This Report not to be cited without prior reference to the Council*

International Council for
the Exploration of the Sea

CM 1976/E:44
Fisheries Improvement Committee

ICES WORKING GROUP ON POLLUTION BASELINE AND MONITORING STUDIES IN THE OSLO COMMISSION AND ICNAF AREAS

REPORT OF THE SUB-GROUP ON THE FEASIBILITY OF EFFECTS MONITORING

CONTENTS

---

* General Secretary, ICES, Charlottenlund Slot, DK-2920 Charlottenlund, Denmark.

ABSTRACT

This report first discusses selected recent literature on the effects of pollutants on marine organisms under six headings - biochemical, morphological, physiological, behavioural, population/community and genetic, and examines the ways in which these effects are measured experimentally, including the use of bioassay procedures. It then goes on to consider effects in the light of their potential value in monitoring programmes. While some symptoms could probably be measured in the sea, there would in most cases be great difficulty in recognising them as effects of specific pollutants, and in distinguishing pollution - linked from naturally-caused events. This is particularly so in population and community studies and the difficulty in using population-related observations is discussed. Most behavioural and genetic effects also seem difficult to apply in a monitoring context. However certain biochemical, morphological and physiological effects measured on individuals may be useful, and bioassay procedures are pertinent.

The relevance of existing effects data to current baseline and monitoring programmes is discussed, and it is concluded that effects and monitoring studies cannot easily be linked at present, because whereas monitoring programmes tend to provide information on residues in organisms, most effects studies relate effects to environmental concentrations of contaminants. There is an urgent need to link environmental concentrations, body burdens and affects.

In spite of the difficulties it is considered that a start should be made in assessing the possibility of effects monitoring, and the following four-part approach is suggested.

1. Observations on some or all of the following items should be included in on-going biological survey programmes to begin the building up of a picture of the well-being of organisms in various geographical areas: liver/somatic and gonad/somatic indices; vertebral deformities; tumours, lesions etc; gill damage; general morphology.

2. Since some of the best monitoring data currently available refer to residues in organisms, it is suggested that experiments should be conducted to link effects with residues, as well as with levels in the water or sediment, and that monitoring programmes should be designed to include relevant tissues or organs.

3. Suitable bioassay techniques should be adapted from the wide range of procedures available, to identify regions of poor water quality and to provide a test of the applicability of experimental results to field situations.

4. A number of effects are recognised as potentially useful in monitoring, but none are thought to be sufficientlyunderstood at present to justify their immediate addition to monitoring programmes. It is suggested however that some of them are worthy of urgent consideration, and that five topics, namely, scope for growth, gill damage, vitellogenesis, lysosomal enzymes and steroid metabolism should be subjected to a concentrated research effort for not less than two years (linked via field validation trials to on-going research programmes) to evaluate their use as monitoring tools.

These proposals recognise that for adequate effects monitoring no single procedure will be sufficient in itself, but it is felt that the work outlined above could lead in the short term to a closer association between the chemical and biological data that together are necessary for the identification of areas at risk from pollution, and in the longer term to a suite of techniques for the detection, measurement and evaluation of effects in the field.

MEMBERSHIP AND TERMS OF REFERENCE

The sub-group consisted of a core of six members, but additional advisors were drawn in either to attend specific meetings or to contribute verbally or by correspondence. (Appendix I).

The terms of reference were as follows:

(1) To review the present state of knowledge relating to the effects of marine pollutants on living resources and their exploitation.

(2) To examine how such effects may be demonstrated or measured experimentally; how the results of such work may be interpreted and applied to their detection and evaluation in the field, including their relevance to data from current baseline and monitoring programmes.

INTRODUCTION

The sub group was asked to review the present state of knowledge on the effects of marine pollutants on living resources, as well as to examine the experimental demonstration and measurement of these effects and their interpretation and evaluation in field and monitoring situations. We have excluded problems of radio-activity from our considerations, since these are well covered elsewhere.

Initially, it may be useful to recognise what is understood by the term "effects". One basic effect of pollution can be the accumulation in organisms of contaminants present in the water, the sediment, or the food. These residues can usually be measured with reasonable accuracy, and to this end a number of survey programmes have been set up in various parts of the world.

For the purpose of this report, however, the concept of "effects" is taken to be more extensive, and to imply a stage at which the organism demonstrates some response to its body burdens or to environmental contamination.

A preliminary appraisal of relevant literature indicated that a comprehensive review would be a lengthy task beyond the current resources of the sub-group. We have therefore tried to focus mainly on ecologically meaningful effects and those most likely to be useful in monitoring, by selecting recent studies dealing with contamination levels which might be encountered in the field, and with effects which would seem likely to set the organism at some disadvantage, or pose a threat to the long term survival of the population. Clearly, this involves considering whole organisms or populations, but if effects can be recognised at lower levels of organisation, in tissues or cells, it may be possible to detect a threat at an earlier time, or by a more convenient method. Further, the examination of biochemical or cytological events might enable the effects of specific pollutants or classes of pollutants to be identified in situations of environmental complexity where the response of the individual organism or the population allows the assessment only of a general syndrome of effect, with the impact of the specific causative agent masked by other events. We have therefore dealt with a wide range of organisational levels.

Turning again to the terms of reference, we have taken a broad view of "living resources" and while paying due attention to fish and shellfish of commercial importance, we have taken the view that any adverse effect on the food web can have implications for exploitable living resources.

In considering the measurement and demonstration of effects we have recognised the value of the bioassay approach, and have devoted a section to this.

The report therefore takes the form of a selective discursive review of recent work on pollution effects on marine organisms, followed by a consideration of the relevance of such effects to field situations and the possibilities of their inclusion in a biological monitoring programme.

EFFECTS OF MARINE POLLUTION ON ORGANISMS

Since knowledge of effects is usually gained in practice by experimental demonstration or measurement, we have combined the first of our terms of reference with part of the second to produce a single section. Our prime concern is not so much with specific pollutants as with their effects, so this section is structured under 6 main heads - biochemical, morphological, physiological, behavioural, population/community and genetic effects.

BIOCHEMICAL EFFECTS

Pollutants will, depending on concentration, affect many biochemical processes within organisms. Some of these processes will effect a change in the physiological performance of the organism. For some others, however, the organism may compensate for a change in one biochemical system by altering a related process in such a way that no decline in physiological performance occurs. In the context of monitoring the effects of pollution, the former category of biochemical response, where a change is reflected in altered physiological performance, is the more useful. In these circumstances the biochemical change may occur more rapidly than the physiological response and so provide earlier warning of a potential pollution effect. In addition, knowledge of the biochemical effects of pollutants may facilitate more understanding of the direct causes of a change in physiological or population condition. In what follows we review briefly certain aspects of biochemical change that are likely to result in overt physiological damage to the organism, and which show promise as monitors of the action of pollutants.

One such category is change in the chemistry of the blood plasma and/or in the formed elements of the blood. Such changes are well recognised in man and are the basis for the medical speciality of clinical chemistry. Attempts are currently underway to adapt the techniques of this speciality for use in the study of pollution effects in marine organism, and some information is available on the effects of various pollutants on the blood of certain species of fish. However, as with many other aspects of biological effects of pollutants, we need much more base-line data on blood chemistry, in order to describe normal seasonal and population variation before the significance of pollution-induced changes can be properly assessed for effects monitoring.

With regard to metals, for example, Larsson (1975) has demonstrated that cadmium in the water, at levels of 5 and 50 ppb, produced blood anaemia (ie significant reductions in haematocrit and haemoglobin) in 4 weeks at the high level and 9 weeks at the low level, in eel, perch and flounder. Nine weeks exposure at 50 ppb resulted in a marked reduction of blood glucose. At higher levels, 0.1-1.0 ppm of cadmium, plasma electrolytes were affected, potassium and calcium being reduced and magnesium and inorganic phosphate elevated. On the other hand, Calabrese _et al_ (1975) exposed winter flounders to 5 and 10 ppb of cadmium and detected no haematological effects, whereas Strik _et al_ (1975) observed an increase in haematocrit and haemoglobin concentration in rainbow trout after 15-22 days exposure to 10 ppm chromium. This type of variability in the dose/response relationships in different species at different metal concentrations argues for the need for more fundamental experimental studies.

Haematological responses of fish to organic pollutants have also been documented. Endrin at 1 ppb caused elevated serum levels of sodium, potassium, calcium and cholesterol in the northern puffer (Eisler and Edmunds, 1966) while glucose in fish was increased by 2 ppb dieldrin (Silbergeld, 1974). Brown trout fed with PCB (5 mg/kg) appeared normal after six weeks, but there was a significant increase in haemocrit, haemoglobin and cholesterol (Larsson, 1973). Other effects of PCB on the blood chemistry of European eel and of rainbow trout are described by Johansson-Sjöbeck et al (1975) and recent studies of haematological effects in fish toxicity experiments have been reviewed by Strik (1974).

Another relevant line of investigation, also involving the measurement of aspects of blood chemistry, arises from the fact that a phospho-protein (vitellogenin) concerned with yolk formation occurs in the blood serum of fish; the annual cycle throughout the year has been studied in the flounder (Korsgaard-Emerson et al, 1975). It might be possible to use deviations from the annual cycle as a measure of the normality or otherwise of vitellogenesis, and ultimately of larval development, in a given fish stock.

Another category of biochemical effect of pollution concerns the function of specific enzymes. The effects of phosphorus and carbonate insecticides on the enzyme cholinesterase are well-known (Cornish, 1971) and we discuss later the effects of DDT on the ATP-ase enzymes. Other studies have attempted to show more general effects of pollutants on the total activity of certain enzymes in various tissues, but the results are often variable and sometimes contradictory. Jackim (1974) has shown thatsome variability in reports in the literature may be due to pollutants (heavy metals) having variable effects on enzyme activity according to the time the animal was exposed to the contaminant. For example, copper stimulated Fundulus liver ribonuclease after 24 hours exposure, but induced a 20% reduction in enzyme activity after 72 hours exposure. The enzyme delta-aminolevulinic acid dehydratase is functional in the synthesis of haemoglobin, and has been shown to be inhibited by mercury (transitory inhibition at 0.02 ppm) and by lead (lasting inhibition at between 1 and 50 ppm) by Jackim (1974). This enzyme (in Salmo) was only slightly affected by a relatively large oral dose of PCB, and there was no change in either haematocrit or haemoglobin level (Westman et al, 1975). Another study by Heitz et al (1974) indicated few effects of acute oil exposures on a variety of enzymes in mullet, shrimp and oysters. In the mullet, for example, there were no changes in the maximum activity of 13 enzymes after the fish had been exposed to 4-75 ppm crude oil for 4 days.

In addition to the possible effects on maximum enzyme activity (V_{max} is often taken as an indication of the amount of enzyme present), the kinetics of an enzyme may be altered by pollutants. There is a danger here, however, that in vitro studies might lead to trivial interpretations, simply because the factors affecting enzyme function in vivo are so complex that their simulation in vitro may prove impossible. In any case, there has been very little study of possible kinetic effects of pollutants on enzymes, in spite of the fact that an alteration in the regulatory enzyme could well be expressed physiologically at the level of the whole organism. We discuss later in this review the possible benefits to be gained from coupling toxicological studies of enzymes to studies of genetic variation.

One enzyme complex that does hold more promise than most for effects monitoring concerns the "detoxification" system for xenobiotics. This enzyme complex is well-known in mammals (Conney and Burns, 1972) and has been reported also in fish (Payne 1976) and invertebrates (Khan et al 1972; Lee, personal communication). The general term mixed-function oxidase is applied to this complex of enzymes. In particular, the initial hydroxylation of the aromatic ring of a xenobiotic hydrocarbon is carried out by aryl hydrocarbon hydroxylase. Induction of increased enzyme activity occurs in mammals, fish and invertebrates after exposure to many foreign organic compounds, including polycyclic aromatic hydrocarbons. Payne (1976 l.c.) found that fish from petroleum-polluted areas had higher aryl hydroxylase activity in liver and gill tissue than in the same species of fish from an unpolluted area; he suggested that assays of this enzyme could be used as a monitor of petroleum pollution. However, more research is needed here also, particularly to explore the role of these enzymes in the normal processes of steroid metabolism. Recently, Forlin and Lidman (1975) have described a technique for preliminary fractionation of this enzyme complex from rainbow trout, which is useful under field conditions.

