COOPERATIVE RESEARCH REPORT

No. 75

ON THE FEASIBILITY OF EFFECTS MONITORING

CONTENTS

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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 at present 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 effects.

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. An attempt could be made to build up a picture of the well-being of organisms in various geographical areas, and as a start observations on some or all of the following items could be included in on-going biological survey programmes: 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 is thought to be sufficiently understood at present to justify its immediate addition to monitoring programmes. It is suggested, however, that any promising approach identified should be subjected to a concentrated research effort (ideally linked via field validation trials to on-going research programmes) to evaluate its use as a monitoring tool. Among possible topics for such study, scope for growth, gill damage, lysosomal enzymes and steroid metabolism are particularly discussed in the report, but the list is far from exclusive.

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.

ORIGIN OF THE SUBGROUP

The ICES Working Group on Pollution Baseline and Monitoring Studies in the Oslo Commission and ICNAF Areas at its first meeting in January 1975 finalised plans for a survey of pollutants in marine fish, initiated arrangements for a baseline survey of metals in sea water and recommended the establishment of a Working Group to examine the problem of using sediments in monitoring operations. The Group also noted "that its membership and present concern with baseline and monitoring exercises did not permit it to pay sufficient attention within the full group to the study of the effects of pollutants on living resources and their exploitation. The Group was concerned that the expansion of the membership necessary to prosecute such work might make it unduly large or unwieldy. Some members felt that the assessment of effects on living resources, as distinct from those of a public health nature, were not sufficiently far advanced to permit their use in a surveillance programme, and that much more basic work needed to be undertaken before this stage was reached. However, the Group was concerned that some action in this field should be initiated as soon as possible; after discussion they agreed to invite Dr McIntyre, with the assistance of a small number of experts in the field, to examine the problems involved in dealing with this aspect of the work" (C.M.1975/E:2).

MEMBERSHIP AND TERMS OF REFERENCE

The sub-group consisted of a core of six members, but additional advisers 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 of 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 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 organisms 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 given a broad definition to "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. It does not attempt a fully comprehensive account of pollution problems in general, nor does it necessarily rule out alternative approaches to effects studies.

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 indicate certain aspects of biochemical change that are likely to result in overt physiological damage to the organism, and which in some cases show promise as monitors of the action of pollutants.

Changes in the chemistry of the blood plasma and/or in the formed elements of the blood are clearly relevant. 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 organisms, and some information is available on the effects of various pollutants on the blood of certain species of fish. This approach has been particularly well studied in relation to pulpmill effluents. McLeay (1973) subjected juvenile coho salmon to sublethal concentrations of kraft pulpmill effluent under laboratory conditions for 12 hours and 25 days. He noted that the level of plasma glucose increased during the shorter period and decreased during the longer, while for blood cells he recorded changes in hematocrits and lymphocytes.

Haematological responses of fish to specific 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).

With regard to metals, Larsson (1975) has demonstrated that cadmium in the water, at levels of 5 and 50 ppb, produced blood anaemia (i.e. 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 flounder 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.

Another relevant line of investigation, also involving the measurement of aspects of blood chemistry, arises from the fact that a phosphoprotein (vitellogenin) concerned with yolk formation occurs in the blood serum in fish; the annual cycle throughout the year has been studied in the flounder (Emmersen and Petersen, 1976). 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.

However, with the blood chemical approach, as with other aspects of biochemical effects of pollutants, we need considerably more baseline data on the normal seasonal and population variation and on the effects of general stress before specific pollution-induced changes can be recognised and their significance properly assessed in the context of effects

monitoring. It is encouraging to note that such information is building up (Sanders, 1966; Wardle, 1972; Phillips, 1974; McLeay, 1975; Bridges et al., 1975; Soivio and Oikari, 1976; Johansson-Sjöbeck and Steviem, 1976).

