibited by 90 μg/l of copper. Abel (1976) and Dorn (1976) observed reduced feeding rates in *Mytilus* when exposed to methyl mercury and other metal compounds or ions at concentrations of 40–1000 μg/l. Production of faecal pellets by the zooplankton *Acartia tonsa*, *Calanus plumchrus* and *Metridia pacifica* was reduced on exposure to copper as low as 5 μg/l within 4–14 days (Reeve *et al.*, 1976). Feeding of larval lobsters *Homarus americanus* was inhibited by 190 μg/l of crude oil (Wells and Sprague, 1976). A concentration of 520 μg/l of hydrocarbons depressed the ingestion rate of the zooplankton crustacean *Eurytemora affinis* by 38 %, and 10 and 50 μg/l inhibited its feeding (Berdugo *et al.*, 1977). The condition index and free amino acid pools of the deposit-feeding detritovore bivalve, *Macoma inquinata*, were reduced in oil-contaminated sediments (Roesijadi and Anderson, 1979). The polychaete *Arenicola marina* exhibited a reduction in sediment working rate when exposed to low concentrations of certain oils (Gordon *et al.*, 1978). Rate of feeding in suspension- and deposit-feeding bivalves or of faecal pellet production in zooplankton and faecal casts in deposit-feeding worms can provide an index of physiological effects of environmental pollution. However, there is a natural variability in feeding rate induced by slight alterations in food availability or variations in environmental conditions, which makes the establishment of a baseline for the rate of feeding extremely difficult.

3.1.2 Growth

A reduction in growth of aquatic animals is a non-specific response and can arise as a result of stress due to a combination of many factors. Normal growth of a healthy animal is the net result of several physiological processes that are in harmony and not seriously disturbed by a pollutant or other stresses. Brett (1979) reviewed the various factors involved in the growth of fishes. A reduction in growth can occur as a result of lack of food, reduced feeding rate, decline in the proportion of organic matter ingested which is absorbed, and an increase in loss of energy through respiration or excretion. Natural and man-made changes in the environment can contribute to one or more of these factors and to a reduction in growth rate.

Measurements of growth rates in fish exposed to different concentrations of a pollutant are sometimes confounded by non-linearity of the response. For example, Pacific salmon species exposed to low concentrations of kraft pulpmill effluent have exhibited a higher growth rate than the controls free of effluent (Webb and Brett, 1972; McLeay and Brown, 1974; Mason and Davis, unpublished data). It is unclear whether low levels of such wastes act as a disinfectant, reducing the stress from microflora, or serve as a nutrient for the test organisms, or introduce some other unknown effect. Otolith ageing techniques for larval fish (Brothers *et al.*, 1976; Struhsaker and Uchiyama, 1976) allow an estimate of growth rate during the elapsed interval from otolith formation, and show promise for comparing relative growth rates in different areas.

An indirect or physiological estimation of growth is based on measurements of feeding, respiration and assimilation rates (Bayne, 1975; Bayne and Widdows, 1978). Such an estimate of the instantaneous growth rate, or the scope for growth, can be directly related to the body burden of toxic substances in animals (Gilfillan, 1980). It integrates a number of physiological measurements which together can provide useful information on the health of the animal (Bayne *et al.*, 1979). Scope-for-growth measurements in a series of studies on *Mytilus edulis* showed a good correlation with other measurements of stress (Bayne *et al.*, 1976) and with fecundity and the survival of eggs and larvae (Bayne *et al.*, 1978). Studies on field-exposed, soft-shell clams from three different oil-spill sites (Gilfillan and Vandermeulen, 1978; Gilfillan, 1980) gave a correlation between the scope for growth and elevated body burdens of hydrocarbons. Widdows *et al.* (1981) demonstrated a reduction in scope for growth of mussels along a gradient represented by a rise in body burden of both petroleum hydrocarbons and metal ions. Edwards (1978) exposed the brown shrimp *Crangon crangon* to progressively increasing levels of the water-soluble fraction of North Sea crude oil at 10°, 15° and 20°C. He found a reduced scope for growth with increasing oil concentration at each temperature, but the greatest reduction occurred at 20°C. Exposure of the copepod *Acartia tonsa* to 250 μg/l of oil in a large enclosure substantially reduced the scope for growth (cited in Bayne *et al.*, 1980, as a personal communication by E. Gilfillan).

3.1.3 Reproduction

Perhaps the most important function in terms of maintenance of a stock of aquatic organisms, or even a species, is successful reproduction. Adverse effects on various physiological functions during the life history of an organism can culminate in reproductive failure. A chemical that causes sterility in males and/or females can have an irreversible negative impact on reproduction. Cadmium, for example, has been demonstrated to produce testicular injury in brook trout (Sangalang and O'Halloran, 1972) with unfavourable consequences for reproduction. If a stock of fish survives under an environmental stress but fails to reproduce, the perpetuation of that stock is at stake. Unfortunately, it has not been possible to document conclusively instances of reproductive failure that have threatened stocks of aquatic animals exposed to chemicals in the field. It has been known for some time that such organochlorines as DDT concentrate in the gonads of fish and are present in high concentrations in the eggs, resulting in a high mortality of hatched alevins (Burdick *et al.*, 1964). Similar effects have been found with water-soluble fractions of fuel oil (Rossi and Anderson, 1978). Polychlorinated biphenyls and DDT have been implicated in reproductive failure of Baltic Sea seals (Helle *et al.*, 1976). Acidification of lakes by acid precipitation has led to loss of fish populations (Beamish, 1974). However, it has not always been clear as to the mechanism by which reproduction fails, and whether such reproductive failure can be corrected by a pH increase in acidified lakes. Laboratory experiments with embryos and alevins of fish exposed to waters of low pH are beginning to provide a better understanding of mortality of these early life stages in acidic waters (Daye and Garside, 1980).

On a long-term basis, a decline in fecundity in aquatic animals may be as important as total reproductive failure. Such a decline may be a reflection of general deterioration in ecological fitness of an animal. Production of gametes by the female and sperm by the male may be adversely affected by a pollutant, with resulting loss of reproductive potential. A reduction in scope for growth has led to a decline in fecundity of *Mytilus edulis* (Bayne *et al.*, 1978). The act of fertilization can be interfered with by such pollutants as oil, which appear to disrupt the chemosensory process that attracts the sperm to the egg (Inman and Lockwood, 1979). Reproductive success must be based not only on the egg-hatching phase in aquatic organisms but also on the early post-embryonic and larval phases. The latter phases are considered to be sensitive to pollutants, and accordingly a great deal of toxicological work has been done with larval stages (Calabrese *et al.*, 1973; Rosenthal and Alderdice, 1976; Wells and Sprague, 1976). Woelke (1965, 1967, 1972) has developed bioassay techniques with the embryo of Pacific oyster, *Crassostrea gigas*, to measure water quality. Eggs and larvae of Pacific herring, *Clupea harengus pallasi*, were exposed to various copper concentrations in laboratory experiments (Rice and Harrison, 1977), and embryo mortality occurred at 35 μg/l of copper, whereas larvae succumbed to 300 μg/l. Armstrong and Milleman (1974) conducted toxicity tests with the insecticide Sevin using the early life stages of *Mytilus edulis*, and demonstrated the wide range in sensitivity from the unfertilized egg to the early veliger stage. Linden (1976) examined the effects of oil on the larvae and adults of *Gammarus oceanicus*.