Pollutants, including both metals and hydrocarbons, are known to affect some processes of steroid metabolism. For example, Canadian workers (Uthe, personal communication) have shown that trout exposed to PCB to produce levels of 30 ppm in back muscle and 80 ppm in eggs did not successfully hatch their eggs, and study of the exposed males suggested that there was hydroxylation of testosterone. Also, cadmium inhibits the biosynthesis of ketosterone from precursor both in vivo and in vitro. The capacity to synthesise steroid hormones may, with further development of the method, prove a feasible technique in effects monitoring.

Finally, we draw attention to the potential uses of studies on lysosomal enzymes as affected by pollutants. Vertebrate lysosomes (membrane-bound intracellular vesicles) are known to accumulate a variety of environmental contaminants, including metals and organic pollutants (Allison, 1965). Such accumulations may be measured cytochemically and their effects detected as a result of the concommitant labilisation of the lysosomal membranes and the release of hydrolase enzymes into the cytoplasm. Such processes ultimately lead to pathological damage to the cells as a result of autolysis by the lysosomal enzymes. It has proved feasible to provide a quantitative index of lysosomal labilisation in Mytilus and to demonstrate population differences in this index (Moore, personal communication). This study holds considerable promise for providing an index of pollutant effect, with evidence also of concommitant disadvantage to the individual.

MORPHOLOGICAL EFFECTS

If anomalies due to pollution are to be detected a good knowledge of normal histology is required, but unfortunately this is available only for a few species of marine vertebrates and invertebrates and for certain selected tissues.

Couch and Nimmo (1974) give examples of histological changes brought about by a variety of pollutants on selected species of Mollusca and Crustacea.

The gill membrane thickness of fish seems to offer a useful criterion of initial damage due to unfavourable water conditions. Much information is available on normal membrane thickness for many species (Steen and Berg, 1966; Newstead 1967; Kempton 1969; Hughes 1972; de Jager and Dekkers 1975) and this may be used to assess damage. The toxic action of zinc at acute levels was shown by Skidmore (1970) and Skidmore and Tovell (1972) to involve damage to gill tissues and subsequent severe aterial hypoxia. Skidmore (1964) reviewed this topic and concluded that copious secretion of mucus restricted respiration and led to mortality in fresh water fish. Lloyd (1960) observed a cytological breakdown of the gill epithelium of trout occurring about 21 hours after exposure to 20 ppm zinc. At about 4 ppm zinc the gill lamellae became swollen before death.

Histopathological changes induced by phenol in gill and liver tissues of the bream have been described by Walluga (1966). Phenol is highly corrosive and invokes immediate necrosis of epithelium on contact. However, Kristoffersson et al (1973) could not find demonstrable effects at subacute levels in brackish water pike. Cope (1966) reported fusion of gill lamellae caused by pesticides.

Varanasi et al (1975) reported that small concentrations of lead and mercury in the water produce significant alterations in the properties of epidermial mucus of rainbow trout, which remain even after a period of depuration. Chow et al (1974) have demonstrated that waterborne lead and mercury accumulate in the epidermal mucus of fish. The intact epidermal mucus is important not only for the hydrodynamics of the skin surface (Rosen and Cornford, 1971) but also for the resistance of the skin against infections and diseases. Haider (1975) showed that sublethal concentrations of lead acetate damaged the chemoreceptors of the common catfish and the tench, and histological examination revealed erosion of the terminal buds. Gardner and La Roche (1973) reported degeneration in the anterior lateral line and olfactory sensory structure following short-term exposure of Fundulus to sublethal levels of copper. Mercury levels of 0.5 ppm caused severe cytoplasmic and nuclear degeneration of all cellular elements comprising the lateral line canals (Gardner 1975).

Of over 900 commercial pesticide formulations, only about 30 have been tested in the laboratory for histological effects on the liver of fish and fewer than 20 species have been examined (Couch, 1975). Some information is available on controlled field research. Severe degenerative liver lesions were reported from bluegills (Lepomis macrochirus) exposed to 0.05 and 0.037 ppm heptachlor in ponds (Andrews et al 1966), but these concentrations were close to the 24 hour LC_{50}. Couch (1975) summarises Eller's (1971) report on endrin-induced changes in the liver of fish, noting that certain of the induced changes resembled prehepatomatous lesions: (1) liver cord disarray, (2) presence of mitotic cells in liver, (3) binucleate cells, (4) swollen cells, (5) pleomorphic cells (6) bizarre cells with enlarged nuclei, and (7) intrazonal and periportal inflammatory foci.

At present, there exists no published report on the effects of PCB's on fish livers, and little information seems to be available on the histopathological effects of DDT on the highly susceptible crustaceans. Noticeable changes in gonadal and mantle tissues of oysters (Crassostrea virginica) were observed following a 12 week exposure to lead concentrations of 0.1 to 0.2 mg/l. Although fish eggs are known to be relatively resistent to stress, embryonic malformations were found in many species (herring, gar pike, flounder and plaice) by a number of authors using various concentrations of heavy metals, detergents, oil emulsifiers, dinitrophenol and sulphuric acid (Dethlefsen 1974, Westernhagen et al 1974, 1975, Westernhagen and Dethlefsen 1975, Rosenthal and Mann 1973, Rosenthal and Sperling 1974, Alderson 1972, Kuhnhold 1972). Table 1 summarises various reports of morphological effects of pollutants on marine organisms. One problem is that most of these effects occur in experiments when excessively high levels of pollutants are used, or in the field in limited areas where abnormal concentrations are found, such as on sludge dumping grounds. The question is how to evaluate these data in terms of sublethal levels.

Physical anomalies observed in natural populations of marine and freshwater fishes are well documented and bibliographics are presented by Dawson (1964, 1966, 1971). Most of the observations do not deal with anomalous populations but with a few aberrant individuals. Rippy and Hare (1969) investigated an epidemic involving Atlantic salmon in the Miramichi river. The fish were severely affected by the bacterium Heromonas liquifacians, but increased concentrations of copper and zinc were also detected and the authors regarded those as contributing factors to the epidemic. Gardner (1975) observed spontaneous lesions in adult Brevoortia tyrannus (Atlantic menhaden) obtained from Narragansett Bay and from the site of a menhaden "fish kill". Tissue abnormalities in fish kidneys (mainly larger vacuolated cells in the glomeruli) were found by Mount and Putnicki (1966), investigating a fish kill due to endrin. Hubbs (1959) reported on high incidences of vertebral deformities in two natural populations of fishes inhabiting warm springs. Kroger and Guthrie (1971) observed thousands of cases of crooked vertebral columns in juvenile Atlantic menhaden, Brevoortia tyrannus, but these have not been related to environmental conditions. Crabs and lobsters from the sewage sludge and dredge spoil disposal areas of New York Bight often showed skeletal erosions on the tips of the dactylopotites and the ventral sides of the chelipeds. In addition, their gills became fouled with granular material and a dark brown coating covered the filaments (Young and Pearce, 1975).

In general, lesions have been reported in fish taken from polluted waters (Halstead 1972) and it has been suggested (Pac. Sci. Congress, Vancouver 1975) that easily detectable skin tumours among bottom living fish might be used as an early warning device in monitoring programmes. Valentine (1975) investigated the presence of high frequencies of skeletal anomalies in a wild population of the barred sand bass (Paralabrax nebulifer), from southern California. These anomalies involved the gill rakers, bones of the opercular series, cranial asymmetries, various fin anomalies and deformed vertebral columns. Barred sand bass of this population always possess more anomalies than Mexican barred sand bass do. Gill raker deformaties appeared to increase both in frequency and severity with age; they are not congenital. Valentine also found that anomalies in this fish are restricted to structures which contain a considerable amount of calcium; there appears to be an excellent association between gill raker anomalies and internal and external anomalies. After taking all other possibilities into account, Valentine assumed that the induction of skeletal anomalies in this non-migratory fish, may have been caused by a ubiquitous pollutant or pollutants that interfere with calcium metabolism. Such agents would include various chlorinated hydrocarbons. Pesticide concentrations are expected to be high in southern California marine waters and organisms. According to Schmidt et al (1972) White's Point sewage outfall (Los Angeles harbour) discharges an estimated 97 kg per day DDT and 100 kg per day PCB's. Duke and Wilson (1971) found DDT and its metabolites in concentrations as high as 1026 ppm in livers of fish taken from water adjacent to Los Angeles (mean concentration for 71 samples reached 318 ppm). Valentine argued that if these anomalies were associated with the accumulation of toxicants which produced metabolic instabilities, then similar anomalies should be observable in other marine teleosts from the same locality. So far, two additional species which have been examined, the California grunion (Leuresthes tenuis) and the barred surfperch (Amphistichus argenteus) show anomalies parallel to those for barred sand bass. The causative mechanisms producing skeletal anomalies in these marine teleosts are of course as yet unknown, but further research would seem to be justified. In this connection observations by Bengtsson (1974) are relevant. He examined minnows exposed to 0.2 and 0.3 ppm zinc in freshwater over 270 days and found 70% of the fish had suffered vertebral damage. Zinc is known as a metabolic antagonist to calcium.

TABLE 1 Some morphological effects

Morphological effect	Pollutant	Effective Concentration & Exposure time	Species	Reference
Successive hydrant reduction	Heavy metals	Hg 0.006 ppm Cu 0.06 ppm Cd 0.3 ppm	Eirene viridula	Karbe (1972)
swelling of the mollusc foot	Orthodichloro-benzene	10g/sq.ft. 3 hrs contact	oysters	Fujiya (1960)
necrotic tissue in stomach	copper	0.1-0.5 ppm (2 weeks)	oysters	Fujiya (1960)
muscular weakness	copper	0.15 ppm several days	Asterias	Loosanoff et al (1960)
abnormal leukocytic infiltration into the gonads	mixtures of DDT, Toxaphen parathion	5 ppb each (24 weeks)	oysters	Lowe et al (1971)
deformaties of telson and uropods	mercury	0.018 ppm (48 hrs)	newly hatches Palaemonetes larvae	Shealy and Sandifer (1975)
branchial oedema	Corexit 7664	0.19 mg/1 30 days	Gammarus oceanicus	Wildish (1970)
vertebral damage	zinc	0.2-0.3 ppm (270 days)	Phoxinus	Bengtsson (1974)
infections	mixture of DDT, Toxaphen parathion	1 ppb each 30 weeks	oysters	Lowe et al (1971)
increased red blood cell numbers	phenol	9 ppm (1 week)	Abramis brama	Walluga (1966)
pathological changes in the intestinal tract	cadmium	50 ppm 1 h	Fundulus heteroclitus	Gardner and Yervich (1970)
alterations in gonadal and mantle tissue	copper	0.1 to 0.2 ppm 12 weeks	Crassostrea virginica	Water Quality Criteria (1972) p. 250
degeneration of gill cilia	heated effluent water of a power station	?	Mytilus edulis	Gonzalez and Yervich (1976)

PHYSIOLOGICAL EFFECTS

Although it is the success of the population, rather than of the individual organism, that is ultimately important to the species, the vitality of the population is nevertheless a function of the survival, reproduction and growth of its individuals. To understand the effects of environmental alterations, we must know the typical responses of individuals. Furthermore, although the response of the individual is itself the result of changes in biochemical and other processes, it is the integrated outcome of these changes that is of the most direct use to the ecologist who seeks to understand the results of environmental change. This section therefore deals primarily with physiological effects at the level of the whole organism.

Physiological response to a change in the environment may be adaptive, leading to a more efficient functioning of the individual in the ecological system, or it may be harmful, resulting in a less efficient performance. These two aspects of physiological response must be distinguished when seeking to measure the effects of pollution, and the criteria for distinguishing between them are to be found in the ultimate expressions of biological performance, namely in survival, reproduction and growth. We must therefore measure "effects" both in terms of physiological response and in terms of ecological performance. The closer the link between the response and its expression as an alteration in growth or reproduction, the more useful will measurements of that response be for assessing the effects of environmental change.