Another type of biochemical effect of pollution concerns the function of specific enzymes. The effects of phosphorus and carbonate insecticides on the enzyme acetyl cholinesterase, which is involved in nerve-impulse conduction, are well-known (Cornish, 1971) and the determination of fish-brain acetyl cholinesterase activity to detect organophosphorus pollution has been proposed (Weiss, 1964) although studies of blue gills suggest that the technique may not be sufficiently accurate for a monitoring tool (Gibson et al., 1969). We discuss later the effects of DDT on the ATP-ase enzymes (p. 13). 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 that some 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 a regulatory enzyme could well be expressed physiologically at the level of the whole organism. We discuss later in this report the possible benefits to be gained from coupling toxicological studies of enzymes to studies of genetic variation.

One enzyme complex that may hold promise 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) 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.

The effect of pollutants on complex enzyme systems through the use of pulse labelling techniques has been studied with respect to the metabolism of the steroid hormones. The importance of such hormones in maintenance of homeostasis within the animal and in preparing it for normal life changes (e.g. reproduction) are well known, so that alterations in hormone levels induced by pollutants must be looked at seriously. For example, brook trout (Salvelinus fontinalis) exposed to PCBs to produce levels of 30 ppm in back muscle and 80 ppm in eggs did not successfully hatch their eggs and study of exposed males indicated marked stimulation of testosterone occurred in the gonads (Freeman and Idler, 1975). Similarly, brook trout exposed to 25 ppb cadmium for 24 hours showed marked suppression of normal androgen synthesis (Sangalang and Freeman, 1974). Methyl mercury-treated seals showed depressed synthesis of both cortisol and aldosterone compared with controls (Freeman, Sangalang, Uthe and Ronald, 1975).

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 concomitant 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 on concomitant 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 for only a few species of marine organisms and for certain selected tissues.

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.

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. Information is available on normal membrane thickness for several 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 arterial hypoxia. A review of this topic (Skidmore, 1964) 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.

Table 1. Some morphological effects of pollutants.

Morphological Effect	Pollutant	Effective Concentration & Exposure Time	Species	Reference
Abnormal vasculature and haemorrhagic necrosis of gonads	cadmium	1ppb for 41 days or 10 ppb for 1 day	Brook trout	Sangalang and Freeman 1974
pathological changes in the intestinal tract	cadmium	50 ppm 1 h	Fundulus heteroclitus	Gardner and Yevich 1970
vertebral damage	{ zinc cadmium	0, 2-0.3 ppm (270 days) 7.5 ppb (70 days)	Phoxinus "	Bengtsson 1974 Bengtsson et al. 1975
alterations in gonadal and mantle tissue	copper	0.1 to 0.2 ppm 12 weeks	Crassostrea virginica	NAS/NAE 1974
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
deformities of telson and uropods	mercury	0.018 ppm (48 hours)	newly hatched Palaemonetes larvae	Shealy and Sandifer 1975
successive hydrant reduction	heavy metals	Hg 0.006 ppm Cu 0.06 ppm Cd 0.3 ppm	Eirene viridula	Karbe 1972
increased red blood cell number	phenol	9 ppm (1 week)	Abramis brama	Walluga 1966
branchial oedema	Corexit 7664	0.19 mg/l 30 days	Gammarus oceanicus	Wildish 1970
swelling of the mollusc foot	Orthodichloro- benzene	10g/sq.ft. 3 hrs contact	oysters	Fujiya 1960
infections	mixture of DDT, Toxaphen parathion	1 ppb each 30 weeks	oysters	Lowe et al. 1971
abnormal leuko- cytic infiltration into the gonads	mixtures of DDT, Toxaphen parathion	5 ppb each (24 weeks)	oysters	Lowe et al. 1971
degeneration of gill cilia	heated effluent water of a power station	?	Mytilus edulis	Gonzales and Yevich 1976

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

Hughes and Perry (1976) introduced the concept of relative diffusing capacity (morphometrically estimated by measuring the diffusion distance between water and blood, together with determinations of the gill area) as a useful tool in comparing the gills of fish treated in different waters.