A thorough review of the effects of chemicals on reproduction in fish has been given by Donaldson and Scherer (1983). They concluded that no agreement appears to exist on the relative significance of criteria for reproductive impairment from effects on gametes over egg and larval development to maturation of adults. Therefore, they were unable to state unequivocally which tests would be of the highest priority, or whether tests on more than one of the life stages should be conducted concurrently.

3.1.4 Respiration

The rate of oxygen consumption is a measure of physiological stress. Interpretation of data on oxygen consumption is confounded by variability due to intrinsic and extrinsic causes. Some of the variability can be eliminated under controlled laboratory conditions, but it is much more difficult to deal with variability in field studies. Measurements of oxygen consumption in the field can be made rather easily in many animal species. Abnormal values would indicate that something is wrong and further tests are required, inasmuch as the oxygen consumption measurement is a convenient screening technique for other physiological testing. For example, a depression in oxygen consumption may indicate gill damage and the need for pathological studies. Much of the published work has been done in the laboratory on fish. Scott and Major (1972) have measured the effects of copper on the respiration and heart rate in the common mussel, *Mytilus edulis*.

Three activities associated with respiration are cough frequency, opercular movement and heart-beat frequency. They have all been used to measure a response to a pollutant, especially in freshwater. The cough response, which is a periodic back-flushing of the gills, may be induced by physical or chemical action on the gill epithelium by suspended solids or a dissolved chemical. The method of measurement is comparatively simple, involving the installation of a transducer in the buccal cavity of fish attached to a recorder, which registers a spike in the pressure record with every cough. Carlson and Drummond (1978) reviewed the literature on this technique. Measurements of opercular frequency have been made on many fish species exposed to a variety of pollutants (Heath, 1972), but changes in opercular frequency are not always associated with concomitant changes in oxygen consumption. Changes in heart-beat frequency are generally related to changes in oxygen consumption, although some regulation can be achieved by altering stroke volume. Of the three responses, cough appears to be most sensitive and most readily induced response by a wide range of pollutants. It has been used effectively for continuous monitoring of effluent quality, but it is not suitable as a sensitive indicator of health of a natural fish population. Pumping rate and heart-beat frequency have been used to monitor environmental effects in bivalve molluscs (McMahon and Wilkens, 1977), but further studies are needed to prove the reliability of such techniques.

3.1.5 Osmotic and Ionic Regulation

All aquatic species are capable of osmotic and ionic regulation. A pollutant can impair this capability. The ability to osmoregulate against a salinity gradient is qualitatively the same within any one species, but it varies quantitatively from one individual to another according to the nutritional condition and level of stress from a pollutant. Theoretically, the ionic concentration or osmotic pressure of blood or haemolymph of an aquatic organism should bear a constant relationship to that of the water in which it is immersed, unless a stress, such as a pollutant, is applied.

The effects of pollutants on osmoregulation are well documented (Thurberg *et al.*, 1973). Sodium regulation in the amphipod, *Gammarus duebeni*, for example, has been shown to be affected by methylmercury and lindane during changes in salinity of the seawater they were held in (Inman and Lockwood, 1979). The effects of copper on osmoregulation of brackish-water isopods were demonstrated by Jones (1975). There are difficulties, however, in field situations in applying techniques that measure osmoregulation as an indicator of stress. When an active osmoregulating organism is exposed to a man-made stress and the process is interrupted, the organism quickly dies. As a result, it is almost impossible to find an 'osmotically sick' organism in a polluted estuary. Another problem is that good osmoregulators are also active swimmers and can rapidly move away from a stressful situation. While Lynch *et al.* (1973) were able to measure the different components in the serum of the blue crab, *Callinectes sapidus*, collected in Chesapeake Bay, and to correlate them with salinity and temperature, they were unable to correlate their measurements with the site of collection.

3.1.6 Haematology

Blood in aquatic organisms has often been considered as a good medium in which to measure the effects of stress (Black *et al.*, 1966). Exposure to pollutants should change blood characteristics, and attempts have been made to measure these in fish (Fujiya, 1961; Iwama *et al.*, 1976; McLeay and Gordon, 1977; Noggle, 1978). Haemoglobin, haematocrit, numbers of red and white blood cells, total plasma protein, glucose, lactate and pyruvate have all been measured in fish blood with some degree of success in relating them to pollutant exposure. Environmental copper and zinc concentrations have been related to levels of basophils in oysters (*Crassostrea gigas* and *C. virginica*) (Ruddell and Rains, 1975). Serum ion composition has been measured in ocean scallops (*Placopecten magellanicus*) under different field conditions (Thurberg *et al.*, 1978). Although haematological measurements are attractive because of the relative ease with which they can be performed, they have not yielded the kind of diagnostic results sometimes expected from tests on pollution effects.

3.1.7 Excretion and Nitrogen Balance

The products of excretion of an aquatic organism can provide an indication of metabolic disorders and a fundamental nutritional disturbance that may arise from a pollutional stress. They include such nitrogen compounds as urea, amino acids and purines, which represent an energy deficit for an individual when excreted. Excretion rates that exceed normal values may be an indication of stress, and rates of urine flow in fish have been used as a measure of a pollutant effect, as reviewed by Lloyd and Swift (1976). An index of the catabolic balance among protein, carbohydrate and lipid substrates can be obtained from the ratio of oxygen consumed to nitrogen excreted (O to N ratio). A predominance of carbohydrate and/or lipid catabolism over protein degradation yields a high O to N ratio. Bayne (1975), Widdows (1978), and Bayne and Widdows (1978) discussed the use of the O to N ratio as an index of stress. Widdows et al. (1981) demonstrated a good correlation between the O to N ratio in mussels, *Mytilus edulis*, and a pollution gradient in Narragansett Bay, Rhode Island; but they also showed that the O to N ratio varies seasonally under natural conditions, so that when glycogen stores are used up protein catabolism plays an important role in energy metabolism.

3.2 Biochemistry

Biochemical changes in an aquatic organism are not regarded as specific enough in themselves to be used alone for diagnosis of the stress of a pollutant without supporting evidence from other types of measurements, such as those available from physiological, behavioural, histological or pathological studies. Some methods for the study of the biochemical composition of marine invertebrates, for example, are given by Giese (1967), but these do not necessarily provide a good diagnostic tool for determining the effect of a pollutant. The biochemical techniques reviewed here are those that were regarded as most suitable for biological monitoring by the Biochemistry Panel at the ICES Workshop on Biological Effects of Marine Pollution and the Problems of Monitoring (Lee *et al.*, 1980). As in physiological measurements, there is a great need to know about what is the range of biochemical variations under normal environmental conditions. With increasing stress, an organism changes from the normal to the reversible strain phase, when it can still recover if the stress is removed, and finally into the irreversible phase which terminates with death.

3.2.1 Blood Chemistry

Some blood chemistry techniques for measuring stress of pollution were discussed in the physiological section. The techniques presented here are confined to routine chemical serum assay. Detailed investigations in crustaceans indicate there is great natural variability in such assays. Only two appear to be suitable:

(1) total serum lipid and protein concentration as a measure of starvation-induced stress; and
(2) extracirculatory tissue enzymes in the serum as an indicator of cellular leakage.

Decreasing food intake as a result of stresses on the organism or a lack of food can lead to starvation and a reduction in serum lipids and proteins. The necessity of locating the tissue of enzyme origin and the ability of an organism to metabolize such an enzyme and remove it from the circulatory system complicates enzyme measurement (de Bruin, 1976).

