Review of the Problems [and Discussion]

M. Waldichuk, D. J. Crisp, R. J. Pentreath, H. Williams and K. W. Wilson

Phil. Trans. R. Soc. Lond. B 1979 **286**, 399-424
doi: 10.1098/rstb.1979.0037

Phil. Trans. R. Soc. Lond. B. **286**, 399–424 (1979)
Printed in Great Britain

Review of the problems

By M. Waldichuk
Department of Fisheries and the Environment, Fisheries and Marine Service,
Pacific Environment Institute, West Vancouver, B.C., V7V 1N6, Canada

Sublethal effects of pollution may be significant to survival of a stock of marine fish or even a species. Such effects sometimes lead to reproductive failure and have been identified so far only in freshwater systems. Atlantic salmon have disappeared from many streams in Europe and eastern North America, partly as a result of pollution in their freshwater spawning areas and in their estuarine nursing grounds. Reductions in populations of marine fishes due to pollution solely have not yet been demonstrated. However, Baltic Sea seals, where reproductive failure is apparently associated with high concentrations of DDT and polychlorinated biphenyl in the blubber, may have suffered a decline owing to the presence of these organochlorines.

Sublethal effects of pollutants have been studied in the laboratory, essentially under four categories: (1) physiology (growth, swimming performance, respiration, circulation); (2) biochemistry/cell structure (blood chemistry, enzyme activity, endocrinology, histochemistry); (3) behaviour/neurophysiology; and (4) reproduction. Not all pollutants elicit meaningful responses in all categories, and a response is not always linear with pollutant concentration. For application to survival of populations the response has to be ultimately related to a healthy progression through a full life cycle, including successful reproduction.

In recent time, physiological studies have moved into polluted marine environments with mobile laboratories having continuous sampling capability, to observe effects of pollutants *in situ* on marine organisms. The Controlled Ecosystem Pollution Experiment (Cepex) in Saanich Inlet, British Columbia, endeavours to investigate the effects of low concentrations of pollutants on marine organisms in large plastic silos having a slow replacement of water.

Nature of the problems and their importance

The adverse effects of pollutants on aquatic organisms have been generally identified with their acute and lethal impact. Mortality is an end point that can be readily recognized and quantified. A fish kill is dramatic evidence that something in the fish's environment has been grossly unsatisfactory. This condition could have been transient in the particular locality, with a sudden introduction of a lethal dose of a highly toxic substance, such as chlorine or potassium cyanide, or the fish may have migrated into the area of lethal conditions. The latter could arise from the presence of poisonous materials or from defects caused by certain processes such as the decomposition of organic materials depleting the dissolved oxygen. The presence of lethal conditions is usually quickly corrected by authorities under existing laws. The public generally will not tolerate for too long the presence of dead fish, and legislators are forced to take early action if there is inadequate legislation on the statute books to correct the matter.

Much as it is undesirable to have acutely toxic conditions and dead fish, perhaps a much more important consequence of pollution to guard against is the sublethal effect. Conceivably a fish population or even a species could be wiped out without anyone except the most discerning observer noticing its disappearance. There may be no overt symptoms of distress. Fish may complete their life cycle, apparently in a healthy state, except for one final step: they fail to reproduce. It is obvious that a population that does not go through the reproduction stage successfully will not propagate the race. The effect is often subtle and insidious, as it may occur gradually over a long period of time. Even careful scientific investigation may fail to identify the cause of a decreasing trend in the fishery until it is too late. Natural fluctuations in fish abundance and changes due to exploitation may obscure a decline in a population caused by a pollutant.

There is ample evidence of populations of fishes and other aquatic species declining and sometimes disappearing in freshwater environments (Brown, Shurben & Shaw 1970). The decline of the fine species of fish and increase of the coarse species has been well documented in the North American Great Lakes, especially in Lake Erie. The disappearance of Atlantic salmon from many polluted streams in Europe and in eastern North America is well known. Declines of Pacific salmon in the western United States of America have been attributed to pollution and loss of habitat through man's developments (Hester 1976). More recently, populations of fish have undergone high mortalities in some lakes and disappeared from other lakes suffering from acid runoff and precipitation in the Scandinavian countries (Leivestad & Muniz 1976; Milbrink & Johansson 1975), in east central Canada (Beamish & Harvey 1972; Beamish 1974, 1976) and in the northeastern part of the U.S.A. (Schofield 1965, 1976). No doubt one could make a long list documenting the loss of fish stocks in rivers and lakes in many parts of the world, particularly in the developed countries. However, the disappearance of fish from a particular freshwater body, especially a small lake or stream, is never regarded as a serious loss, because economically it may be quite insignificant. The policy is sometimes adopted of 'writing off' a particular stream for fisheries and utilizing it almost exclusively as a sewer for disposal of wastes. There have been exceptions where survival of a unique species has been threatened by a development in fresh water, and action has been taken to stop it, as in the well publicized case in the U.S.A. where a dam threatened a fish species and construction was prevented or at least delayed under the Endangered Species Act.

With respect to the marine environment, if there is a clear indication of a decline in stocks due to pollution, we cannot afford to take as lenient an attitude as we have sometimes taken towards our freshwater systems. The declining trend in a particular stock of marine fish or invertebrate species may be difficult to reverse if it is due to pollution. The world oceans are vast and there are some species that have a world-wide distribution. The stocks from different areas often intermingle, so that if one stock declines, it may become replenished by another stock. However, there are discrete stocks of fish and invertebrates in some parts of the world, and these undergo little migration and intermingling with other stocks. They are the most vulnerable to extinction, whether it be by overfishing or by severe alterations of their environment. It is worthwhile to examine marine situations where sublethal effects of pollutants have been identified and where they could potentially have serious consequences.