Small concentrations of lead and mercury in the water (Varanasi et al., 1975) produce significant alterations in the properties of epidermal 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, and this can be important since the intact epidermal mucus is required 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, while Gardner and La Roche (1933) reported degeneration in the anterior lateral line and olfactory sensory structure of Fundulus following short-term exposure to sublethal levels of copper. Gardner (1975) investigated the histological changes in the skin of estuarine and marine teleosts (Fundulus heteroclitus, Menidia tyrannus) exposed to various chemical pollutants. Although the free neuromasts appeared to be unaffected at all levels of pollutants tested, the lateral line and mandibular canals were extremely vulnerable with oedema and necrosis of the epithelium of the canal. For example, mercury levels of 0.5 ppm caused severe cytoplasmic and nuclear degeneration of all cellular elements comprising the lateral line canal. Most of the observations referred to above relate to gills or tissues associated with the skin, but internal organs are also affected. In experiments with cadmium, Sangalang and Freeman (1974) recorded abnormal vasculature and haemorrhagic necrosis in the gonads of brook trout exposed to 1 ppb Cd for 41 days (or 10 ppb for 1 day).

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 from controlled field research in this context. Severe degenerative liver lesions were reported from blue gills (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 PCBs 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 resistant 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; Kühnhold, 1972).

Turning to field observations in general, physical anomalies in natural populations of marine and freshwater fishes are well documented, and bibliographies are presented by Dawson (1964, 1966, 1971). Most of the observations do not deal with anomalous populations but with a few aberrant individuals.

Pippy and Hare (1969) investigated an epidemic involving Atlantic salmon in the Miramichi river. The fish were severely affected by the bacterium Heromonas liquifaciens, but increased concentrations of copper and zinc were also detected and the authors regarded those as contributory 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, and Hubbs (1959) reported on high incidence of vertebral deformities in two natural populations of fishes inhabiting warm springs. Thousands of cases of crooked vertebral columns have been recorded in juvenile Atlantic menhaden, but these have not been related to environmental conditions (Kroger and Guthrie, 1971). Field experiments on pulp mill effluents in Japanese waters have demonstrated widespread histological damage to the fish Sparus macrocephalus (Fujiya, 1961). Dumping grounds are obvious sites for the study of adverse effects, and crabs and lobsters from the sewage sludge and dredge spoil disposal areas of New York Bight often show skeletal erosions on the tips of the dactylopodites and the ventral sides of the chelipeds. In addition, their gills become fouled with granular material and a dark brown coating covers the filaments (Young and Pearce, 1975).

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 deformities appeared to increase both in frequency and severity with age; they are not congenital. Valentine also found that anomalies in 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 an ubiquitous pollutant or pollutants that interfere with calcium metabolism. Such agents would include various chlorinated hydrocarbons. Pesticide concentrations are reported 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 PCBs. 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 ppm zinc (c.f. the 96 hr LC_{50} dosage of 3.2 ppm) in freshwater over 270 days and found 40% of the fish had suffered vertebral damage. Zinc is known as a metabolic antagonist to calcium. Later, in similar experiments on the same species with cadmium, Bengtsson et al. (1975) demonstrated vertebral damage at concentrations as low as 7.5 µg/l for 70 days.

In general lesions have been reported from fish taken from polluted waters (Halstead, 1972), and in the Baltic, Ljungberg (1976) considers that the high frequencies of skin tumors in pike in coastal areas with a marked degree of pollution might indicate a possible relationship between the occurrence of the disease and the increasing pollution levels, especially since pike tumors appeared as a previously unrecognised disease about 1950. It has been proposed that fish tumors might be used as indicators of carcinogen concentrations in estuaries (Stick and Action, 1976) and this approach is demonstrated for another marine taxa by the work of Ishio et al. (1972a,b). They showed that aromatic substances isolated from marine muds cause the growth of tumors in the seaweed Porphyra tenera which is eaten and extensively cultivated in Japan. It was possible to identify the carcinogens responsible and to trace their source to an industrial complex.

Some recent studies with bivalve molluscs suggest high incidences of neoplasia (invasive cellular abnormalities, often associated with the circulatory haemocytes) which may be related to environmental pollution (Barry and Yevitch, 1975). In one population as many as 40% of individuals showed signs of a haemopoietic neoplasm; in another population an incidence of 2% appeared to be correlated with high hydrocarbon levels in neighbouring muds. More specifically, there is some indication that low concentrations of aroclor 1254 and Mirex may enhance naturally occurring Baculovirus infections in pink shrimp (Couch, 1976) and it has been suggested that viruses, including tumor viruses, may be latent in shellfish and may be activated in the presence of certain chemicals or by environmental alterations such as thermal addition (Rosenfield, 1976).