3.2.2 Adenylate Energy Charge

The adenylate energy charge (AEC) is a measure of the metabolic energy available to an organism from the adenine nucleotide pool. The technique for measuring AEC has potential for application to the evaluation of stress in virtually all aquatic organisms arising from a pollutant. The response is rapid and has been demonstrated to occur before physiological and behavioural changes arise (Giesy *et al.*, 1978). Some limitations of the AEC-measuring technique have been discussed by Ivanovici (1980), which may preclude its use as an early-warning indicator in routine monitoring. Although most of the early work on AEC has been confined to invertebrates, recent studies (Vetter and Hodson, 1982) have shown it to be applicable as an indicator of hypoxic stress in estuarine fish.

3.2.3 Metal-binding Proteins

A mechanism for detoxifying metals by animals is through metal-binding proteins of the metallothionein type. Such proteins have been found in fish and various invertebrates (Olafson and Thompson, 1974; Noel-Lambot, 1976; Talbot and Magee, 1978; Jennings *et al.*, 1979). While a metal is bound to the protein, it is believed that the exposed animal is protected from the toxic intracellular effect of the metal. However, when the binding capacity of the metal-binding protein is exceeded, then the toxic metal spills over into the metalloenzyme pool (Bremner, 1974; Brown *et al.*, 1977). This was demonstrated by Brown and Parsons (1978), who found that at 5 μg/l of mercury there was a large increase in mercury in the enzyme protein pool and a concomitant decrease in growth in the exposed fish. It has been suggested that the ratio of metals in metallothioneins to metals in enzyme pool proteins might be used as an early warning of possible pathological effects. A major problem with metallothioneins as indicators, however, is that they are involved in a specific response to certain metals only.

3.2.4 Mixed Function Oxygenase (MFO) Induction

Mixed function oxygenase reactions, mediated by cytochrome P450, play a vital role in the metabolism of various chemicals by aquatic organisms. The activity of MFO can be accelerated by increased exposure of organisms to chemicals in the laboratory, and levels of MFO activity in the field can be used as a measure of pollutant effects (Burns, 1976; Payne, 1976; Stegeman and Sabo, 1976; Kurelec *et al.*, 1977; Stegeman, 1978). However, the effective use of MFO activity is limited to some specific compounds, such as hydrocarbons and halogenated biphenyl isomers (James and Bend, 1982). Moreover, the interactive effects of other classes of pollutants could seriously interfere with the response and affect the interpretation of results (Ahokas *et al.*, 1976; Krasny and Holbrook, 1977). Invertebrates exhibiting an MFO induction (Lee and Singer, 1980) are believed to produce a slower response than fish. There are seasonal and sex-related differences in MFO induction in fish (Stegeman, 1980), and this may also be true for invertebrates (Singer and Lee, 1977). Low temperature reduces the MFO induction response in fish. These factors contribute to the natural variability of MFO induction in the field. Nevertheless, elevated MFO levels have been used to measure effects of chronic oil pollution (Burns, 1976; Payne, 1976; Stegeman and Sabo, 1976) and acute contamination (Kurelec *et al.*, 1977).

3.2.5 Steroid Metabolism

The chronic effects of sublethal pollution in aquatic organisms can usually be detected by alterations of normal steroid hormone metabolism, such as glucocorticosteroids, mineralocorticosteroids and sex steroids, which can be measured. Steroidogenesis is affected by chemical pollutants; this has been documented for birds (Peakall, 1967), as well as for aquatic species (Sangalang and O'Halloran, 1973; Freeman and Idler, 1975; Freeman *et al.*, 1975). The uses of steroid hormones to measure the effects of pollution have been described by Freeman and Sangalang (1977) and Uthe *et al.* (1980). Alterations of steroid hormone metabolism as an indicator of pollution effects can only be used in those species for which steroid hormone research has provided the essential basic information.

3.2.6 Lysosomal Damage

The destabilization of the lysosomal membrane, with consequent activation of lysosomal hydrolases, appears to be sensitive to a wide range of physical factors and chemical agents and produces a fast response (Moore, 1980). The level of destabilization is quantitatively related to the degree of stress in the mussel, *Mytilus edulis* (Bayne *et al.*, 1976, 1978, 1979; Moore *et al.*, 1978) and in the hydroid, *Campanularia flexuosa* (Moore and Stebbing, 1976). This index may also be correlated with deleterious effects on reproduction. There is no seasonal variability, although lysosomal stability is reduced temporarily by the spawning process (Bayne *et al.*, 1978). The lysosomal technique for measuring stress can be applied to a wide range of phyla (Moore, 1980), but its success depends on the presence of lysosome-rich cells or tissues.

3.2.7 Ratio between Taurine and Glycine

The ratio between taurine and glycine has been correlated with an energy deficit arising from an excess of energy respired over that consumed in laboratory studies of *Mytilus edulis* (Bayne *et al.*, 1976). In marine invertebrates, the free amino acid pools appear to be closely integrated with the physiological functions when organisms are exposed to environmental stressors. A high ratio between taurine and glycine is indicative of environmental stress, and in *M. edulis*, it parallels low physiological condition, as measured by lysosomal stability and scope for growth (Bayne *et al.*, 1979). Alterations in free amino acid concentrations and a reduction in condition index were exhibited by the bivalve, *Macoma inquinata*, exposed to oil-contaminated sediment (Roesijadi and Anderson, 1979). Changes in free amino acids, however, are complex and variable in different species and phyla, as well as in natural environmental conditions; for example, freshwater species are very low in amino acids.

3.2.8 Other Biochemical Changes

Several other biochemical changes in aquatic organisms exposed to chemical pollutants could be measured as an index of stress, but these have not been evaluated sufficiently to indicate how useful they might be. These include variations in lipid and/or carbohydrate metabolism, RNA/DNA changes, and membrane, immunological and neurochemical responses.

3.3 Morphology and Pathology

It has been recognized for a long time that pollution imposes a stress on aquatic animals, which lowers their resistance and renders them more vulnerable to disease and parasites. Pathological conditions in organisms are being increasingly correlated with pollution (see for example, Dethlefsen, 1980), at least in terms of prevalence of a disease in relation to pollution on a geographical basis. These diseases include neoplasms, skin ulceration, fin erosion, skeletal deformations, liver lesions, gill damage, genetic and embryological anomalies, parasitic infections, as well as immunological and haematological conditions; there is a considerable potential in some of these pathological conditions to serve as an index of pollution effects. Others require further research and evaluation. However, a great deal of skill and experience is necessary to achieve a statistically valid system of sampling, as well as a meaningful microscopic and histological examination. A review of pathobiological effects of pollution, incidence of pathological effects in marine species observed in the field, and recommended approaches to monitor pathobiological effects of pollution was prepared by the Pathobiology Panel at the ICES Workshop on Biological Effects of Marine Pollution and the Problems of Monitoring (Sindermann *et al.*, 1980). The following is essentially a summary of that Panel's findings.

3.3.1 Skin Ulcers

Skin ulcers have been found in fish species of coastal waters and a cause–effect relationship with pollution has been suspected. Cod, *Gadus morhua*, in northern European waters, especially around Denmark and in the German Bight have exhibited an 'ulcus syndrome' (Dethlefsen, 1978; Jensen and Larsen, 1978) which has been linked to the degree of organic-carbohydrate water pollution and potential fish pathogens, for example, *Vibrio* spp., in water. It has been suggested that a high incidence of the ulcer or red disease of the European eel, *Anguilla anguilla*, caused by *Vibrio anguillarum*, is possibly also associated with pollution. During a winter survey of the German Bight, it was found that the percentages of ulcer-infected dab, *Limanda limanda*, were higher in inshore than in offshore waters (Dethlefsen, 1978).