With these considerations in mind, much recent research on the physiological effects of pollutants is seen to be irrelevant to the immediate needs of environmental monitoring. Even those aspects of physiological response which show some promise still require more research effort to bring our understanding to the point where indices of performance can be proposed for deployment in the field. Nevertheless, we can identify some aspects of research into physiological responses that will eventually serve as environmental monitors, while urging that more immediate actions be taken, as identified later in this report.

Recent research is briefly considered under four headings - respiration, osmotic and ionic regulation, nitrogen excretion and reproduction and growth.

Respiration Most studies involve the measurement of rates of consumption of oxygen from the medium (respiration rate) either by intact organisms or by isolated tissues. Such measurements are simple to make, but interpretation is often controversial.

Collier et al (1973) and Thurberg et al (1973) recorded reduced respiration rates by isolated gill tissues of three species of crabs when exposed to cadmium, but they found that copper had no effect. Scott and Major (1972) and Brown and Newell (1972) noted that copper depressed respiration rate of bivalve gill tissue (the latter authors also noting that zinc had no effect). Thurberg et al (1974) recorded increased respiration rates by bivalve gill tissue exposed to silver. Dunning and Major (1974) recorded a transient depression of respiration rate by gill tissue exposed to water soluble extracts of oil.

Where a depession in the rate of oxygen uptake by gill tissue is recorded, this is usually associated with cellular damage, or with narcotic effects of the pollutant. In another section of this report we identify morphological effects on gill tissues as a promising area for future monitoring. However, as the quoted papers demonstrate clearly, interpretation of the changes in respiration rate of isolated tissues is extremely difficult, because such changes differ amongst different contaminants, amongst different species, and even from one experimental condition to another. (Thurberg et al, 1974).

The variability in respiration rates is even more pronounced when the respiration of the whole animal is measured. A minimum requirement in such studies is for activity level and nutritional states of the individual to be quantified. Fry (1947) called the difference between an animal's standard and active metabolic (or respiration) rates the "scope for activity". This is a measure of the capacity the animal has, under particular environmental conditions, for all its metabolic activities in addition to the maintenance of its standard metabolic state. As environmental conditions change, standard and active respiration rates will change, often in different ways, so that the scope for activity may serve as an index of the animal's response to the environment, especially when considered in tandem with behavioural effects. Application of this concept to pollutant research (see Brett, 1964) promises to be rewarding.

In studies of fish, the activity level can be measured simultaneously with respiration rate. Waiwood and Johansen (1974) found that both activity and oxygen consumption by Catostomus increased in response to sub-lethal levels of methoxychlor. They suggested that the increased ventilation (= breathing) rate that accompanies hyperactivity, itself a response to the pollutant, may result in increased uptake of the insecticide. Sparks et al (1972) and Morgan (1974) recorded increased ventilation rates by fish in response to zinc, copper and cadmium. These responses might be the result of tissue hypoxia, brought about by gill damage (Skidmore, 1970). This points, once more, to the possibility of assessing pollution effect in terms of gill morphometrics, as discussed in the previous section.

Anderson et al (1969) record that the respiratory responses of fish to pollutants are variable with respect to (a) the concentration required to elicit a significant change in rate, (b) the direction of change (either stimulation or suppression) and (c) the magnitude of the response. The same is true for invertebrates. Hargrave and Newcombe (1974) attempted to relate activity by a snail to its rate of respiration, in response to oil and a low-toxicity dispersant. MacInnes and Thurberg (1973) demonstrated disturbance of both activity pattern and metabolic rate in another species of snail caused by copper and cadmium. Other studies, too numerous to list (but see Dorn, 1974; Dunning and Major, 1974; Anderson et al 1974) have demonstrated altered rates of oxygen consumption in response to sub-lethal concentrations of contaminants. There may be some merit in establishing, under standard conditions, the minimum level of a pollutant that stimulates a change in respiration rate, but there is little merit, at this time, in proposing respiration rate measurements per se as part of a monitoring programme. However, oxygen consumption is an index of energy expenditure and is therefore a component in the "energy budget" of the individual. When placed in the context of an integrated assessment of the "scope for growth", therefore, disturbed respiration rates acquire more significance, as discussed later in this report.

Osmotic and ionic regulation The regulation by fish, and certain invertebrates, of blood osmolality and of blood and cellular ionic concentrations are mechanisms vulnerable to certain contaminants. This has been well documented as an effect of polychlorinated hydrocarbons (Kinter et al 1972; Nimmo and Blackman 1972). A loss in capacity for osmo-regulation at reduced salinity by certain crustaceans when exposed to copper or cadmium has been recorded by Thurberg et al (1973) and Jones (1975). In other studies it is the individual's tolerance of reduced salinities that is affected by certain pollutants (Caldwell, 1974; Nimmo and Bahner, 1974). Renfro et al (1974) recorded depressed ion transport in osmo-regulatory systems in fish resulting from exposures to mercury.

Such studies are popular, because of the known capacity of DDT and PCB's to inhibit the Na^+, K^+ activated, Mg^{2+} dependent ATPase enzymes (NaKMg ATPases) (Yap et al, 1971; Desaick et al 1972). These enzymes are important in ion transport, and Kinter and his colleagues (Janicki and Kinter, 1971; Kinter et al

1972) have postulated that ATPase inhibition by DDT and by PCB's impair the capacity of fish to osmo-regulate. Caldwell (1974) confirmed *in vitro* inhibition at ATPase by methoxychlor in crabs, but failed to demonstrate impairment of osmotic or ionic regulation.

Inhibition of gill ATPases may be incidental to the ultimate lethality of insecticides to fish (Davies *et al*, 1972) and crabs, but there is, nevertheless a marked effect on an important physiological function, and this clearly requires further research for clarification. The capacity to osmo-regulate can be quantified (Siebert *et al*, 1972) and therefore shows promise as a tool in assessing sub-lethal effects of environmental change. For example, animals of the same species, from different sites, could be exposed to a standard series of diluted seawater and their capacities for osmo- and ionic- regulation measured and compared. However, further research into the natural variability of these physiological responses is needed before they can be used as monitors in field programmes.

Nitrogen excretion. The balance between different end-products of nitrogen metabolism may be disturbed by certain environmental stressors, including heavy metals. Measurement of the rate of excretion of various nitrogenous end-products (ammonia, amines, purines) may therefore indicate the effects of a pollutant (Corner, 1972). A different approach may be to determine the O:N ratio of oxygen consumed to nitrogen excreted as atomic-equivalents. This ratio can provide an index of the balance within the individual between the catabolism of carbohydrate, lipid and protein substrates, a balance that may be disturbed by pollution. The O:N ratio has been documented for planktonic crustacea (Corner, 1972) and benthic molluscs (Bayne, 1975); in both groups further research is needed to clarify the normal range for this index, and the circumstances in which it may change in a predictable way. As with so many other aspects of physiological response, the measurement of the O:N ratio holds promise for future use, rather than the possibility of immediate application.

Reproduction and Growth. Most studies of the effects of pollutants on reproduction and growth have been concerned with either effects on the development of eggs and larvae, or with effects on growth over the entire life cycle of the individual, from "egg to egg". For example, Brown and Ahsanullah (1971), Saliba and Ahsanullah (1973) and Grosch (1974) studied cultures of *Artemia*; Biesinger and Christensen (1972) studied *Daphnia*; Karbe (1972) and Stebbing (1976) have developed bioassays using clones of colonial hydroids; D'Agostino and Finney (1974) discuss long-term effects on reproduction and growth in *Tigriopus japonicus*; and Reish and his co-workers (Reish, 1976) have established bioassays with cultures of small and rapidly reproducing polychaetes. These procedures are "bioassays" in the classical sense, and their possible role in monitoring the effects of pollution is discussed later in this report. In the present section we comment upon another aspect of the physiological study of growth, namely the "scope for growth".

Warren and Davis (1967) defined the scope for growth as the difference between the energy value of the food consumed by an animal and the energy value of all the "uses and losses of food other than growth", under particular environmental conditions. In estimating the scope for growth, therefore, food consumption (Ac), loss due to faeces (Aw) and the energy equivalent of respiratory (or heat) loss (Am) are measured, and the scope for growth is calculated as:

$$\text{Scope} = Ac - Aw - Am$$

The scope for growth is readily converted to an index of growth efficiency as:

$$\text{Efficiency} = \frac{(Ac - Aw - Am)}{Ac}$$

This scope for growth, and its derivative, growth efficiency, provide an integrated assessment of the energy balance of the individual, and incorporate within one value the variable environmental influences on assimilation and respiration. It is an extremely useful ecological index, since it considers both the food relationships and other physiological responses to the environment, and expresses these in terms of the potential for growth, which is an ultimate expression of ecological well being. In situations where growth may be difficult to measure directly, or where measures of growth may be unable to detect the subtle effects of an environmental change, an assessment of the energy "status" of the individual, provided by the scope for growth, may yield the information necessary on which to base judgements of environmental impact. Although in its simple form, as given, the scope for growth does not distinguish between energy made available for somatic growth and for reproduction, a more complete assessment of energy balance is possible by including estimates of fecundity.

Warren and Davis (1967) and Brocksen, et al (1968) have examined some of the says in which the scope for growth in fish may vary with environmental change. Bayne et al (1973) and Bayne (1975) have discussed scope for growth in a bivalve mollusc as a function of environmental stress, while Gilfillan (1975) and Gilfillan et al (1976) have shown how the scope (estimated in terms of carbon rather than calories) declined in two bivalve species as a result of exposure to crude oil. This index shows considerable promise as a monitor of the effects of environmental change on marine animals. More research is needed on the natural variability in the scope for growth of different species. However, where sufficient basic information is available, such as for the bivalve molluscs Mytilus edulis and Mya arenaria, estimates of the scope for growth can be and are being used in field programmes to assess environmental condition in work in progress at Plymouth (Bayne) and in Maine, USA)Gilfillan).

BEHAVIOURAL EFFECTS

Effects on behaviour are often ignored in toxicity studies but they can be of vital importance to the survival of the animal or the population. Some laboratory studies are indicated below under appropriate headings.

Maintenance of equilibrium Subtle behavioural effects can follow exposure of fish to low methyl mercury concentrations (Spyker et al 1972), and in particular some functions which are controlled by the nervous system, such as maintenance of equilibrium may be affected (Lindahl and Schwanbom, 1971). Under the influence of 25 ppm phenol in water of 5 to 6‰ salinity, pike (Esox lucius) lost their balance within 90 minutes (Kristoffersson et al 1973).

Swimming behaviour and orientation Bengtsson (1974) showed that the addition of zinc (0.24 ppm) produced an initial hyperactivity in minnows over a period of several days, followed by a period when the fish displayed hypoactivity. Besides quantitative alterations in activity there were also changes in the distribution between diurnal and nocturnal activity. Waller and Cairns (1972) demonstrated that zinc concentrations down to 2.9 ppm can be detected by monitoring fish movement patterns in tanks. Kleerekoper et al (1972) have examined the interaction between temperature and copper ions as orientation stimuli in the locomotor behaviour of the gold fish.

Avoidance behaviour Scherer and Nowak (1973) developed an apparatus for recording avoidance movements of fish in order to investigate what concentrations in water would repel or attract, and whether the avoidance thresholds could eventually be detrimental. The method was used to evaluate escape reactions of rainbow trout at sub-lethal concentrations of oil drilling fluids (Lawrence and Scherer, 1975). On the other hand, examples are known of pollutants which are apparently not detected by fish, or which even act as attractants (Sprague and Drury, 1969).

Westlake and Kleerekoper (1974) have shown that avoidance and locomotor response of the fish is dependent on the slope of the gradient of copper ions. A steep gradient may elicit avoidance escape; a shallow gradient may produce 'attraction'. Avoidance tests to acid gradients were conducted by Ishio (1960) with freshwater fish using a gradient tank. Fish do avoid high concentrations of carbonic acid of about 18 ppm (Carassius) and of 240 ppm (Lepomis), whereas Carassius approaches fairly low pH values (2.8 pH) compared to Lepomis (5.8). Sprague (1964) showed that salmon parr actually avoided thresholds of 0.1 of the incipient lethal level with copper and 0.14 in tests with zinc, or 0.07 of copper - zinc mixtures.