On the other hand, Rosenfield (1976) considers that no chemical factor has been identified as causal in cases of prolyactive lesions in molluscs while Wellings et al. (1976) found no evidence of a direct relationship between tumors of Pleuronectidae and pollution in Puget Sound. This is supported by observations of Mearns and Sherwood (1976) that skin tumors in Dover sole in Southern California were not enhanced by municipal waste water, although they felt the disease could be associated with some specific but widely distributed agent which may or may not be associated with industrial wastes. They did, however, suggest that fin rot may be affected by waste water, and could be used as an indicator of environmental damage.

Clearly, the literature on lesions and tumors in marine fish and benthic invertebrates is at best confusing and at worst contradictory. More screening of populations and more experimental work are needed, but the presence of easily detectable neoplasms in marine organisms holds some promise for future effects monitoring programmes, and the work of the Registry of Tumors in Lower Animals provides a valuable data base in this context (see p. 29).

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 rate 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 depression 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 and Petersen (1969) recorded 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; Major, 1977) 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 M 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, but in other experiments (Schmidt et al., 1977) no such effects were demonstrated.

Such studies are popular, because of the known capacity of DDT and PCBs 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 PCBs 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 ATPase 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 sea water 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 culture 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 (p. 22). 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:

$$Scope = Ac - Aw - Am$$

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

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

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 ways 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 the field programmes to assess environmental condition in work in progress at Plymouth (Bayne) and in Maine, USA (Gilfillan).

Behavioural Effects

This type of effect is often ignored in toxicity studies but could be of vital importance to the survival of the animal or the population. It should be noted, however, that this heading covers a wide range of reactions, from, on the one hand, an effect directly on the behaviour of an organism, to, on the other, a reaction (or lack of it) which could be explained in terms of toxic side effect. 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 (Kristoffersen 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) demon-
strated 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. Effects
of pulpmill effluent have been intensively studied, and Howard (1975),
using concentrations of bleached kraft pulpmill effluent showed that
juvenile coho salmon did not achieve their maximum critical swimming
speeds in effluents above a threshold concentration between 10 and 20%
of the 96 hr LC_{50}. Swimming in fish can also be affected by dieldrin.
Cairns and Scherer (1964) showed that an exposure to 0.0017 mg/l (one-
ninth of the 24 hr LC_{50}) for twelve weeks affected the cruising speed
(and the oxygen consumption) of the pumpkin seed sunfish (Lepomis
gibbosus).

Interference with chemical signals

In marine organisms, many essential behavioural responses (concerning.
among other things, feeding and mating) are triggered by substances
which occur at very low concentrations in the water. These chemical
signals can be masked by certain pollutants, and oil in particular is
known to have this effect. Studies by Kettredge (1974) showed that water-
soluble extracts of crude oil altered the chemoreception and the sex
pheromone responses of a shore crab (Pachygrapsus crassipes), the effect
being apparently due to the polynuclear aromatic hydrocarbon fraction of
oil. Similarly, in experiments with the gastropod Nassarius obsoletus,
Jacobson and Boylan (1973) illustrated how very low levels of Kerosene
(1-4 ppb) in sea water can interfere with feeding behaviour.