3.3.2 Fin Erosion

Fin erosion appears to be one of the most universal diseases in fish associated with degraded estuarine or coastal environments (Perkins *et al.*, 1972; Carmody *et al.*, 1973; Mahoney *et al.*, 1973; Nakai *et al.*, 1973; Shelton and Wilson, 1973; Murchelano, 1975; Wellings, *et al.*, 1976; Overstreet and Howse, 1977; Sherwood and Mearns, 1977; Dethlefsen, 1978). It is a non-specific disease, but there is increasing evidence of its association with pollution. There appear to be two types of fin erosion:

(1) dorsal and anal fin erosion in demersal fish, possibly as a result of direct contact with contaminated sediment; and
(2) general fin erosion, but predominantly in the caudal fin, in pelagic nearshore fish species.

The cause of fin erosion is complex but is probably a combination of such factors as chemical stress acting on the mucus and epithelium, low dissolved oxygen concentration in water and secondary bacterial invasion at least in some instances. Special care is required in surveys of fin erosion to take into account season, habitats, susceptible species, size of fish and migratory characteristics. Net damage to fins during trawling is sometimes confused with fin erosion. Contaminant levels in the environment and in fish should be measured concurrently with surveys of fin erosion to establish a relationship between fin erosion and environmental contamination.

3.3.3 Skeletal Deformities

Skeletal abnormalities in fish often involve the spinal column and include such anomalies as spinal fusions and flexures, vertebral compression or flattening and head and fin anomalies. Although such abnormalities occur in natural populations, they are found more frequently in polluted conditions. Three species of inshore fish in waters adjacent to Los Angeles and San Diego in Southern California showed a higher incidence of skeletal abnormalities, particularly gill raker deformities, than noted in the relatively clean waters along the Baja California peninsula. The cause was suspected to be pollution from metals and organochlorines. Similar skeletal anomalies have been reported in fish from polluted bays in Japan, as well as the New York Bight and Biscayne Bay, Florida.

Laboratory experiments showed that minnows developed scoliosis when exposed to the organochlorine pesticide Kepone, and spinal curvatures and vertebral fractures have been reported in fish exposed to metals (Bengtsson, 1974, 1975, 1979; Bengtsson *et al.*, 1975). Although visual observation can provide information on the more obvious skeletal deformities, X-ray examination is required for the less apparent deformities such as vertebral fusions. Fish species examined should be of the less migratory type, or at least their migration patterns should be well known.

3.3.4 Neoplasms

Neoplasms or tumours occur in all classes of aquatic cold-blooded vertebrates and bivalve molluscs. Chemicals in the environment are suspected of inducing some of these tumours or strongly influencing their induction. Liver tumours (hepatocellular carcinoma) have been reported in tomcod, *Microgadus tomcod*, from the Hudson River in New York State, English sole, *Parophrys vetulus*, from the Duwamish River near Seattle, Washington, and epidermal papillomas and/or carcinomas, have been reported in white croaker, *Genyonemus lineatus*, near sewage outfalls along the coast of California. Haematopoietic neoplasms have been noted in several species of oysters and mussels taken in estuaries along eastern, western and southern coasts in North America, western Europe and eastern Australia.

Most of the species for which neoplasms have been reported live and/or feed at the bottom where the concentration of chemicals is usually the highest. Studies conducted in Puget Sound (Malins *et al.*, 1980) showed that the incidence of liver lesions could be related to the total concentration of metals in the sediments, but there might be also a synergistic interaction with other pollutants. Stich *et al.* (1977) showed no relationship, however, between the incidence of tumours in the English sole, *Parophrys vetulus*, and the concentration of benzo(a)pyrene in the sediments.

It is essential to verify suspected neoplasms by histopathological examination to eliminate lesions that mimic neoplasia. 'Papillomas' of flatfish and gobies and the parabranchial body 'tumours' of cod are now considered to be protozoans rather than host cells, and the condition has been diagnosed as a parasitic disease rather than a neoplasm.

3.3.5 Cytological, Cytogenetic and Embryological Anomalies

Quantitative studies of cytotoxic, cytogenetic and teratogenic changes in developing fish eggs during exposure to chemicals are a promising approach to measuring pollution effects. Information is available on chromosome mutations, mitotic irregularities, abnormal differentiation at the cell level, development rate as deduced from mitotic index and on gross embryo malformation. Examination of alterations in the outer egg envelope (chorion) using a scanning electron microscope during exposure to a chemical provides useful data. Available techniques have been useful in studying the effects of oil on pelagic fish eggs at the sea surface (Longwell, 1977). Moribundicity indices for mackerel, *Scomber scombrus*, embryos have been calculated from combined cytological, cytogenetic and embryological data for several sites in the New York Bight. Egg health appears to be related to levels of heavy metals and indicator toxic hydrocarbons in the surface microlayer.

Application of cytological, cytogenetic and embryological anomalies of early-stage fish eggs to monitoring of effects of pollution requires an adequate sample size, suitable controls in uncontaminated waters and appropriate physical and chemical measurements in the study area taken simultaneously.

3.3.6 Immune Response

Fish generally are able to develop typical antibodies with a high degree of specificity. This does not appear to be so in invertebrates. According to limited evidence, pollutants cause some degree of immunosuppression. Robohm and Nitkowski (1974) demonstrated that exposure of fish to cadmium reduced the phagocytic response. Significantly higher antibody levels to 36 bacteria have been found in flounder of the polluted New York Bight area than in samples from a cleaner control area (Robohm *et al.*, 1979). There is some evidence for genetic selection of high antibody responders in summer flounder, *Paralichthys dentatus*, from polluted waters. Surveys of antibodies to facultative microorganisms, such as *Vibrio*, *Pseudomonas* and *Aeromonas*, are needed, taking into account seasonal variations.

3.3.7 Lymphocystis and Viral Infections under Pollutant Stress

Lymphocystis is a disease affecting many species, but flatfish are the most common victims. It is caused by an iridovirus and may be transmitted by parasitic copepods in some cases. There is some evidence of a higher prevalence of the disease in polluted waters, including heated effluents, which suggests that it might be a useful indicator for pollution. Certain virus diseases exist in a latent state and may be provoked into patency by environmental stressors. A baculovirus disease of shrimp and a herpes-like disease of oysters have been recently shown to respond in this way to environmental stressors. Such latent or patent viral infections may prove useful in pollution monitoring programmes.

3.3.8 Potential Use of Morphological and Pathological Effects as Indicators of Pollution

An increasing number of diseases and other pathological changes in aquatic organisms are being correlated with pollution. The potential for using pathological conditions as a monitoring tool is considerable, but some techniques are better developed than others. The Pathobiology Panel of the ICES Workshop (Sindermann *et al.*, 1980) rated the available techniques for readiness to be applied as follows: skin ulcerations, fin erosion, skeletal anomalies, and neoplasms can now be used in pilot-monitoring programmes; other approaches show promise but require further research. These include genetic and developmental anomalies of fish eggs and larvae, immunological changes and increased prevalence of such viral diseases as lymphocystis. The remaining approaches have potential for the future but require much developmental work and consist of gill hyperplasia, scale disorientation, changes in parasite frequencies, haematological changes, epidermal changes and liver pathology. The Panel concluded that: 'There is no single disease or pathological sign that could serve as a universal indicator of pollution.' It recommended that pathology be considered an important component of a series of approaches used to monitor effects, including physiology, biochemistry, genetics and ecosystem analysis, and should be conducted in concert with physical and chemical measurements of pollution.