Frequency of gill movements and heart beat Many investigations have measured ventilation frequency and/or coughing frequency in fish exposed to sub-lethal concentrations of pollutants; however some fish (trout) often respond by changing ventilatory depth more than frequency (Heath 1972). Drummond et al (1973) demonstrated that the increase in cough frequency of brook trout (Salvelinus fontinalis) to copper is a promising short-term indicator of the long-term effects of heavy metals. The lowest concentration of methylmercuric chloride that caused a significant increase in cough frequency in brook trout was 3 ug/l. In the large mouth bass (Micropterus salmonides) as little as 1.0 mg/l of anitmony in the form of tartar emetic caused projectule vomiting (Jernejcic, 1969). Among invertebrates, oysters are sensitive to chlorine concentrations of 0.01 - 0.05 ppm and react by reducing pumping activity. (Galstoff, 1946).

Embryonic and larval behaviour Alteration in heart rate during embryonic development under the influence of different environmental stressors is frequently reported in the literature:

fish embryos	pollutant	effect	reference
herring	oil emulsifiers	reduction in heart rate	Linden (1974)
herring	Dinitrophenol	reduction in heart rate	Rosenthal and Stelzer (1970)
herring	sulphuric acid (20% dilution 1:32,000)	initial increase in heart-rate (CO_2 - effect)	Kinne and Rosenthal (1967)
gar pike	cadmium (0.1 ppm)	reduced embryo activity	Westerhagen et al (1975)

Swimming behaviour and activity pattern of newly hatched fish larvae are often affected under different stress situations. The narcotic effect of oil-emulsifiers on herring larvae was demonstrated by Wilson (1972). Similarly, Mironov (1972) reported that newly hatched larvae of Rhombus maeoticus exposed to soluble oil components immediately sank and became motionless and their reaction to touch was weak. Rosenthal and Sperling (1974) found that herring larvae obtained from eggs which were incubated in 0.5 to 1.0 ppm Cd had difficulties in keeping their equilibrium. This failure of behaviour was attributed to the fact that the otic capsules are not well developed under Cd exposure.

Observations of behavioural effects have also been made in the field. For example adult Atlantic salmon (Salmo salar) apparently avoided sublethal copper-zinc pollution in an eastern Canadian river (Sprague and Saunders 1963).

More recently, Anderson (1971) reported field observations on the movements of sexually ripening autumn salmon (Salmo salar) into and through a polluted river estuary using ultrasonic tracking. He correlated locomotory movements with chemical assays of the effluents and the hydrography of the estuary. Preliminary results indicate that in addition to normal movements there was a considerable delay around known points of industrial pollution. Within the industrialized port of the Miramichi estuary, upstream movement was noticeably slower than in an adjacent unpolluted estuary, but above the industrialized part the fish moved rapidly upstream.

Gardner (1975) noted that adult menhaden (Brevoortia tyrannus) collected from a "discharge quarry" which received nuclear generating station effluent generally displayed erratic swimming patterns associated with an apparent loss of equilibrium. These fish showed certain morphological alterations.

Howell and Shelton (1970) observed that the deposition of China clay in two bays in south-west England. They noted this had a marked effect on bottom fauna, and suggested that the area of high turbidity was avoided by herring shoals. Evidence for such avoidance behaviour by whitebait in the same area is recorded by Wilson and Connor (1976).

Apathy was a marked behavioural effect in a ring seal (Pusa hispida) which showed extremely high mercury concentrations (Tillander et al 1972) up to 197 mg/kg being found in the flesh, and about 210 mg/kg in the liver (Henriksson et al 1969).

Snow and Stuart (1963) observed many paralysed cockles on an oyster ground in Tillamook Bay (Oregon) that had been treated 30 min previously with MGS-90 (Sevin) to control burrowing ghost and mud shrimp.

POPULATION AND COMMUNITY EFFECTS

Huntsman (1948) has pointed out that to explain changes in numbers of animals in a population it is essential to understand how individual organisms respond to their environment in terms of survival, reproduction, growth and movement, a point which is emphasised throughout this report. But from a strictly biological as well as a fisheries point of view it is the population and not the individual that is important, and it is argued here that unless an effect has consequences at the population level it is insignificant.

This effect at the population level is generally non-specific and thus will be unlikely to indicate the cause of the change. A large number of field investigations that have as their basis the identification and enumeration of the species occurring in a community are documented. Many of these studies are concerned with the relatively sedentary benthos on the basis that these species will be unable to avoid adverse conditions and thus the status of such populations at any point of time is likely to reflect the conditions that prevailed over a relatively long preceding period. They are also important components of the marine food web.

In such studies the most obvious effects are of course those in the immediate vicinity of continuous pollution sources such as effluent pipes or on dumping grounds. Some such discharges are not toxic but alter the community by a smothering effect or by altering the sediment. Sludges from aluminium works containing oxides of aluminium, iron and silicon are an example and there are descriptions of the totally azoic zones produced by the blanketing effect of such material (Bourcier 1969). Other inert materials such as fly ash and china clay act in a similar way and are well documented (Probert, 1975).

Effluents from pulp mills have a more direct effect and have been extensively studied in America as well as in western Europe. At sites in Scandinavia and Scotland changes in benthic communities have been followed which could apparently be related to variations in the pulp mill effluents (Pearson and Rosenberg, 1976).

Considerable research effort has been focussed on effluents from oil refineries. Chronic pollution from this source probably has an adverse effect on marsh grass, but it may be difficult to distinguish between this and the influence of natural events. (Dicks 1976). Animals are also affected, and around refinery outfalls certain gastropod molluscs and barnacles are considerably reduced in numbers (Crapp 1971). When such effluents are discharged into water where rapid mixing and dispersal take place, it is difficult to detect any effect, but if during a period of crises, discharge is redirected into more stagnant regions, kills of invertebrates and fish are observed (Baker 1976). Large macro-algae tend to be somewhat resistant to oil, protected by their mucilage covering, but at high concentration of effluent, only Enteromorpha and blue green algae survive. The polychaete Nereis diversicolor is very resistant to oil, but even this species is absent in the immediate vicinity of effluents.

Off sewage outfalls there may be a localised increase in primary production (Eppley et al 1972), but the main effect is on the benthos where a reduction of species diversity usually occurs, and again is well documented for several regions (McIntyre and Johnston 1975).

The major problem of population/community monitoring, bearing in mind the non-specific nature of the response, is to distinguish pollution induced changes from those due to other causes. The understanding of fluctuations in exploited populations is difficult enough even when the substantial background of fishery statistics is available, so the magnitude of the problem for most natural populations may be appreciated. Changes in species diversity have been proposed as indicators of pollution, and while such changes are certainly associated with gross pollution, the difficulty remains of detecting and relating them at sublethal levels before the effect is extreme. The use of very small metazoans (meiofauna) which have short generation times and which in some cases are particularly sensitive to stress may be helpful, and may permit the detection of changes in taxa higher than species. Thus it has been suggested (McIntyre 1976) that pollution in a sandy beach which had not yet affected macrofauna may be detected in the meiofauna by a reduction in number and species of small crustaceans and an increase in numbers of turbellarians and nematodes. However this approach requires extensive knowledge of non-polluted habitats and even then does not necessarily produce a direct link with a particular type of pollutant.

Another approach is to try to identify species that act as biological indicators of specific conditions. For example, Capitella capitata has often been found in large numbers around sewage outfalls and is considered to be one of the best indicators of gross organic enrichment in coastal seas (Reisch, 1972). However, Eagle and Rees (1973) describe a situation where the appearance of this species seemed due to changes in the sediment unrelated to organic enrichment, and this emphasises that such observations must be interpreted with care, and not in isolation from other studies.

Finally, the possibility of using primary production for monitoring is at present under active consideration. The production index, mg ^{14}C/mg chlorophyll, can be used to indicate changes in production from time to time or place to place, while the use of radio carbon bioassay to detect pollution, and of turbidostats to measure effects are in the process of evaluation.

On the experimental side these higher level effects can only be demonstrated by large scale long term experiments, which could confirm that observations made on single or small numbers of individuals by themselves also apply to larger groups in more natural conditions. Such experiments go some way towards answering doubts about extrapolation of small scale laboratory experimental results to the field.

Thus, tank experiments (Saward et al 1975, McIntyre 1976) demonstrated that for the Tellina-plaice food chain, low levels of pollutants such as copper, mercury and lead at less than 10 times background, could produce effects at each trophic level studied and that there were interactions which produced a community effect in terms of depression of important processes.

In experiments with large plastic bags in the sea (Davies, Gamble and Steele, 1975) it has been shown (Lee and Takasashi 1975) that major changes are produced in pelagic populations as the result of fuel oil extracts. The normal phytoplankton populations of diatoms were replaced by microflagellates, which in turn were followed by large increases in the populations of tintinnids and rotifiers which were presumably feeding on the small flagellates. There was also a decline in the larger planktonic carnivores and marked effects on the bacteria in the system. It is interesting that experiments with copper in the bags (Topping and Windom, in the press) produced very similar effects on population structure, and there is perhaps a suggestion in that this is a general reaction to stress.

GENETIC EFFECTS

It is generally accepted that by far the greater proportion of all mutations are deleterious. At the population level this will result in lower overall fitness and reduced ability to exploit the environment. In individuals, fertility, fecundity, viability, growth rate etc., all aspects of fitness, may be decreased, leading to a smaller population size and/or smaller total biomass. The utility of these parameters for the detection of the consequences of pollutant-induced mutation is rather limited however. Even for the well-studied fish populations under commercial exploitation it has not been possible to reach universal agreement on the long-term effect of fishing stress, and it is known thatother factors, particularly climate, can elicit larger, masking fluctuations to confuse the situation in these populations. It is unreasonable, therefore, to expect to be able to detect the integrated response of a population which might be attributable to the mutagenic effects of current levels of pollution.

It is becoming widely accepted, however, that mutation is involved in carcinogenesis, possibly as an initiating step. Therefore an increased incidence of malignant tumours could be taken as indirect evidence of an increase in mutation rate. It would, perhaps, be possible to screen fish landed from the more contaminated inshore waters for superficial tumours to detect any long-term change in incidence. To detect a change with a reasonable degree of certainty, however, the sample size would need to be very large.

Another category of mutation which should be considered covers those changes resulting from chromosome breaks; these include deletions, duplications, inversions and translocations. In germ cells, many of these rearrangements will lead to reduced fertility and/or fecundity and therefore may not be detectable in practice. Reciprocal translocations induced in spermatogonia can be detected in cytological preparations of meiotic cells. However a considerable amount of effort would be required to develop the techniques necessary to monitor marine organisms for this type of chromosome damage. In somatic cells the chromosome aberrations become apparent at cell division; many are incompatible with normal mitosis and the affected cells die and those aberrations consistent with the survival of the dividing cells are very difficult to score. Thus in those tissues in which cell division takes place much of the damage is eliminated. For those tissues in which cells do not normally divide and which therefore accumulate damage, the problem is to stimulate mitosis so that chromosome abberrations can be scored. In man and the higher vertebrates the small lymphocyte is an example of a cell type which has a very long intermitotic period and which can be stimulated to divide <u>in vitro</u>. This system has been used to great advantage to investigate the mutagenic effect of radiation and to a lesser extent many other agents. To date, however, the technique has not given any consistent success with fish lymphocytes despite considerable effort.

In summary, the prospects for monitoring the mutagenic effects of marine contaminants do not appear too promising; there are, however, one or two aspects of the problem which might repay further investigation.

In addition to the possible mutagenic effects of marine contaminants, we should be aware of the occurrence of normal genetic variation in marine populations. Protein polymorphisms, expressed both through the structural genes (those coding for enzymes) and the regulatory genes, are widespread in animals (Johnson, 1974) and appear to be balanced under ambient environmental conditions. In the context of monitoring for environmental contaminants, it is important to appreciate that the normal genetic variability may or may not cause differences in the general adaptive fitness of the individuals under conditions of environmental stress or specific sensitivity or insensitivity to pollutants. Should changes in protein polymorphisms prove to be adaptive, however, they will result in differential survival, which will be manifest in changes in the genetic profile of a population. Genetical screening of the distribution of polymorphisms in a population may therefore indicate the extent to which the organisms are being influenced by environmental disturbance.