Avoidance behaviour

Scherer and Nowak (1973) developed an apparatus for recording avoidance
movements of fish 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, 1974). 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
responses of fish are 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 con-
ducted 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 (<u>Salvelinus</u>
<u>fontinalis</u>) 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 µg/l. In the large mouth bass
(<u>Micropterus salmonides</u>) as little as 1.0 mg/l of antimony in the
form of tartar emetic caused projectule vomiting (Jernejcic, 1969).
Lunn <u>et al.</u> (1976) investigated short-term sub-lethal changes in
coughing rates of young rainbow trout exposed to various organo-
chlorine pesticides including DDT. Threshold for the coughing
response was found to be between 52 and 140 ppb p,p' - DDT. The
authors suggest that this response might prove useful as a sub-
lethal water-quality index. On the other hand, Hargis (1976) showed
that sub-lethal changes in pH values caused significant changes in
the respiratory-cough response in rainbow trout, so this response
may not be reliable as an indication of pollutant-caused stress in
studies where experimental pH has not been specified or controlled.
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

Alterations 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 <u>et al.</u> (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 <u>Rhombus maeoticus</u> 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 difficulty in keeping
equilibrium. That was attributed to the fact that the otic capsules
are not well developed under Cd exposure. A detailed review on sub-
lethal effects of environmental stressors on marine fish eggs and
larvae has recently been published (Rosenthal and Alderdice, 1976).

Field observations

Field observations of behavioural effects have also been made. For
example, adult Atlantic salmon (<u>Salmo</u> <u>salar</u>) apparently avoided sub-
lethal 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 (<u>Salmo</u> <u>salar</u>) 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 the deposition of China clay in two bays in southwest 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 are documented that have as their basis the identification and enumeration of the species occurring in a community. 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 found in the immediate vicinity of continuous pollution sources such as effluent pipes or on dumping grounds. Some such discharges are not toxic but affect the community by smothering 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 (Probert, 1975).

Effluents from pulp mills have a more direct effect and have been extensively studied in North 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 bar-
nacles 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 macroalgae
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 (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. Reduction in
species diversity has often been proposed as an indicator of pollution
(e.g., see Wilhm and Dorris, 1966) but a broad generalisation may be
disputed (Logan and Maurer, 1975). Marked changes in diversity are
certainly associated with several types of gross pollution, but if this
approach is to be useful, it must be sensitive enough to detect slight
changes and relate them to pollution before the effect becomes extensive,
and for this a range of population parameters, rather than only diversity,
is required. 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 using
taxa higher than species. Thus it has been suggested (McIntyre, 1977)
that pollution which had not yet adversely affected macrofauna in a sandy
beach may be detected in the meiofauna by a reduction in number and
species of small crustaceans and an increase in numbers of 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 identify species that could 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 (Reish, 1972). However, Eagle and Rees (1973) describe a situation
where the appearance of this species seemed due to changes in the sedi-
ment unrelated to organic enrichment, and this emphasises that such obser-
vations 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 demon-
strated 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, 1977) 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 et al., 1975) it has been shown that major changes are produced in pelagic populations as the result of fuel oil extracts (Lee and Takasashi, 1975). The normal phytoplankton populations of diatoms were replaced by micro-flagellates, which in turn were followed by large increases in the populations of tintinnids and rotifers 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, 1977) 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 is harmful. 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 that other factors, particularly climate, can elicit larger, masking fluctuations to confuse the situation. It is unreasonable, therefore, to expect 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 recognised, however, that mutation is involved in carcinogenesis, possibly as an initiating step. Therefore an increased incidence of malignant tumors might 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 tumors to detect any long-term change in incidence. To detect a change with a reasonable degree of certainty, however, the sample would need to be very large. A slightly different approach involves measurement of the mutagenicity of animal tissues, using a standard bioassay of effect on mutation rate. Such a procedure was employed by Parry et al. (1976) to compare the mutagenicity of mussel tissues from various populations, the tissue extracts affected a bioassay based on the metabolism of yeast cells. Where chemical analysis of likely mutagenic agents accumulated in animal tissues is difficult or time-consuming, an approach of this type may be warranted.

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 transloactions 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 effected 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 these tissues in which cells do not
normally divide and which therefore accumulate damage, the problem is to
stimulate mitosis so that chromosome aberrations 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 in vitro. 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.

One relevant study utilizing the technique of observing abnormal con-
figurations within cells is described by Longwell (1976). He examined
fish eggs collected in New York Bight, and of 452 mackerel eggs, found
that less than 20% had these dividing cells free from chromosome
and division abnormalities. In some of the samples the frequency of
abnormalities was substantially higher than an expected background level.
There was a suggestion that some of these high levels were associated
with dumping grounds, but further information is required on the
distribution of spawning fish and the dispersal of eggs before a con-
clusive evaluation can be reached.