3.4 Genetics

The presence of a chemical agent in the aquatic environment in sublethal concentrations will exert a selective pressure on aquatic species. As a result, changes in the composition of the gene pool may occur where a shift is made in the average phenotype to those with an adaptive advantage. On this basis, Luoma (1977) explicitly recommended genetic monitoring. Pollutants can also act directly upon genetic material to produce mutations, and it is recognized that gene mutations tend to be recessive and deleterious, thereby reducing the general fitness of the population (Muller, 1950). Genetic responses should be evoked by pollution in all aquatic organisms ranging from bacteria to marine mammals. The following is a brief résumé of the report of the Genetics Panel in the ICES Workshop on Biological Effects of Marine Pollution and the Problems of Monitoring (Beardmore *et al.*, 1980).

A high degree of genetic variability appears to be the normal state of most outbreeding species (Nevo, 1978). Electrophoretic assay has been used for gene pool studies in a variety of marine species (Nevo *et al.*, 1977, 1978; Battaglia and Beardmore, 1978; Battaglia *et al.*, 1980), and may be used to study adaptive shifts, as carried out on the fish *Fundulus heteroclitus* to study the effects of thermal pollution (Mitton and Koehn, 1975). The level of genetic variation, as measured by the mean heterozygosity, appears to be lower in populations in polluted areas than in the same species in comparatively unpolluted areas. The adaptive response of some species able to exploit polluted areas has been demonstrated, for example, *Nereis* adaptation to high levels of metals in sediments (Bryan, 1973) and *Capitella capitata* tolerance to polycyclic aromatic hydrocarbons (Lee and Singer, 1980). Based on the observations that summer flounder, *Paralichthys dentatus*, from highly polluted areas in the New York Bight have antibacterial serum antibody levels that are higher and more diverse than summer flounder from relatively unpolluted areas, a hypothesis has been made that this difference stems from the presence of greater numbers of bacteria in the polluted areas and that this is reflected in greater production of antibodies by fish in such areas.

Many chemicals entering aquatic systems from man-made sources can cause changes in genetic material (Payne and Martins, 1980). Mutations of genes or chromosomes may give rise to disturbances in cell differentiation. Gametes and zygotes may be affected by chromosomal damage that arises spontaneously, and many chromosomal aberrations lead to early zygote death (Longwell and Hughes, 1980). The latter authors showed that the incidence of chromosome abnormalities in the fertilized eggs of mackerel in the New York Bight varied widely, suggesting that the increased evidence of chromosomal mutation might be generated by pollution. It is believed that the frequency of induced simple chromosomal aberrations bears a simple relationship to dose.

According to Ames and Hooper (1978), up to 90% of chemical carcinogens are mutagens. It has been demonstrated (Matsushima and Sugimura, 1976; Khudoley and Syrenko, 1978) that tumours can be produced in fish, bivalves and echinoderms with some known mutagens. A rapid method for determining mutagenic activity has emerged with development of Ames strains of bacteria. Parry *et al.* (1976) have developed sensitive methods for detecting mutagens, based on the fact that mitotic gene conversion is increased by mutagens.

It has been concluded by the GESAMP Working Group on Monitoring Biological Variables Related to Marine Pollution (GESAMP, 1980) that none of the effects measurements discussed by the Genetics Panel at the ICES Workshop on Biological Effects of Marine Pollution can be recommended at present. Chromosomal damage assessment was considered useful but costly in terms of trained personnel and high quality microscopes, and it may be relatively insensitive. The mutagenicity assay was regarded as useful for testing chemicals but difficult to assess in terms of the criteria used for selecting variables for monitoring biological effects. It was noted, however, that genetic effects measurements have long-term potential and may be useful after further development and evaluation in water pollution studies.

3.5 Behaviour

The simplest form of behavioural response is exemplified by avoidance in choice tests with aquatic species. The Y-shaped trough, with a chemical substance in one area and uncontaminated water in the other, for example, offers aquatic organisms a choice between clean and contaminated water during movement.

Sprague and Drury (1969) demonstrated in the laboratory that rainbow trout, *Salmo gairdneri* R., and Atlantic salmon, *Salmo salar* L., will avoid low concentrations of certain pollutants. Avoidance of kraft pulpmill effluent in seawater of various salinities by pink salmon, *Oncorhynchus gorbuscha*, acclimated to seawater, was investigated by Greer and Kosakoski (1978). Kleerekoper (1976) conducted rather complex experiments to show how the behavioural responses in fish could be influenced by the multiple type of alterations in the aquatic environment, with a gradient in metal concentrations superimposed on a thermal gradient. The blue carb, *Callinectes sapidus*, burrows into the sediments during the winter period, and any pollutant which disrupts this adaptive burrowing behaviour can lead to heavy predation or winter kill due to cold (Olla *et al*., 1980a,b). The combination of high body loads of DDT in this species and the rapid reduction of temperature was found to disrupt the usual burrowing behaviour and cause extensive mortality in areas of DDT contamination (Koenig *et al*., 1976).

Chemoreception can be interfered with in aquatic organisms by pollutants, and this can have serious consequences in searching for food, seeking mates for reproduction, possibly avoiding predators and in homing on parent streams during spawning migrations (Olla *et al*., 1980a,b; Pearson *et al*., 1981). Pearson and Olla (1977) noted, by measuring changes in the antennular flicking rate occurring with changes in gill-bailing activity, that the blue crab, *Callinectes sapidus*, could detect food extract at a concentration as low as 10^{-12} mg/l. By applying the same technique to Dungeness crab, *Cancer magister*, Pearson *et al*. (1979) showed that the detection threshold for a food extract was 10^{-7} mg/l. At a concentration of 0.27 mg/l of crude oil in Prudhoe Bay, Dungeness crabs changed antennular orientation and increased antennular flicking rate when presented with a clam extract (Pearson *et al*., 1981). Male crabs of the species *Pachygrapsus crassipes*, display certain postural responses to the sex pheromone, crustecdysone (Kittredge *et al*., 1971). Exposure to the water-soluble fraction of crude oil or naphthalene was observed to inhibit both this response and feeding behaviour (Takahashi and Kittredge, 1973; Kittredge *et al*., 1974). Lobsters *Homarus americanus* when exposed to crude oil, required a longer time to start searching for food than either control animals or those exposed to the water-soluble fraction of crude oil (Atema and Stein, 1974).

Equilibrium in fish can be affected by uptake of cadmium (Rosenthal and Alderdice, 1976), and some fish exposed to certain pollutants have difficulty in maintaining a normal swimming position when a torque is applied by a rotating cylinder (Lindahl and Schwandom, 1971).

Most behavioural experiments have been conducted in a laboratory environment. For a fuller appreciation of the effects of sublethal concentrations of pollutants on the behaviour of fish in nature, it is essential to conduct field experiments. Although such studies are difficult to execute, some effort has been made in this direction. Saunders and Sprague (1967) were able to show a significant effect of metals from tailings of a copper–zinc mining operation on the spawning migration of Atlantic salmon. Anderson (1971) demonstrated that a DDT concentration as low as 20 ng/g reduced a conditioned response (propeller tail reflex) in trout, and that very low concentrations of organochlorines affected the learning ability of fish. Birtwell (1977) studied the avoidance of pulpmill effluent by Pacific salmon in an estuary, using a long partitioned chamber suspended in the water column that allowed fish a choice during migration between high effluent concentrations in surface water and relatively low concentrations in deeper water.