However, in any such screening programme, priority should be given to enzymes of known physiological function, or enzymes specifically affected by particular pollutants (see section on Biochemical effects). Variability amongst individuals in their physiological response may in many cases be a function of variability at the genetic level. These studies should therefore be carried out in close co-operation with the monitoring programmes directed at effects on individual organisms. To these they may contribute by providing a link between the effect at the individual level and the possible consequences for the species.

EFFECTS ON LIVING RESOURCES AND THEIR EXPLOITATION

The previous section discussed some of the effects which pollutants can have on a range of marine organisms. We may now focus on species, particularly some fish and shellfish, which are of commercial importance. Although effects on these will not be any different from those on other marine organisms, the implications may be different because they are exploited. The most significant ways in which a resource and its exploitation may be affected are either by its being rendered unwholesome, or by some threat to the survival of the population.

Unwholesomeness may be caused by a readily recognisable taint which imparts an unacceptable colour, smell or taste to the product, or it may result from microbial contamination, or from the more subtle effect of some residue (eg a metal or pesticide) which although not immediately detectable could have a long term adverse effect on the health of the consumer, and which could require complex chemical analysis for detection. In the first category, the green colour of zinc-saturated oysters has been described, and the smell of phenol from the flesh of fish from certain industrialised regions recorded. The most obvious example in this category however, is probably tainting by oil. Some shellfisheries were affected for a short period after the Torry Canyon spill, but for the most part such effects are short term although after the West Falmouth spill of No. 2 diesel oil, local shellfisheries were closed for several years due to oil in the sediments. Apart from effects of spills, large scale mortalities do not seem to occur even in areas of extensive oil exploitation such as Louisiana, although tainting of shellfish is not uncommon there and this can apparently be treated by transplantation to a clean area. In the Gulf Coast of Texas reduced yields of commercial fish have been reported from small creeks contaminated by oil compared with clean areas, but here again the effect is relatively localised.

The other well known effect is from contamination by sewage micro-organisms. Again shellfish are most affected and contaminated areas may be closed to fishing, but again the effect on overall exploitation may not be disastrous since purification techniques, particularly for filter feeders such as Mytilus are well developed so that exploitation can be continued even in contaminated areas.

Contamination of commercial resources by metals and man-made pollutants such as insecticides and PCBs is becoming increasingly well documented by numerous baseline and monitoring surveys. Exploitation of stocks can be affected in those cases where maximum permissible levels in edible tissues have been declared, as for mercury in some countries, so that fish from certain areas may be banned. This can occasionally affect ocean stocks, such as tuna or swordfish, but usually the populations involved are in coastal waters and the source of contamination can be pinpointed. Thus in some places where the fishery has been severely curtailed by mercury contamination, control measures are producing an improvement.

Turning to the second type of effect on commercial species - the threat to population survival, we are less able to provide relevant examples. As indicated earlier in this report, on dumping grounds where large amounts of inert material settle, or on accumulating sewage sludge grounds or even in wider areas of gross enrichment such as the Oslo Fjord, radical changes in populations can occur which may include commercial species, but these examples are of limited geographical extent. More serious perhaps are some cases where a fishery for species such as the oyster or the herring has declined or been eliminated and some correlation between this and industrialisation seems possible but difficult to disentangle from fishing effects. Evaluation of those effects, if they are effects, can probably best be approached by a mixture of survey and experiment as discussed later.

Apart from such inshore regions, it seems that, as concluded at the NATO conference on the North Sea, no adverse effects of pollution on offshore fisheries can be unequivocally demonstrated (Goldberg 1973).

BIOASSAY APPROACH TO EFFECTS STUDIES

Bioassays are means of evaluating the nature or potency of a material (or a sample of water) by means of the reaction that follows its application to living matter (see Tarzwell, 1971 and the three-part review paper of Sprague (Water Research Vol. 3, 793-821, 1969; vol. 4, 3-32, 1970; vol. 5, 254-266, 1971), and more recently the procedings of the Workshop on Marine Bioassays (M.T.C. 1974)). They can be divided according to their applications:

(1) Bioassays in the strict sense are used to measure the abundance or activity of a substance by the biological response it elicits. In marine science some recent examples are:

AUTHOR	SUBSTANCE ASSAYED	ORGANISM USED
Davey et al., 1973	Cu complexing capacity of sea water	Thalassiosira
Gold, 1964	vitamin B_{12}	Cyclotella nana
Natarafan & Dugdale, 1966	thiamine	Cryptococcus
Whitfield & Lewis, 1976	metal complexing capacity of sea water	Euchaeta

(2) Measurement of the biological effects of specific toxins or contaminants. These are often lethal, short-term bioassays which are used largely to determine the relative toxicity of contaminants (Portmann & Wilson 1971). The more sensitive bioassays of this kind using larvae (Connor, 1972) are sometimes responsive to the levels of contaminants that are found in polluted waters, but sublethal bioassays usually have greater sensitivity (Karbe, 1972; Grey & Ventilla, 1973).

(3) Direct biological measurement of water quality by maintaining the bioassay organism in samples brought in from the field. This is possible only with the most sensitive techniques. Although Wilson & Armstrong (1961) and Johnston (1964) showed that this approach was feasible some years ago, only Burrows (1971), Kobayashi et al. (1972) and Woelke (see Walden, 1976) seem so far to have published work on the bioassay of polluted water.

Rationale for direct bioassays

Emphasis has been given in pollution studies to the levels of contaminants present, although we are chiefly concerned with the effects of the contamination upon the organisms living in it. It is self-evident that a contaminant that has no effect is of no significance. The misplaced emphasis is because analytical techniques have been sensitive to the levels of contaminants present in sea water, while bioassay techniques have not. We require biological methods because it is not possible to predict the ecological consequences of pollution from chemical data alone. There are a number of reasons for this:

(a) The extent to which the interaction of contaminants with each other and with the other constituents of sea water changes their biological activity.

(b) Analyses (particularly of metals) do not necessarily tell us the chemical form of the contaminants, which can change their toxicity markedly (Steeman Nielsen & Wium Anderson, 1970).

(c) Chemical analyses can only tell us about the specific contaminants analysed. Bioassays integrate all the variables - known and unknown - that influence water quality, thus providing a means of detecting previously unsuspected contaminants.

Operational bioassays

Many bioassays have been developed and proposed for use in the measurement of water quality. We draw attention here to four only of these which seem to offer most promise in monitoring programmes. Woelke (1968) has developed a sensitive bioassay based on the early developmental stages of oysters, and has used this assay extensively in monitoring the toxicity of pulp and paper mill effluents (see Walden, 1976). A bioassay using sea urchin eggs and early developmental stages has been developed and employed by Kobyashi (1971) and Kobyashi et al (1972). Reish has, over many years, maintained laboratory cultures of the polychaete Capitella and described various responses of this organism to waters of different qualities (Reish, 1972, 1976; Reish and Barnard, 1960). These studies illustrate the potential utility of adopting a "standard" organism, maintained under standard conditions, as an agreed bioassay organism for use in a wide variety of situations. The same rationale is found in a bioassay developed by Stebbing (1976) which employs a colonial hydroid, cultured as a clone in the laboratory. The growth rate and pattern of the hydroid serve as a quantifiable response to waters of different qualities.

Vertebrates also have been used in such bioassays, and mention should be made of the work of Baxter on herring eggs. These are stripped from ripe females, attached to plates, fertilized artificially, and used for experiments in which hatching and subsequent larval development are studied in relation to added pollutants or to water collected from different sea areas. (Baxter and Steele 1973, Baxter 1974).

Finally behavioural responses are obviously appropriate to bioassay techniques. Stirling (1975) has studied the effects of various pollutants on the burrowing reactions of a bivalve mollusc and proposes that this could be used as a general test. In a swimming test, Lindahl and Schwanborn (1971) placed a fish in a narrow tube in which water revolves around the direction of flow with linearly accelerating velocity. The critical rpm at which the fish starts to rotate with the water is used as a measure of the ability of the fish to resist the torque acting upon it, and thus as an indication of its resistance to pollutants or adverse water quality.

Future Developments

It is suggested that with these improved bioassay techniques now being developed it should be possible to direct the pollution chemist to areas where water quality is demonstrably poor, rather than to leave him unaided to direct his work solely on the basis of known effluent inputs. However, this kind of approach can be expected to provide no more than correlations in space and time between contaminants and measured effects. The onus for providing a causal relationship between them depends on the use of bioassay techniques in collaboration with chemists in experiments which may involve the "manipulation" of the sea water. Other non-experimental biological measurements are almost invariably subject to ambiguities due to uncontrolled variables.

There is in principle no limit to the kinds of biologically important contaminants that can be identified with a sensitive bioassay technique, so long as each can be removed without otherwise changing the sea water. Such separations are not yet possible, but a number of techniques may be borrowed from analytical methods to remove groups of substances from sea water.

1. Ion exchange resins can be used to remove metals and organics from sea water.

2. U/V photo-oxidation of sea water breaks down organic constituents and should therefore improve water quality where petroleum or other toxic hydrocarbons are present at significant levels. However, there is also the possibility that the break-up of organo-metallic complexes may make the metals more toxic.

3. Activated charcoal can be used in the same way to remove organics.

4. Addition of EDTA should, by complexing the metal ions, improve the quality of metal-contaminated water.

At present such bioassay techniques offer a most promising means of identifying biologically active contaminants in the sea.

DETECTION AND EVALUATION OF EFFECTS IN THE FIELD

Having reviewed the effect which pollutants may have on organisms, and how these effects may be demonstrated and measured experimentally, we may now consider whether they are likely to occur in the sea and if so, how they may be detected and their significance determined.

An examination of recent data on contamination in sea water (eg Jones 1975) indicates that, in general, levels in the open sea are low. There may be significant inputs of some contaminants such as lead by atmospheric deposition (Goldberg 1972), and elevated measurements of others may be expected in limited regions of exploitation or dumping or in such areas as the mid Atlantic ridge where high levels of mercury (1.4 ug/l) in deep water are ascribed to natural input (Carr et al 1974), but for the most part, concentrations in open ocean water may be taken as indicating background levels. In the light of the data presented in the first part of this report it would seem that for most contaminants the levels recorded in the open ocean are unlikely to produce readily detectable effects and are thus too low to cause immediate concern. Thus conclusion is supported by analysis of fish from the open sea, which for the most part have very low levels of contaminants in their tissues (ICES 1974).

In well documented coastal regions on the other hand, enhanced levels of contaminants, often associated with identifiable human activity, are common, and concentrations in the water may be reached which have been shown experimentally to produce adverse effects. We may therefore reasonably suspect that in some areas, sublethal effects may be caused in the field. Unfortunately, any significant change in an organisms circumstances or environment, might produce a reaction which could be confused with a pollution effect. Certain of the symptoms referred to in the earlier section could be observed in the field, but it would seem almost impossible to link these observations causally with specific pollutants, since whole-animal responses tend to be associated with generalised effects, and only by a more detailed examination, for example, at the biochemical level, could a tie up with a specific pollutant be established. It would be useful even to detect such symptoms and identify them as adverse. Clearly, if several of the effects listed in our review were found in organisms at a single location, we would have detected an "effects black spot" which would merit further study. But usually the problem would be to identify individual effects which were clearly adverse. For this same frame of reference is required. Thus we might agree that an effect was adverse which significantly reduced reproduction or growth, or which altered behaviour to make the organisms more vulnerable, and it might be possible to grade the various approaches according to their usefulness in producing meaningful extrapolation about the population. Further, however, we wish to select those effects which would be useful in a monitoring context, remembering that the complexity of living material and of the environment make it difficult to detect and evaluate biological effects in the field. In considering what criteria are relevant to judging the value of an effect in a monitoring programme, the following questions may usefully be asked of any proposed effect.

1. <u>What is its interpretative potential in terms of (a) long term individual survival (b) population survival (c) species survival (d) effects on communities?</u>

The fact that these are attributes related to individuals or populations does not place other types of effect (biochemical, physiological etc) outside our consideration, but rather emphasises that the latter must be shown to be demonstrable and meaningful at the individual, populations and community levels.