In summary, the prospects for monitoring the mutagenic effects of marine
contaminants do not appear too promising at present; 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 environ-
mental contaminants it is important to appreciate that the normal
genetic variability may or may not cause differences in the general
adaptive fitness of the invididuals 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 cooperation 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, parti-
cularly some fish and shellfish, which are of commercial importance.
Although effects on these will not in principle be any different

from those on other marine organisms, the implications may be different
because they are exploited. The most significant ways in which such a
resource 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 produce, or it may
result from microbial contamination, or from the more subtle effect of
some residues (e.g., a carcinogen, a metal or a 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 context of obvious taint, 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
striking example in this category however, is probably tainting by oil.
Some shell-fisheries 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 shell-fisheries
were closed for several years due to oil in the sediments (Blumer et al.,
1970). Apart from effects of spills, large-scale mortalities do not
seem to be recorded 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.

Another well known effect is from contamination by sewage microorganisms.
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
produce 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 and cleaned up. Thus in some places where the fishery has
been severely curtailed by mercury contamination, control measures are
producing an improvement. Finally, some polynuclear aromatic hydrocarbons
from petroleum have been identified as carcinogens, and the possibility
of such substances building up in commercial species causes concern.
An objective review of this problem however (King, 1977) does suggest
that on the basis of our present knowledge, the risks are insignificant.

Turning to the second type of effect on commercial species - the threat
to population survival, we are less able to provide relevant examples.
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 the effect is relatively localised. 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 or long-
term natural changes. Evaluation of those effects, if they are effects,
can probably best be approached by a mixture of survey and experiment as
discussed later.

Thus, although localised effects may be recognisable in inshore regions, it seems that, as concluded at the NATO conference on the North Sea, no extensive adverse effects of pollution on offshore fisheries have been so far 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) through the reaction that follows its application to living matter (see Tarzell, 1971; Sprague, 1969, 1970 and 1971; 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	Orgamism used
Davey et al., 1973	Cu complexing capacity of sea water	Thalassiosira
Gold, 1964	Vitamin B_{12}	Cyclotella nana
Natarafan and Dugdale, 1966	Thiamine	Cryptococcus
Whitfield and 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 (Portman and 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 sub-lethal bioassays usually have greater sensivity (Karbe, 1972; Grey and 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 and Armstrong (1961) and Johnston (1964) showed that this approach was feasible some years ago, there is less published work on the bioassay of polluted water than might have been expected. (See Burrows, 1971; Baxter, 1974; Kobayashi et al., 1972 and Woelke in Walden, 1976).

Generally speaking, emphasis has been given in pollution studies to the level 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 arises because analytical techniques have been sensitive to the levels of contaminants present in sea water, while bio-assay 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 is unknown.

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

(c)   Chemical analyses can tell us only 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.

Many bioassays have been developed and proposed for use in the measurement
of water quality. We draw attention here to only a few of these which
seem to offer promise in monitoring programmes. Woelke (1968) 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
Kobayashi (1971) and Kobayashi 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, main-
tained 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, as illustrated by 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). Extensive experience
using salmonids in bioassays of, in particular, pulp mill effluents, is
available from Canadian studies which measured among other things, blood
sugar, blood cell numbers and temperature effects (McLeay, 1977; McLeay
and Howard, 1977).

Finally, behavioural responses are obviously appropriate to bioassay
techniques. Stirling (1975) has studied the effects of various pollu-
tants 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
Schwanbom (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.

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 definition of a causal relationship between them
depends on the use of bioassay techniques in collaboration with chemical
measurements 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
contaminant 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.

At present such bioassay techniques offer a promising means of identifying biologically active contaminants in the sea, and are thus essential in most field situations, where the mixture of contaminants or the environmental complexity precludes direct recognition and evaluation of the pollutant.

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 the extent to which they are likely to occur in the sea and how they may be detected and their significance determined.