The structure of an entire community can be affected by loss of a particular species. For example, the limpet, *Patella vulgata*, was found to have its behavioural patterns affected by oil which prevented it from holding fast to the rocks (Dicks, 1973, 1976). Many of such dislodged and paralysed limpets were unable to survive. Since these grazers maintain the balance between algal and animal abundance, the community structure of the rocky intertidal was changed. Pollutants can have an impact on trophic interactions through behavioural responses. If a prey species is less sensitive to a pollutant than the predator species, or if a predator's normal behaviour is changed so that it cannot effectively feed, there will obviously be a change in the predator/prey relationship (Woltering *et al.*, 1978). Larvae of lobster, *Homarus americanus*, were paralysed temporarily by exposure to oil (Wells and Sprague, 1976). Without being able to swim they may become easier prey for predators as they settle in the water column.

Zooplankton exhibit certain behavioural responses to environmental changes that may be useful as an index for measuring effects of pollution. Locomotor activity may increase at low exposure levels and decrease as acute lethal levels are approached (Miller, 1980). With increasing metal concentrations, the animals exhibit an increase in their rate of change in direction (DeCoursey and Vernberg, 1972; Lang *et al.*, 1979). Light-oriented behaviour (phototaxis) may be altered under a pollutant stress. Mercury (Vernberg *et al.*, 1973), oil (Bigford, 1977), insect growth regulators (Forward and Costlow, 1976, 1978) and copper (Miller, 1980) are known to alter phototaxis in several species of invertebrate meroplankton. It is unknown whether pollutants interfere with the use of light intensity as a cue for diel vertical migration in holoplankton (Forward, 1976; Longhurst, 1976).

The Behaviour Panel of the ICES Workshop on Pollution Effects Monitoring qualified their recommendation for the application of behavioural tests by noting that the maximum effectiveness would come with a suite of different behaviours and organisms, the choice of which would depend on the nature of the particular ecosystem and the classes of dominant pollutants (Olla *et al.*, 1980a,b). GESAMP (1980) suggested that the effects of abnormal behaviour, for example, altered distribution of fish due to an avoidance response, may be more readily observed and used than the immediate behavioural response.

3.6 Ecological Studies

It has been emphasized (ICES, 1978) that the response of communities and populations to pollutants is non-specific and is difficult to distinguish from natural changes. The Ecology Panel of the ICES Workshop on Biological Effects Monitoring noted, however, that ecological monitoring provides the only real test on effects of pollutants on communities and populations (Gray *et al.*, 1980). The Panel reiterated that the natural variability in any response must be determined before the effects can be categorically attributed to pollution.

Techniques for studying the marine ecological impact of pollutants have been reviewed by Waldichuk (1973), Gray (1979, 1980) and Gray *et al.* (1980). Population structure requires that year-classes be distinguishable and quantifiable, if possible. Jones (1979) demonstrated how this could be done with such bivalves as *Cerastoderma edule*, which carry annual growth-check marks. The need for long time series of observations in undisturbed aquatic systems has been repeatedly stated, in order to provide a pattern of natural variability against which the chronic effects of man can be measured. The design of a sound sampling strategy is perhaps the most critical to any meaningful ecological monitoring programme (Green, 1979; Gray *et al.*, 1980). Fisheries statistics on stocks, recruitment and harvesting are often the longest sets of data on living resources available, but there are few situations where environmental data have been collected simultaneously with fisheries data. Such data provide a basis for constructing models on the impact of harvesting on fish stocks, but not for the impact of pollution on these living resources. Often the year-to-year variations in fish stocks are so large, because of natural fluctuations in recruitment and fishing pressure, that any effect of pollution, except in the case of unusual incidents, is totally obscured. Outside of fisheries data, there are some long-term records on plankton, such as those for the North Atlantic from the Continuous Plankton Recorder (Longhurst *et al.*, 1972), which have covered a period of over 40 years and show that there are cycles of up to 20 years in oceanic planktonic species. The collection of data is one necessary phase of such long-term studies, of course, but the statistical analysis of these data by advanced techniques (see, for instance, Platt and Denman, 1975) is what eventually will separate out the natural fluctuations and effects of harvesting and establish the effects of pollution.

The acute effects of pollution, such as that from sewage and pulp and paper mill effluents (Pearson, 1975; Pearson and Rosenberg, 1978) and from oil spills (Sanders, 1978) can be measured relatively simply, because the changes in certain communities and populations are quite large. The areas covered by such point-source effects are comparatively localized and the period of study is often less than 10 years. However, even in such situations the judicious selection of the appropriate trophic level for a study of the impact is essential, and application of the most effective statistical approach to conclusively demonstrate the effects of pollution is vital.

The benthos is often the group of animals chosen to monitor biological effects of pollution because they are usually resident in the area, with relatively restricted migratory movements, and they tend to integrate the effects of pollution over time. Biomass changes and the number and diversity of species are usually the simplest types of observations that can be made. The log-normal distribution of individuals among species has been found to be a rather useful analytical technique to describe changes in a wide variety of animal communities in both polluted and unpolluted systems (Krebs, 1972; May, 1975; Gray, 1979, 1980). In a large sample from a heterogeneous community, a log-normal distribution can be expected, since populations tend to increase geometrically. If the interaction effects of many randomly operating environmental factors are summed, they also give a log-normal distribution (May, 1975). While the log-normal distribution of individuals among species has no intrinsic biological meaning, inasmuch as it can be the net result of many different processes, it can provide a representation of changes in communities arising from pollution. Gray (1979, 1980) has demonstrated its application to the benthos of Loch Eil, Scotland, which was subjected to the input of pulp and paper mill wastes (Pearson, 1975).

This is only one example of many approaches that can be taken to measure the effects of pollutants on aquatic ecosystems. It is clear that all approaches, even those measuring the acute effects, require a comparatively long time. The key to analysis of any long time series of data, of course, is an appropriate statistical evaluation. Gray *et al.* (1980) summarize briefly some statistical tools that are available for such evaluation. Green (1979) provides details of statistical methods for environmental studies. Clearly, the response time in ecological studies must be regarded as very slow. Moreover, the signal-to-noise ratio is low, because of the enormous amount of natural variability in the system. For purposes of monitoring biological effects arising from pollution, measurement of many ecological effects can be recommended, more for the purpose of an early-warning system rather than for a rapid measure of effects.

GESAMP (1980), in evaluating various ecological approaches, noted that ecological conditions can be initially assessed by measurements of community biomass, abundance, diversity indices and alterations in the distributions of selected species. More detailed observations can be made at greater expense, if a higher level of training in sampling and statistical procedures is available, by analysis of community and population structure, and estimation of growth rate and fecundity. Although growth rate is an important biological effect, its accurate measurement in invertebrates is usually difficult. Estimates of growth rate from shell length, body length or weight changes often neglect gamete production, which may constitute a large proportion of biomass in adult stages. Gamete production represents a component of growth that is the most likely to be affected by pollution and other forms of stress.