2. <u>How easily can it be correlated with other effects at different levels?</u>

This follows from the first question. For example, an effect at the biochemical level is significant for our purposes only if it can be shown to influence higher levels of organisms, affecting an individuals' physiology and ultimately resulting in a population change.

3. <u>How easily can it be measured, and can many laboratories measure it?</u>

Clearly, if an observation or technique is highly demanding in terms of expertise or facilities, its overall applicability will be much reduced.

4. <u>How amenable is it to observation in the field?</u>

An effect might be confidently expected from information based on laboratory experiment, but might not be easily detectable in the field, because, for example, of the difficulty of recognising a slight reduction in growth or viability or because of avoidance reactions.

5. <u>How easily can it be recognised as a pollutant effect and distinguished from a natural one?</u>

In many respects, especially at the higher levels of organisation, it will not be possible to distinguish between natural and pollution-induced effects. Both will result from complex interactions and the resultant effects may well be apparently the same. This is related to the previous question. The distinction can probably best be made at the lower levels of organisation. For example, it may be possible to attribute some biochemical effects directly to a specific pollutant, but the higher the level (ie in individuals and populations) the less will this be possible. Part of the problem stems from the natural variability of organisms. In monitoring for effects, there is a need to document this variability, for example by using a number of measurement sites to establish the natural range of the factors studied. Sites should be chosen to represent the observed or predicted gradients of contamination, and the greater the number of sites the better the possibility of distinguishing natural variability (the "noise") from real pollution effects (the "signal). See No. 6.

6. <u>How easy is it to find appropriate controls?</u>

For field considerations, this follows from 5 The degree of control may be related to the number of sites available, - the more studied, the more chance there wil be of detecting an anomaly.

7. <u>Can it be measured with precision and accuracy?</u>

There is a need to quantify effects in precise terms, qualitative descriptions usually being of little value for comparative purposes. It may be possible to find methods which help to escape the demands of high precision and accuracy. For example, rather than strive for high precision by measuring all amino acids in a sample, it may be acceptable to concentrate on only two and use ratios for comparative purposes.

8. <u>Is there much background data relevant to the effect?</u>

This is essential for the establishment of the "norm", and the degree of variability. Thus an effect which is well supported by a framework of scientific theory and by relevant experimental and field observation will be much more likely to be valuable in a monitoring programme.

Keeping these questions in mind, we may now consider the effects discussed earlier in relation to monitoring programmes.

At the biochemical level, knowledge of blood chemistry although potentially very useful, at present scores low because of interpretive problems and the difficulty of setting normal values. The situation is further complicated by the very act of capturing a fish, which can cause considerable changes in blood chemistry. A less immediately sensitive test may be desirable. The use of hormones or critical enzyme systems may be the answer. Appropriate techniques are becoming increasingly available and the biochemical measurements can be interpreted more widely than just, say, changes in steroids, but validation is needed under field conditions and some of the methods are quite intricate.

Considering morphological effects, gills might be regarded as critical organs, since almost any gill damage could be said to be detrimental, and there is much detailed knowledge of the gills of fish and of certain invertebrates (eg Mytilus, Crangon, Cancer). Other possibilities are vertebral deformities, tumours and liver morphology. All these score high in terms of field detection and measurement and are becoming well documented, but their use would be limited in migratory species.

Turning to physiology, while various techniques using eggs were considered (egg analysis, energy content of mature gonad, assays of egg quality) the best measure was thought to be "scope for growth". This has been studied in fish and invertebrates, and can be applied in the field using water from specific sites, or in the laboratory under controlled conditions. However, we need to know the range of values over the seasons, so that perhaps a couple of years work would be needed to validate the scope for growth technique in terms of monitoring.

On the topic of behaviour, it was felt that much of the work reviewed, although clearly relevant to the question of pollution effects, would be difficult to apply in the field. One approach, however, that of giving an organism a task to perform and evaluating the response, falls into the bioassay type of study, the usefulness of which has already been noted.

When our concern is the resources, the ultimate interest in biological effects is clearly with effects on populations and communities, and in this we are dealing, for macro-organisms at least, with a different time scale, probably in terms of years. While there is much in the literature on species diversity and on the identification and evaluation of population changes, we have tended in our discussions to emphasise the difficulties of interpretation, of separating the signal from the noise of natural variability, and our reaction in this report has been to highlight other approaches what may be more fruitful.

The best hope for the population approach is initially perhaps with relatively non-mobile populations of invertebrates in coastal waters where higher levels of pollution may be effected. In this context the programme of Dundee University (communicated to us by Dr A M Jones) at Scapa Flow in Orkney is of interest. This involves a study of eight species of mollusc (both gastropods and lamellibranchs) which are sampled at regular intervals in an area of potential pollution to determine growth rates, population structure (by length frequency analysis) and shell/body characteristics (including the shell parameters of height, length, width, breadth, weight and aperture length, as well as the soft body parameters of wet and dry weight). Seasonal variations in these relationships are also analysed in a control area and expressed as regression equations. The supposition is that it will be possible thus to detect any pollution effects. While this may allow the detection of changes, the problem of interpretation remains, and the field studies would need to include chemical analyses of the organisms and the environment and to be coupled with laboratory experiments before the effects could be unequivocally linked with specific pollutants.

Finally, we should consider the relevance of such laboratory and field work as may be loosely classified under the general heading of "bioassay". The classical work of D P Wilson who studied the effects of water type on the hatching and rearing of echinoderm and polychaete larvae has been followed in more recent years by a variety of approaches and techniques, involving for example hydroids, brine shrimps, polychaetes, mollusc larvae and herring eggs. The use of caged animals (shellfish in particular) which may be exposed to natural conditions in the field and later examined for body burdens and general condition is also worth assessing. These offer the possibility of testing organisms and water in a variety of combinations which appropriately used could make useful contribution to an effects study programme.

It is inherent in our recommendations on the evaluation of the effects of pollution in the field that no single procedure can suffice. On the contrary, the complexity of biological material and of organisational responses to environmental change signifies that the greater the variety of processes monitored the greater the possibility of making meaningful evaluations of environmental well-being. Our suggestions constitute a reasonable minimum of techniques to be incorporated, in the short- and medium-term, in a viable monitoring programme.

RELEVANCE TO BASELINE AND MONITORING PROGRAMMES

It is clearly appropriate to consider the relevance of effects studies to current baseline and monitoring programmes, and indeed this is part of our terms of reference. Such programmes are at present being pursued in various parts of the world by a variety of agencies, and include analysis of contaminants in the water, the sediment, and the main compartments of the biota, although few programmes encompass the full range of components. The best example of internationally coordinated work on a wide geographical scale is probably to be found in the ICES programmes, in which, although data on water concentrations of contaminants are now beginning to accumulate and the possibility of routine measurements in sediment is being examined, the bulk of the current information relates to the levels of a number of metals and organohalogens in selected tissues of fish and shellfish. A major aim of these surveys is to provide data of public health relevance, and recent results indicate that in the North Sea offshore, the levels are in general low and safe, while higher levels in coastal waters require closer surveillance. As these data build up, they should constitute a valuable information base, and ideally it should be possible to couple effects studies to this, thereby providing an indication of the well-being of the resource. Unfortunately, existing data are such that a coupling of this kind is seldom possible. Monitoring surveys on fish for example give reliable information on residues in muscle or sometimes liver, but most experimental work relates concentrations in water directly to effects, residue data often not being provided, particularly for fish of commercial size. Thus the application of most of the existing effects data to the residue information from monitoring programmes often necessitate an attempt to link the monitoring residue data with water levels in the sampling area and so with water level of the experiment.

The ideal sequence of information is: level in water → residue in tissues and whole body → effects, and if a reasonable coupling of effects data with chemical monitoring information is to be provided, this end should be kept in mind when both effects studies and the monitoring programmes are designed. For example an experimental study of the effects of copper in young flatfish in an experimental food chain (Saward et al 1975) showed that when effects could be detected in the fish, measurable residues were not found in the muscle tissue, but only in the liver, suggesting that this would have been the appropriate organ for a monitoring study of the fishes health.

CONCLUSIONS AND RECOMMENDATIONS

It is clear from the foregoing discussion that the major problem in building effects into a monitoring programme is the complexity of biological material and the normal variability of the environment, which make it difficult to detect and evaluate effects in the field. Of considerable importance is the fact that in most cases the basic biochemical, morphological, physiological and behavioural information is lacking which would allow us to recognise or evaluate abnormal characteristics or individuals in a population. This is not to say that such abnormalities would necessarily be a result of contamination. Pollution can be usefully regarded as simply another environmental variable which may induce, especially at the higher levels of organisation, effects which are difficult to distinguish from those caused by natural stressors. However, as described earlier, the identification of effects "black spots" would allow for the location of more detailed studies including experimental programmes designed to investigate the causative agent.

This difficulty of detecting and evaluating effects in the field has been recognised by other study groups dealing with the same topic, and it is perhaps one reason why reports on effects tend to indicate general areas for future research, but are usually lacking in detailed practical proposals. While we do not wish to gloss over the difficulties, we suggest that if the possibilities of effects monitoring are to be properly evaluated, a start should be made now, even if only on exploratory lines. We have therefore attempted not just to list promising topics for long term consideration, but also to identify projects which might be integrated into current programmes and could thus provide data for immediate evaluation without a major extension of effort. In making proposals, we consider that in this complex field, no single approach is likely to be adequate in itself, but that we should aim to produce a suite of complimentary procedures, each reinforcing the other, and involving several animal groups and levels of organisation.

This report emphasises that for an effect to be significant in the present context, it must have implications at the population level, so it may appear that diversity indices, details of community structure, and other population-related observations would be of particular relevance. Such indices may indeed be correlated with pollution measurements, and their use is at present being actively examined in the fresh water field as a tool in water quality control. However, there are considerable problems in applying these techniques in the sea, where the major stress on commercial populations is usually fishing. We have therefore focused on individual organisms as offering the most promise of advance at present.

As indicated above, there are in existence regular or routine biological sampling projects undertaken by several institutes as part of their on-going programmes, the most relevant of which are designed to provide data for fish or shellfish stock management. We have considered what type of observation might be built into such programmes to produce effects monitoring data without demanding a substantial increase in effort. The following topics are initially suggested, if only to begin the building up of a picture of the well being of organisms in various geographical areas.

1. Liver/somatic and gonad/somatic index.
2. Skeletal deformities.
3. Tumours, lesions etc.
4. Gill damage.
5. General observations on morphology.

The application of these is likely to be most informative in the case of sessile species such as the mussel and oyster, and of organisms which are motile but relatively restricted in their habitat (crabs and some other decapod crustaceans) as well as non-migratory fish like the flounder and small coastal species. However, even migratory species in many cases spend considerable periods of time in relatively circumscribed areas, so that the relating of effects to a broad region might be possible. In relation to the list of criteria given earlier, these items score high in terms of ease of measurement and suitability for field observation. Their interpretative potential will best be judged in the light of the data as they accumulate.

It is suggested that individual countries engaged in programmes which could incorporate observations of the above type might usefully begin to collect records. Eventually an assessment of accumulated data would indicate whether any spacial or temporal patterns are apparent which would justify an internationally coordinated programme, involving standardised operating procedures and in some cases the organisation of collections for processing at appropriate centres (eg for gill studies, halfheads might be collected and processed at suitable laboratories, while for the evaluation of tumour data, the facilities of the existing "tumour bank" might be utilised).

While the above approach is relevant to existing biological surveys, there is also the question of whether a degree of biological awareness might be injected into programmes designed to provide chemical data. This is specifically referred to in our terms of reference, in which we were asked to consider relevance to baseline and monitoring programmes, and has been discussed under that head. For example, the possible "well-being" of material presently being analysed chemically might in part be assessed by relating its body or organ burdens of contaminants to values obtained from acute toxicity experiments or to measurements from valid experiments on sublethal levels. Unfortunately, this proposal reveals a serious information gap in marine studies. Data are accumulating on concentrations of pollutants in sea water and on residues in organisms, and much experimental evidence is available relating water concentrations to effects, but there are surprisingly few studies which connect all three - water levels, residues and effects in an unequivocal way. Further much of the residue data refer to concentrations in specific tissues rather than total body burdens. In this field, the need for further data is clear, and it is recommended that experimental programmes be set up to link effects with tissue residues which are being or could be measured in chemical monitoring programmes.