An examination of recent data on contamination in sea water (e.g., 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 µg/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 thus too low to cause immediate concern. This 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, sub-lethal effects may be caused in the field. Unfortunately, any significant change in an organism's 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, however, even to detect such symptoms and identify them as adverse. Obviously, 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 some 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 makes 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. **What is its interpretative potential in terms of (a) long-term individual survival (b) population survival (c) species survival (d) effects on communities?**

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, population and community levels.

2. **How easily can it be correlated with other effects at different levels?**

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

3. **How amenable is it to observations in the field?**

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.

4. **How easily can it be recognised as a pollutant effect and distinguished from a natural one?**

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 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 (i.e., 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 Question 5.

5. **How easy is it to find appropriate controls?**

For field considerations, this follows from 4. The degree of control may be related to the number of sites available, - the more studied, the more chance there will be of detecting an anomaly.

6. **Are there much background data relevant to the effect?**

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.

7. <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.

8. <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 in some cases 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.

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

At the biochemical level, knowledge of blood chemistry although potentially very useful, at present scores low because of interpretative 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 (e.g., <u>Mytilus</u>, <u>Crangon</u>, <u>Cancer</u>). 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. Their use would be limited in migratory species, but for certain sessile species general "screening" for evidence of pathological conditions (presence of granulomas, evidence of neoplasma) may provide useful evidence of environmental deterioration.

Turning to physiology, while various techniques using eggs were considered (egg analysis, energy content of mature gonad, assays of egg quality) one of the most useful measures 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 extremely 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 population 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, and while these approaches obviously have considerable value we have tended in our discussions to emphasise the difficulties they bring of interpretation, of separating the signal from the noise of natural variability. Our reaction in this report has been to highlight other approaches that may be fruitful.

One hopeful approach at the population level is the study of relatively non-mobile invertebrates in coastal waters where higher contaminant concentrations may exert effects. In this context the programme of Dundee University, Scotland (communicated to us by Dr A M Jones) at Scapa Flow in Orkney is of interest. This involves a study of eight species of molluscs (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 recognise 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 we have 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 conditions is also worth assessing. These offer the possibility of testing organisms and water in a variety of combinations which appropriately used could make a useful contribution to an effects study programme.

It is inherent in our recommendations on the evaluation of pollutant effects 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 programme, 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 (ICES, 1977). 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, as has been pointed out by Holden (1973) in his review of the effects of pesticide on fish, existing data are such that a coupling of this kind is seldom possible. Monitoring surveys on fish 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: concentration in physical environment $\longrightarrow$ residue in tissues and whole body $\longrightarrow$ 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 fish health.

CONCLUSIONS AND RECOMMENDATIONS

It is clear from the foregoing discussion that the major problem in building effects studies 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 usefully be regarded as simply another environmental variable which may induce, especially at 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 causative agents.

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 taxonomic 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 observations. 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 spatial 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 (e.g., for gill studies, halfheads might be collected and processed at suitable laboratories, while for the evaluation of tumour data a link with the highly successful Registry of Tumours in Lower Animals (see Harshbarger, 1975) might be investigated).

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 the 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 sub-lethal levels. Unfortunately, this proposal is at present difficult to implement because of the paucity of information linking environmental

concentrations, biological residues, and effects, as discussed in the previous section. 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 "bioassay" procedures, 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 emphasized 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 (i.e., 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, lysosomal 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 topics such as these linked, via field validation trials, with on-going programmes.

In summary, we consider that the possibilities 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 utilised 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 sub-lethal 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.

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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 Dr D F Alderdice, Dr H C Freeman, Professor J S Gray, Mr A V Holden, Dr A M Jones, Dr M S Mounib, Dr M Parker, Dr R J Pentreath, Mr A Preston, Dr G B Sangalang, Dr A R D Stebbing and Dr D S Woodhead. It should be emphasized, however, that the editorial committee (McIntyre, Bayne, Rosenthal and White) accept responsibility for the views expressed in the final report.

Indication of spine colours

Liaison Committee Reports .... Red

Reports of Advisory Committee on Marine
Pollution .... Yellow

Fish Assessment Reports .... Grey

Pollution Studies .... Green

Others .... Black

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