4 SELECTION OF BIOLOGICAL VARIABLES FOR MEASUREMENT

Most of the overview-type sources of information (ICES, 1978; GESAMP, 1980; McIntyre and Pearce, 1980) used in the preparation of this paper deal with biological variables and measurement techniques useful for monitoring purposes in the marine environment. Such techniques may not always be the best for rapid measurement of the effects of chemicals on aquatic animals, inasmuch as the response time may not be as important in monitoring as in providing an immediate diagnosis of a pollution effect. However, response time as well as 12 other criteria (Table 1) were used in selecting variables for biological monitoring by a GESAMP Working Group on Monitoring Biological Variables Related to

Table 1 Criteria[a] for selection of biological variables for inclusion in a water pollution monitoring programme. Adapted from GESAMP, 1980 and reproduced with permission

Category A. Fundamental scientific aspects of assessing the biological impact of an environmental change

(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 of 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.

Category B. Efficiency of biological measurements and their practical value as indices of impact

(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?

Category C. Administrative questions which may be important in selecting indices for inclusion in monitoring programmes

(1) *Cost*: How expensive is the measurement of the variable in terms of equipment purchase, 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?

[a] Neither the categories nor the criteria in each category are listed in order of priority.

Marine Pollution (GESAMP, 1980). It is believed that this is a good basis for initial selection of measurements to determine effects of chemicals on aquatic animals.

The GESAMP (1980) Working Group noted that a technique for measurement of effects will not score high by all the criteria given in Table 1 at any one level of biological organization, because some criteria, such as 'ecological significance' and 'sensitivity'/'specificity' may be inversely related over the range of organizational levels. As an example, the Working Group cited the case where the effects at higher levels of organization (population, community) are generally more significant ecologically but relatively insensitive and non-specific, in contrast to lower levels of organization (cellular and molecular), which are usually more sensitive and specific but ecologically less significant. In a biological effects monitoring programme, there may be a requirement for highly specific or for general, non-specific biological effects measurements, or both. The Working Group felt that it is necessary to include biological effects at several levels of organization, in order to meet the basic requirements of both generality and specificity. Such an approach is probably also essential for selecting methods for measurement of the effects of chemicals as indicators of ecological damage.

Based on the 13 criteria given in Table 1, the GESAMP (1980) Working Group assessed 37 biological variables in 7 categories covered by the 7 panels in the ICES Workshop on Biological Effects Monitoring (McIntyre and Pearce, 1980). The variables were allocated a score on a 3-point scale, based on the assessment. These are reproduced in Table 2, with 'primary production' deleted as being irrelevant to this exercise. A total of 21 measurement techniques are listed as being highly recommended for biological effects monitoring. This number would probably be reduced to about one-half for comparatively routine measurements that could provide a rapid assessment of the effects of chemicals on aquatic animals. But one would be rather foolhardy to attempt selecting one universally applicable technique to make such an assessment.

An estimate of risk associated with introduction of a given chemical into an aquatic system can be acquired by a step-sequence procedure, as outlined by Lloyd (1980). Four stages in such a procedure have been recommended:

(1) screening tests,
(2) predictive tests,
(3) confirmative tests, and
(4) monitoring.

In the first stage, the physical and chemical properties of a substance and its patterns of use and discharge are obtained. The maximum aquatic concentration likely to occur is estimated. The species that must be protected are then reviewed. The ratio of the maximum predicted environmental toxicant concentration to the maximum acceptable toxicant concentration provides an estimate of the possible degree of risk.

Table 2 Biological variables that can be measured for an estimate of pollution effects and their ranking. Adapted from GESAMP, 1980 and reproduced with permission

Measurement	Ranking[a]	References[b]
(1) PHYSIOLOGICAL EFFECTS		
Respiration	3	1–3
Feeding rate	1	4–11
Body condition index	1	10, 12
Scope for growth (+ growth efficiency)	1	13–21
Oxygen/nitrogen ratio	1	13, 14, 20, 22, 23
(2) BIOCHEMICAL EFFECTS		
Mixed function oxidase	2	24–34
Metallothionein	2	35–40
Lysosomal stability	1	16–18, 41–43
Steroids	3	44–49
Energy charge	2	50, 51
Blood chemistry	2	52–59
Taurine/glycine ratio	1	16, 17
(3) MORPHOLOGICAL AND PATHOLOGICAL EFFECTS		
Gill deformity	3	60–62
Liver structure	2	61, 63
Gametogenic cycle	2	61, 64, 65
Liver (as % body weight)	1	61, 63
Ulcers	1	61, 66
Fin erosion	1	61, 62, 67–75
Neoplasia/tumours	2	61–63, 73, 76
Asymmetry	1	61, 77–80
Early developmental stage	2	61, 64, 81–89
(4) GENETIC EFFECTS		
Chromosomal abnormalities	3	65, 90
Mutagenicity assays	3	90–94
(5) BEHAVIOURAL EFFECTS		
Torque test	2	95, 96
(6) ECOLOGICAL EFFECTS		
Community biomass	1	97–102
Abundance	1	97–104
Diversity	1	97–104
Alterations in distribution	1	97, 100–103
Growth rate	1	97, 105–115
Reproduction (gonad as % body weight)	1	97, 110, 116
Population structure	1	97, 117
(7) BIOASSAY		
Bivalve/echinoderm larvae	1	118–125
Microalgae bioassays	1	118, 126
Hydroid bioassays	1	118, 127
Other organism bioassays	1	118, 128–132

Further testing beyond stage 1 may be unnecessary if the degree of risk is considered acceptable or if screening tests demonstrate a totally unacceptable risk. Predictive tests may be necessary to obtain a more accurate measure of the minimum concentrations likely to cause unacceptable harm to aquatic biota. Multispecies tests should be given priority if one bears in mind the protection of the aquatic ecosystems. Selection of appropriate test methods which have a high predictive capability (EIFAC, 1975; Mayer and Hamelink, 1977; Cairns *et al.*, 1978) is essential. Confirmative tests involve the testing of the validity of results from the predictive tests under simulated or actual field conditions. Monitoring provides a means of observing the effects of discharging a particular substance on water quality and on biota. Concentrations of the substance may be measured in

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Footnote to Table 2

[a] *Basis for ranking system*

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

2 Recommended only for selective use, because it is more costly or requires further field testing before it can be used routinely;