Another approach which could be of immediate application involves the use of procedures of the "bioassay" type, which could be employed in the field and experimentally in the laboratory to give information on water quality and on the health of certain species. These procedures might include the use of phytoplankton, hydroids, invertebrate larvae and adults, and fish eggs and larvae in "reciprocal bioassay" (standard water with natural organisms:

natural water with standard organisms, etc) as well as the use of caged animals. It should be possible to produce a group of standardised techniques (thus ensuring comparability) from which suitable selections could be made for a range of circumstances and requirements. This approach scores high in many of the proposed criteria. The methods are well researched and currently available. They can be done in most laboratories and might be designed with suitable controls to distinguish natural from pollutant effects. It should be emphasised however, that these procedures do not in themselves constitute "effects monitoring", but, as indicated earlier, should form only part of a broader approach.

A complete formulation of a comprehensive effects monitoring programme is not immediately possible. Of the effects listed in the first part of this report, knowledge of many which would appear to be potentially most useful in a monitoring context is at an early stage of development, and we cannot realistically recommend that any are ready to be incorporated immediately (ie within the next two years) in existing monitoring programmes. We consider however, that some of the topics already discussed are worthy of urgent consideration and would list scope for growth, gill damage, vitellogenesis, lysosomeal enzymes and steroid metabolism as prime candidates for development. Concentrated research effort of not less than two years, combined with field validation trials (involving widespread testing, where appropriate, with many species, different life stages and in different areas), would be needed to assess the value of these approaches as monitoring tools. We therefore recommend intensive study of these topics linked, via field validation trials, with on-going programmes.

In summary, we consider that the possibility of biological effects monitoring have not been fully explored and we propose a four-part approach. First, current biological survey programmes could be employed for an immediate effort by building into them certain specified types of observations. Second, for current baseline and monitoring programmes, it is suggested that experimental work on effects and residues should be directed at linking these programmes with effects data to provide information of the health of the stocks. Third, existing expertise on bioassay techniques should be utilized to validate extrapolation from experimental studies to field situations. Fourth, on the longer term, a number of topics worthy of development are identified. The aim is to develop a suite of techniques for the detection of the effects of sublethal concentrations of pollutants in the field.

Finally, the sub-group was aware of its limitations of time and expertise. It considers that a workshop of perhaps around 50 invited specialists could usefully consider and debate these problems.

REFERENCES

Alderson, R. 1972. pp 312-315, In: Marine pollution and sealife. ed. M Ruivo. London: Fishing News (Books).

Allison, 1965. pp 178-204, In: "Lysosomes in Biology and Pathology" eds. J T Dingle and H B Fell, North Holland, 2.

Anderson, J M. 1971. Proc. Roy. Soc. Lond. B. 172, 302-320.

Anderson, J M and Peterson, M R. 1969. Science, 164, 440-441.

Anderson, J W, Neff, J M, Cox, B A, Tatem, H E and Hightower, G M. 1974. pp 285-310. In: Pollution and physiology of marine organisms, eds F J Vernberg and W B Vernberg. Academic Press.

Andrews, A K, van Valin, C C and Stebbings, B E. 1966. Trans. Am. Fish. Soc., 95, 297.

Baker, J M. 1976. pp 201-225, In: Marine ecology and oil pollution, ed. J M Baker Applied Science.

Baxter, I G. 1974. International Council for the Exploration of the Sea. CM 1974/E:35.

Baxter, I G and Steele, J H. 1973. International Council for the Exploration of the Sea. CM 1973/E:29.

Bayne, B L. 1975. pp 213-238, In: Proc. 9th European Marine Biology Symp. ed. H Barnes, Aberdeen Univ. Press.

Bayne, B L, Thompson, R L and Widdows, J. 1973. pp 181-194, In: Effects of temperature on ectothermic organisms, ed W Wieser, Springer-Verlag.

Bengtsson, B E. 1974. Oikos, 25, 135.

Biesinger, K E and Christenden, 1972. J. Fish. Res. B Canada, 29, 1691-1700.

Bourcier, M. 1969. Têthys, 1, 779-782.

Brett, J R. 1964. J. Fish. Res. Bd Canada, 21, 1183-1226.

Brocksen, R W, Davies, G E, and Warren, 1968. Jour. Wildlife Manag. 32, 51-75.

Brown, B and Ahsanullah, M. 1971. Mar. Poll. Bull. 2, 182-188.

Brown, B and Newell, R. 1972. Mar. Biol. 16, 108-118.

Burrows, E H 1971. Assessment of pollution effects by the use of algae. Proc. Roy. Soc. Lond. B., 177, 295-306.

Calabrese, A, Thurberg, F P, Dawson, M A and Wenzloff, D R. 1975. pp 15-21, In: Sublethal effects of toxic chemicals on aquatic animals eds J H Koeman and J J T W A Strik. Elsevier.

Caldwell, R S. 1974. pp 197-223, In: Pollution and physiology of marine organisms, eds. F J Vernberg and W B Vernberg. Academic Press.

Carr, R A, Jones, M M and Russ, E R. 1974. Nature (Lond.) 253, 489-490.

Chow, T J, Patterson, C C and Settle, D. 1974. Nature (Lond.), 251, 159-161.

Collier, R, Miller, D, Dawson, M A and Thurberg, F P. 1973. Bull. envir. contam. toxicol., 10, 378-382.

Conney, A M and Burnes, J J. 1972. Science, 178, 576-586.

Connor, P H. 1972. Mar. Poll. Bull. 3, 190-192.

Cope, O B. 1966. J. Appl. Ecol., 3 (Suppl.) 33.

Corner, E D S. 1972. pp 185-201. In: Conservation and productivity of natural waters, eds. R W Edwards and D J Garrod, Academic Press.

Cornish, H. 1971. Critical Reviews in Toxicology, 1, 1-32.

Couch, J A. 1975. pp 559-583. In: The Pathology of fish, eds Ribelin and Migaki Wisconsin Univ. Press.

Couch, J A and Nimmo, D R. 1974. Proc. Symp. on Diseases o Aquatic Animals (Regional) LSU-Sea Grant.

Crapp, G B. 1971. pp 187-203. In: The ecological effects of oil pollution on littoral communities, ed. E B Cowell. Applied Science.

D'Agostino, A and Finney, C. 1974. pp 445-463, In: Pollution and physiology of marine organisms, eds. F J Vernberg and W B Vernberg, Academic Press.

Davey, E W, Morgan, M J and Erickson, S J. 1973. Limnol. Oceanogr., 18, 993-997.

Davies, P W, Friedhoff, J M and Wedemeyer, G A. 1972. Bull. Env. Cont. Toxicol. 8, 69-72.

Davies, J M, Gamble, J C and Steele, J H. pp 251-264, In: Estuarine Research, vol. 1, ed L E Cronin, Academic Press.

Dawson, C E. 1964. Gulf Res. Rep., 1, 308.

Dawson, C E. 1966. Gulf Res. Rep., 2, 169 pp.

Dawson, C E. 1971. Gulf Res. Rep., 3, 215.

De Jager, S and Dekkers, W J. 1975. Netherlands J. Zool, 25, 276-308.

Desaick, D, Cutkomp, H H and Koch, R B. 1972. Biochem. Pharmacol., 21, 857-865.

Dethlefsen, V. 1974. International Council for the Exploration of the Sea. CM 1974/E:6.

Dicks, B. 1976. pp 227-245, In: Marine ecology and oil pollution, ed J M Baker, Applied Science.

Dorn, P. 1974. Bull. Environ. Contam. Toxicol., 12, 86-91.

Drummond, R A, Spoor, W A and Olson, G F. 1973. J. Fish. Res. Bd Canada, 30, 698-701.

Duke, T W and Wilson, A J Jr., 1971. Pestic. Monit. J., 5, 228.

Dunning, A and Major, C. 1974. pp 346-366, In: Pollution and physiology of marine organisms, eds F J Vernberg and W B Vernberg, Academic Press.

Eagle, R A and Rees, E I S. 1973. Mar. Poll. Bull. 4, 25.

Eisler, R and Edmunds, P M. 1966. Trans. Am. Fish. Soc. 95, 153.

Eller, L L. 1971. Amer. J. Pathol., 64, 321.

Eppley, R W, Carlucci, A F, Holm-Hansen, O, Kiefer, D, McCarthy, J J and Williams, P M. 1972. p 74, In: Calif. Co-op. Oceanic Fish. Invest. Rep., 16.

Förlin, L and Lidman, U. 1975. pp 213-220, In: Sublethal effects of toxic chemicals on aquatic animals, eds. J H Koeman and J J T W A Strik. Elsevier.

Fry, F E J. 1947. University Toronto Studies, Biological Series 55; Ontario Fish. Res. Lab. Publ. 68, 62 pp.

Fujiya, M. 1960. Bull. Jap. Soc. Sci. Fish, 26, 462-468.

Galtsoff, P S. 1946. Fish and Wildlife Service, U S. Dept Interior, Res. Rep. no 11.

Gardner, G R. 1975. pp 657-693, In: The Pathology of Fish, eds. Ribelin and Migaki. Wisconsin Univ. Press, Madison.

Gardner, F R and Jevich, P P. 1970. J. Fish. Res. Bd Canada, 27, 2185-2196.

Gardner, F R and La Roche, 1973. J. Fish. Res. Bd Canada, 30, 363-386.

Gilfillan, E S. 1975. Mar. Biol. 29, 53-58.

Gilfillan, E S, Mayo, D, Hanson, S, Donovan, D and Jiang, 1976. Mar. Biol., 37, 115-123.

Gold, K. 1964. Limnol. Oceanogr., 9, 343-347.

Goldberg, E. 1973. (Ed.) IDOE Baseline Conference. New York.

Goldberg, E. 1973. (Ed.) North Sea Science. MIT Press.

Gonzalez, J G and Yervich, P. 1976. Mar. Biol., 34, 177-189.

Gorsch, D S. 1974. Biol. Bull. 145, 340-351.

Gray, J S and Ventilla, R J. 1973. Ambio, 11, 118-121.

Haider, G. 1975. Hydrobiologia, 47, 291-300.

Halstead, B W. 1972. pp 584-594, In: Marine pollution and sea life, ed. M Runo. London: Fishing News (Books).

Hargrave, B and Newcombe, C. 1974. J. Fish. Res. Bd. Canada, 30, 1789-1792.

Heath, A G. 1972. Water Res., 6, 1-7.

Heitz, J R, Lewis, L, Chambers, J and Yarbrough, J D. 1974. pp 311-328, In: Pollution and physiology of marine organisms, eds F J Vernberg and W B Vernberg. Academic Press.

Henriksson, K, Helminen, M and Karppanen, E. 1969. pp 54-59. In: Proceedings of the Nordic Symposium on the problems of mercury, Lidingö, Sweden, Oct 10, 1968 (In Swedish).

Howell, B R and Shelton, R G J. 1970. J. Mar. Biol. Ass. UK, 50, 593-607.

Hubbs, C. 1959. Ecology, 40, 154.

Hughes, G M. 1972. Resp. Physiol., 14, 1-25.

Huntsman, A G. 1948. Ecology, 29, 30-42.

Ishio, S. 1960. Bull. Jap. Soc. Sci. Fish., 26, 894-899.

ICES, 1974. Co-operative Research Report no 39. Charlottenlund, Denmark.

Jackim, E. 1974. pp 59-65, In: Pollution and physiology of marine organisms, eds. F J Vernberg and W B Vernberg. Academic Press.

Janicki, R H and Kinter, W B. 1971. Science, 173, 1146-1148.

Jernejcic, F. 1969. Trans. Amer. Fish. Soc., 98, 698-702.

Johansson-Sjöbeck, M L, Dave, G and Lidman, U. 1975. pp 189-196, In: Sublethal effects of toxic chemicals on aquatic animals, eds. J H Koeman and J J T W A Strik, Elsevier.

Johnston, R. 1964. J. mar. biol. Ass. UK, 44, 87-109.

Johnston, G B. 1974. Science 184, 28-37.