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

[b] *References*

1. Carlson and Drummond, 1978; 2. Heath, 1972; 3. McMahon and Wilkens, 1977; 4. Blaxter, 1977; 5. Abel, 1976; 6. Dorn, 1976; 7. Reeve *et al.*, 1976; 8. Wells and Sprague, 1976; 9. Berdugo *et al.*, 1977; 10. Roesijadi and Anderson, 1979; 11. Gordon *et al.*, 1978; 12. Bayne *et al.*, 1980; 13. Bayne, 1975; 14. Bayne and Widdows, 1978; 15. Gilfillan, 1980; 16. Bayne *et al.*, 1979; 17. Bayne *et al.*, 1976; 18. Bayne *et al.*, 1978; 19. Gilfillan and Vandermeulen, 1978; 20. Widdows *et al.*, 1981; 21. Edwards, 1978; 22. Lloyd and Swift, 1976; 23. Widdows, 1978; 24. Burns, 1976; 25. Payne, 1976; 26. Stegeman and Sabo, 1976; 27. Kurelec *et al.*, 1977; 28. Stegeman, 1978; 29. James and Bend, 1982; 30. Ahokas *et al.*, 1976; 31. Krasny and Holbrook, 1977; 32. Lee and Singer, 1980; 33. Stegeman, 1980; 34. Singer and Lee, 1977; 35. Olafson and Thompson, 1974; 36. Noel-Lambot, 1976; 37. Talbot and Magee, 1978; 38. Jennings *et al.*, 1979; 39. Bremner, 1974; 40. Brown *et al.*, 1977; 41. Brown and Parsons, 1978; 42. Moore, 1980; 43. Moore *et al.*, 1978; 44. Peakall, 1967; 45. Sangalang and O'Halloran, 1973; 46. Freeman and Idler, 1975; 47. Freeman *et al.*, 1975; 48. Freeman and Sangalang, 1977; 49. Uthe *et al.*, 1980; 50. Giesy *et al.*, 1978; 51. Ivanovici, 1980; 52. Black *et al.*, 1966; 53. Fujiya, 1961; 54. Iwama *et al.*, 1976; 55. McLeay and Gordon, 1977; 56. Noggle, 1978; 57. Ruddell and Rains, 1975; 58. Thurberg *et al.*, 1978; 59. de Bruin, 1976; 60. Sindermann, 1979; 61. Sindermann *et al.*, 1980; 62. Dethlefsen, 1978; 63. Malins *et al.*, 1980; 64. Longwell, 1977; 65. Longwell and Hughes, 1980; 66. Jensen and Larsen, 1978; 67. Perkins *et al.*, 1972; 68. Shelton and Wilson, 1973; 69. Nakai *et al.*, 1973; 70. Mahoney *et al.*, 1973; 71. Carmody *et al.*, 1973; 72. Murchelano, 1975; 73. Wellings *et al.*, 1976; 74. Sherwood and Mearns, 1977; 75. Overstreet and Howse, 1977; 76. Stich *et al.*, 1977; 77–79. Bengtssen, 1974, 1975, 1979; 80. Bengtsson *et al.*, 1975; 81. Burdick *et al.*, 1964; 82. Helle *et al.*, 1976; 83. Daye and Garside, 1980; 84. Calabrese *et al.*, 1973; 85. Rosenthal and Alderdice, 1976; 86. Wells and Sprague, 1976; 87. Rice and Harrison, 1977; 88. Armstrong and Milleman, 1974; 89. Linden, 1976; 90. Beardmore *et al.*, 1980; 91. Payne and Martins, 1980; 92. Ames and Hooper, 1978; 93. Matsushima and Sugimura, 1976; 94. Khudoley and Syrenko, 1978; 95. Olla *et al.*, 1980a,b; 96. Lindahl and Schwanbom, 1971; 97. Gray *et al.*, 1980; 98–99. Gray, 1979, 1980; 100. Pearson, 1975; 101. Pearson and Rosenberg, 1978; 102. Krebs, 1972; 103. Sanders 1978; 104. May, 1975; 105. Webb and Brett, 1972; 106. McLeay and Brown, 1974; 107. Bayne, 1975; 108. Bayne and Widdows, 1978; 109–112. Bayne *et al.*, 1976, 1978, 1979, 1980; 113. Edwards, 1978; 114. Brett, 1979; 115. Gilfillan, 1980; 116. Giese, 1967; 117. Jones, 1979; 118. Stebbing *et al.*, 1980; 119. APHA/AWWA/WPCF, 1981; 120–122. Woelke, 1965, 1967, 1972; 123. Maciorowski and Clarke, 1980; 124–125. Kobayashi, 1971, 1974; 126. Smayda, 1974; 127. Stebbing, 1980; 128. Davis, 1976; 129. Davis and Hoos, 1975; 130. Walden, 1976; 131. Åkesson, 1980; 132. Nimmo *et al.*, 1977.

water, sediments and tissues of aquatic organisms. The response of organisms to environmental and tissue concentrations of the substance might be observed. Laboratory data should be available with which one could correlate the environmental or tissue concentrations of the substance with a specific or general response of a selected species.

The estimation of risk in aquatic animals exposed to a chemical or mixture of chemicals is still highly subjective. It depends a great deal not only on good bioassay data but also on good judgment that can only be acquired by experience. Bayne *et al.* (1981) discussed certain practical aspects in the measurement of pollution effects on bivalve molluscs and some possible ecological consequences. Models are often of little help because they seldom take into account all the variables which may be important in the final ecological impact that a substance discharged into the aquatic environment could have. And even in the case where the initial impact may be considered acceptable, long-term effects may manifest themselves in due course, and a good monitoring programme to document any year-to-year changes is essential.

5 CONCLUSIONS

There are a host of tests that can be applied in biological assays to measure the various effects of a chemical on individual aquatic animal species in the laboratory. Some of these tests have potential for application in monitoring the effects of chemicals in field conditions. So far, most of these tests have been applied to commercially important species or to organisms that can be easily maintained in the laboratory or are convenient species to monitor in the field, such as the mussel, *Mytilus edulis*. Predictive testing and extrapolation of results to higher levels of biological organization has not advanced far enough to be universally applicable in studies of aquatic ecosystems. Use of the microcosm approach (Sugiura *et al.*, 1976a,b, 1982; Giesy, 1980) offers good possibilities for evaluating the impact of pollutants on aquatic ecosystems.

The choice of suitable techniques for measurement of the effects of chemicals on aquatic animals as indicators of ecological damage should be guided by the extent of injury the chemicals might be expected to inflict on the community of organisms or the population of a species. It has been argued that unless an effect has consequences at the population level it is insignificant . Clearly, an effect on reproduction can have dire consequences at the population level of a given species. From that point of view, measurement of physiological and biochemical effects of chemicals should provide the type of information that would be most relevant. If the community structure is altered by introduction of a substance, the ecological effect must be considered as significant. Ecological studies require a long time series, extending over several years or even decades, in order that natural fluctuations can be separated from the effects of pollutants. Statistical evaluation of confidence in such data by appropriate methods is the only way that the significance of changes due to a chemical can be established.

There is no single type of measurement that can provide a universally applicable test for the effect of chemicals on aquatic animals. A suite of measurements is required to provide meaningful information on the impact of a chemical or a mixture of chemicals on aquatic living resources. A multifactorial type of model is needed for input of data from a suite of measurements in order to provide an output that can be applied for controlling water pollution and protecting all indigenous aquatic species.

An estimate of risk to aquatic animals from exposure to chemicals over a long-term period cannot be quantitatively achieved at the present time. In most cases of pollution control, a great deal of informed judgment is used in establishing acceptable pollutant levels to meet recognized water quality criteria for protection of aquatic organisms and ecosystems. Such an approach has been generally successful in preventing adverse short-term ecological changes. However, long-term effects sometimes arise which cannot be predicted, especially if there is a synergistic interaction with other man-made pollutants. There is a need for a systematic approach to solving such problems. This may soon be possible with the increasing amount of toxicological and ecological data, and the availability of advanced techniques of statistical analysis and electronic data processing and computations.

After examining 35 different biological variables that can be measured for estimating pollution effects on physiological, biochemical, morphological, pathological, genetic, behavioural and ecological processes in marine organisms, a GESAMP (1980) Working Group on Monitoring Biological Variables Related to Marine pollution recommended 21 types of measurement that could be applied immediately in monitoring programmes in all regions. While no attempt was made to identify any of the specific measurements as providing a good index of the impact of a pollutant on the marine ecosystem, it is clear that the ecological measurements of community biomass, abundance, diversity, alterations in distribution, growth rate, reproduction and population structure should provide a good indication of any ecological damage that might occur.

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