Jones, M. 1975. Mar. Biol., 30, 13-20.

Jones, P G W. 1975. Meeting of sub group on contaminant levels in sea water. ICES. Charlottenlund, 21-23 May.

Karbe, L. 1972. Mar. Biol., 12, 316-328.

Kemptnn, R T. 1969. Biol. Bull., 136, 226-240.

Khan, et al, 1972. Life Science, 11, 405-415.

Kleerekoper, H, Westlake, G F, Matis, J H and Gensler, P J. 1972. J. Fish. Res. Bd Canada, 29, 45-54.

Kinne, O and Rosenthal, H. 1965. Mar. Biol., 1, 65-83.

Kinter, W B, Merkens, L S, Janicti, R M and Guarino, A M. 1972. Env. Health Persper, 1, 169-173.

Kobayashi, N. 1971. Publ. SETO. Mar. Biol. Lab., 18, 379-406.

Kobayashi, N, Nogami, H and Kazuhisa, D. 1972. Publ. SETO Mar. Biol. Lab., 19, 359-381.

Kristofferson, R, Broberg, S and Oikari, A. 1973. Ann. Zool. Fennici, 10, 392-397.

Kroger, R L and Guthrie, J F. 1971. Chesapeake Sci., 12, 276.

Kühnhold, W W. 1972. pp 315-318, In: Marine pollution and Sea life, ed. M Ruivo, London: Fishing News (Books).

Larsson, A. 1973. pp 619-628, In: Comparative physiology, eds. L Bolis, K Schmidt-Nielson and S H P Maddrell. North Holland.

Larsson, A. 1975. pp 3-13, In: Sublethal effects of toxic chemicals on aquatic animals, eds. J H Koeman and J J T W A Strik. Elsevier.

Lawrence, M. and Schorer, E. 1974. Dep. Environ., Fish. Marine Service, Tedr. Rep., No 502, 1-47.

Lee, R F and Takahashi, M. 1977. (In the press), In: Petroleum hydrocarbons in the marine environment. eds, A D McIntyre and K Whittle. Rapp. P.-v. Réun. Cons. int. Explor. Mer, 171.

Linden, O. 1974. Annls. Zool. Fenn. Azf., 11, 141-148.

Lindahl, P E and Schwanbom, E. 1971. Oikos, 22, 354-357.

Lloyd, R. 1960. Ann. Appl. Biol. 48, 84-94.

Lowe, J J, Wilson, P D, Rick, A J and Wilson, A J. 1971. Proc. Nat. Shellfish. Ass., 16, 71-79.

Loosanoff, V L, MacKenzie, C L and Shearer, L W. 1960. Science, 131, 1522-1523.

MacInnes, J R and Thurberg, F P. 1973. Mar. Pollut. Bull, 4, 185-186.

McIntyre, A D. 1976. In: Ecology of marine benthos, ed. B C Coull. vol. 6. Belle W Baruch Library in Marine Science. University of South Carolina Press, Columbia.

McIntyre, A D and Johnston, R. 1975. pp 131-141, In: Discharge of sewage from sea outfalls ed. A L M Gameson. Pergamon Press.

Mironov, D G. 1972. pp 222-224, In: Marine pollution and sea life, ed. M Ruivo. London: Fishing News. (Books).

Morgan, K. 1974. Water Res., 8, 67-77.

Mount, D J and Putnicki, G J. 1966. Trans. 31st N. Amer. Wildl. Conf. 177.

TC. 1974. Marine Bioassays, Workshop Proceedings. Marine Technology Society, Washington.

Natajaran, K V and Dugdale, R C. 1966. Limnol. Oceanogr., 11, 621-629.

Newstead, J D. 1967. Z. Zoolforsch. Mikrosk. Anat., 79, 396-428.

Nimmo, D R and Bahner. 1974. pp 427-443, In: Pollution and physiology of marine organisms, eds. F J Vernberg and W B Vernberg. Academic Press.

Nimmo, D R and Blackman, K B. 1970. Progress Rep. Gulf Breeze, Fla. 1969. US Fish. Wildl. Serv. Circ. 335, 29-31.

Nimmo, D R and Blackman, R R. 1972. Trans. Am. Fish. Soc., 101, 547-549.

Payne, J F. 1976. Science, 191, 945-946.

Pippy, J H L and Hare, G M. 1969. Trans. Am. Fish. Soc., 98, 685-690.

Portman, J E and Wilson, K W. 1971. MAFF Shellfish Information Leaflet, 19, 1-11.

Pearson, T H and Rosenberg, R. 1976. Ambio, 5, 77-79.

Probert, P K. 1975. J. mar. biol. Ass. UK, 55, 19-44.

Reish, D R. 1972. pp 203-207, In: Marine pollution and sea life, ed. M Ruivo, Fishing News (Books). London.

Reish, D R. 1976. FAO technical leaflet (FAO/SIDA Training course in marine pollution).

Reish, D R and Barnard, J L. 1960. Pac. Nat., 1, 1-8.

Renfro, J L, Schmidt-Nelsen B., Miller, D, Bends, D and Allen, J. 1974. pp 101-122, In: Pollution and physiology of marine organisms, eds. F J Vernberg and W B Vernberg. Academic Press.

Rosen, M W and Cornford, N E. 1971. Nature (Lond.), 234, 49-51.

Rosenthal, H and Mann, H. 1973. Arch. Fischereiwiss., 24, 217-236.

Rosenthal, H and Stelzer, R. 1970. Mar. Biol., 5, 325-336.

Rosenthal, H and Sperling, K R. 1974. pp 383-396, In: The early life history of fish, ed. J H S Blaxter. Springer, Berlin.

Saliba, K and Ahsanullah, M. 1973. Mar. Biol., 23, 297-302.

Saward, D, Stirling, A and Topping, G. 1975. Mar. Biol., 29, 351-361.

Scherer, E and Nowak, S. 1973. J. Fish. Res. Bd Canada, 30, 1594-1596.

Schmidt, T T, Risebrough, R W and Gress, F. 1972. Bull. env. contam. toxicol. (US), 6, 235-243.

Scott, D. and Major, C. 1972. Biol. Bull., 143, 678-688.

Siebert, K, Luca, A, Sperling, K and Ebertein, A. 1972. Mar. Biol., 17, 291-303.

Silbergeld, E K. 1974. Bull. Environ. Contam. Toxicol., 11, 20.

Shealy, M J Jnr and Sandifer, P A. 1975. Mar. Biol., 33, 7-16.

Skidmore, J F. 1964. Quart. Rev. Biol. 39, 227-248.

Skidmore, J F. 1970. J. exp. Biol., 52, 481-494.

Skidmore, J F and Tovell, P W A. 1972. Water Res. 6, 217-230.

Sparks, R E, Cairns, J and Heath, A G. 1972. Water Res., 6, 895-911.

Snow, C D and Stuart, N E. 1963. In: Informational Rep. Fish. Comm. 1-9, Portland, Oregon.

Sprague, J B. 1964. J. Water Pollut. Contr. Fed., 36, 990-1004.

Sprague, J B. 1969. Water Res., 3, 793-821.

Sprague, J B. 1970. Water Res., 4, 3-32.

Sprague, J B. 1971. Water Res., 5, 245-266.

Sprague, J B and Drury, D E. 1969. pp 169-179, In: Adv. Water Pollut. Res., Proc. 4th Internat. Conf. Pergamon Press.

Sprague, J B and Saudners, R L. 1963. p 221, In: Proc. 10th Ontario Industr. Waste Conf., Ontario Water Resources Comm., Toronto, Ont.

Spyker, J M, Sparber, S B and Goldberg, A M. 1972. Science, 177, 621-623.

Stebbing, A R D. 1976. J. mar. Biol. Ass. UK (In press).

Steeman Nielsen, E and Wium Anderson, S. 1970. Mar. Biol., 6, 93-97.

Steen, J B and Berg, T. 1966. Comp. Biochem. Physiol. 18, 517-526.

Stirling, A. 1975. Mar. Poll. Bull., 6, 122-124.

Strik, J J T W A. 1974. Haematological effects in fish toxicity studies, In: Ecological aspects of water pollution in specific geographical areas: Study of sublethal effects on marine organisms in the Firth of Clyde, the Oslo Fjord and the Waddensea. Report of a Working Group, WHO, Wageningen, 2-4 Dec. 1974.

Strik, J J T W A, de Ionch, HH, van Rijn van Alke Made, J W A and Wuite, T P. 1975. pp 31-41, In: Sublethal effects of toxic chemicals on aquatic animals, eds. J H Koeman and J J T W A Strik. Elsevier.

Tarzwell, C M. 1971. Proc. Roy. Soc. Lond., B 177, 279-285.

Thurberg, F P, Dawson, M A and Collier, R. 1973. Mar. Biol. 23, 171-175,

Thurberg, F P, Calabrese, A and Dawson, M A. 1974. pp 67-78, In: Pollution and physiology of marine organisms, eds. F J Vernberg and W B Vernberg. Academic Press.

Tillander, M, Miettinen, J K and Koivisto, I. 1972. pp 303-305, In: Marine pollution and sea life, ed M Ruive, Fishing News (Books) London.

Topping, G and Windom, H L. 1977. Bull. Mar. Sci. (in press).

Valentine, D W. 1975. pp 695-718, In: The pathology of fish, eds. Ribelin and Migaki. Wisconsin Univ. Press: Madison.

Varanasi, U, Robisch, P A and Malins, D C. 1975. Nature (Lond.), 258, 431-432.

Waiwood, K and Johansen, D. 1974. Water Res., 8, 401-406.

Walluga, D. 1966. Acta Hydrobiol., 8, 55-78.

Waller, W T and Cairns, J Jnr. 1972. Water Res., 6, 257-269.

Walden, 1976. Water Res., 10, 639-664.

Warren, C E and Davies, G E. 1967. pp 175-214, In: The biological basis of freshwater fish production, ed. S D Gerking. Blackwell.

Webternhagen, H. von and Dethlefsen, V. 1975. J. mar. biol. Ass. UK, 55, 945-957.

Westernhagen, H von, Dethlefsen, V and Rosenthal, H. 1975. Helgoländer wiss. Meeresunters, 27, 268-282.

Westernhagen, H von, Rosenthal, H and Sperling, K R. 1974. Helgoländer wiss. Meeresunters, 26, 416-433.

Westman, I, Johansson-Sjöback, M L and Fänge, R. 1975. pp 111-119, In: Sublethal effects of toxic chemicals on aquatic animals, eds. J H Keeman and J J T W A Strik. Elsevier.

Westlake, G F and Kleerekoper, H. 1974. Water Resources Res. 10, 103-105.

Whitfield, P H and Lewis, A G. 1976. Est. Coast. Mar. Res., 4, 255-266.

Wildish, D J. 1970. Bull. Environ. Contam. Toxicol., 5, 202-204.

Wilson, K W. 1972. pp 318-322, In: Marine pollution and sea life, ed. M Ruivo. London: Fishing News (Books).

Wilson, K W and Connors, P M. 1976. J. mar. biol. Ass. UK, 56, 769-780.

Wilson, D P and Armstrong, F A J. 1961. J. mar. biol. Ass. UK, 41, 663-681.

Woelke, C E. 1968. Northwest Sci., 42, 125-133.

Yap, H H, Desaiam, D, Cutkomp, L K and Koch, R B. 1971. Nature (Lond)., 233, 61-62.

Young, J S and Pearce, J B. 1975. Mar. Pollut. Bull., 6, 101-105.

APPENDIX I

Membership and Contributors

The core group consisted of:

Dr A D McIntyre (Convenor)
Dr Brian Bayne
Mr Grim Berge
Professor R Lange
Dr H Rosenthal
Dr I C White

Dr J F Uthe attended the second meeting by special invitation and contributed extensively. Others who presented written submissions or contributed by discussion were Mr A Preston, Dr D S Woodhead, Dr R J Pentreath, Dr Alan Jones, Dr H C Freeman, Mr A V Holden, Dr G B Sangalang, Dr M S Mounib, and Dr A R D Stebbing. It should be emphasised however, that the editorial committee (McIntyre, Bayne, Rosenthal and White) accept responsibility for the views expressed in the final report.