Some existing marine pollution problems at the lethal and sublethal level

Clear-cut evidence of sublethal and even acute effects of pollution on stocks of strictly marine species of fish is difficult to find. The effects of pollution on anadromous species can be more readily identified. However, declines in these species are usually due to adverse alterations in their freshwater environments or possibly in the estuaries. No marine pollution effects *per se* have been documented as causing declines of salmonids.

Semi-enclosed coastal marine bodies of water are the ones where both acute and subacute effects of pollution are most likely to occur. This includes fjords in the Scandinavian countries, lochs in Scotland, confined estuaries on the east coast of North America and inlets on the Pacific coast of Canada and in Alaska. The rate of water exchange in these partially enclosed, sometimes elongated systems is just not rapid enough to dilute and disperse pollutants which have been introduced, or to replace water that has been adversely altered, in dissolved oxygen content for example. Thus, there have been episodes of kills of herring and other species in inshore waters of the Pacific coast of Canada receiving sulphite pulpmill wastes where the dissolved oxygen content of the water was depleted to near zero (Waldichuk 1958, 1966). Similar fish kills have occurred in other coastal areas of the world. The effects of sublethal stress on fish stocks in these areas, however, have not been extensively documented, if at all.

Species of marine fish and invertebrates most vulnerable to pollution are those that frequent the particular part of the marine environment where a pollutant is likely to occur. This is usually at the physical interfaces between the atmosphere and the water, between the water and the bottom and sometimes between waters of different density. Few fish species or invertebrates are affected by the air–water interface, since they usually swim at some depth below the surface and only break the surface occasionally when capturing food. Therefore, an oil film only affects adult fish to the extent that they may be exposed to droplets of the oil that become mixed into the water column. However, pelagic fish eggs and larvae, especially if they come to the sea surface, can be very much affected by oil (Longwell 1977; Johnston 1977). The main impact of oil, nevertheless, is on invertebrates in the intertidal zone, the water–land interface, where oil may cover epifauna and smother infauna as the tide recedes.

Bottom fish and benthic invertebrates may be extremely vulnerable to pollution of certain types. An immiscible, heavy, toxic fluid, for example, would sink to the bottom and spread over the benthic habitat of fish and invertebrates. Accidental spills of polychlorinated biphenyls always end up on the bottom of coastal waters. Solid inert wastes, such as dredge spoils and mine tailings, would eventually settle to the bottom and cover benthic organisms and their habitats. Organic solids, such as wood wastes from sawmills and pulpmills, may blanket the bottom and decompose, causing anoxic conditions with possible formation of highly toxic hydrogen sulphide. A similar effect can arise from dumped sewage sludge, as noted in the New York Bight (Pearce 1972).

From a sublethal point of view, the long-term effect of ocean dumping of sewage sludge, dredge spoils and mine wastes on bottom populations is of interest. There is some evidence of fin erosion and lesions in one bottom species of fish, the Dover sole (*Microstomus pacificus*), which appears to be related to its proximity to a sewage discharge (Young, Young & Hlauka 1973). Similar incidents of fin erosion and lesions have been reported for bottom fish in the area of sewage sludge dumping in the New York Bight (Pearce 1972). An aspect that might be worthy of investigation in connection with the ecological impact of such waste disposal is the effect on commercially valuable invertebrates, e.g. scallops, lobsters and crabs. These species are less capable of migrating out of the affected area when conditions become unfavourable.

The effects of industrial wastes and domestic sewage in producing tumours in bottom fishes has not been clearly demonstrated, and the present evidence on the coast of British Columbia (Stich, Acton & Forrester 1976) is that there are as many tumours in fishes well removed from a pollution source as there are in fishes exposed to pollutants, which has been found true also on the coast of Japan (Oishi, Yamazaki & Harada 1976). The same conclusion was reached in studies, on demersal fishes in the vicinity of major southern California sewer outfalls, conducted by the Southern California Coastal Water Research Project (Young, Young & Hlauka 1973). However, the sublethal stress of a pollutant on a fish afflicted with tumours is probably greater than on a tumourless fish. The survival and ultimate reproduction by fish with tumours could be substantially lower than by healthy fish without tumours. This aspect has not yet been reported although some studies have considered this problem (Stich *et al.* 1977). The morphological changes in fish induced by pollutants offers one avenue for monitoring the effects of marine pollution on fish.

The bioaccumulation of metals and persistent organochlorines by marine organisms has provided one means of monitoring the presence of contaminants in living marine resources. The effect of human consumption of sea food contaminated in this way was exemplified by the tragedy of Minamata disease with mercury poisoning (Irukayama 1967). Bioaccumulation of mercury in such pelagic species as swordfish and tuna has been attributed to natural sources.

A problem of coastal pollution that is in part natural, but has been aggravated by activities of man in some areas, is nutrient enrichment leading to heavy algal blooms. One of the classic effects of apparent over-enrichment of coastal waters with nutrients, probably phosphates, was the stimulation of the dinoflagellate *Gymnodinium breve*, which developed in unusually high concentrations as a ‘red tide’ along the coast of Florida in 1946–7 and led to massive fish kills (Ketchum & Keen 1948). The source of nutrients that caused the over-enrichment was not positively identified, but it was believed to be phosphate deposits on land in Florida, which had been leached by runoff, thereby enriching coastal waters. Similarly caused fish kills associated with over-enrichment from sewage discharges were reported for the Ravenna coast of Italy on the Adriatic Sea during the latter part of the summer of 1976 (M. Cenerini, personal communication). The Japanese consider red tide, induced by excessive nutrients, as second only to oil pollution damage to their fisheries, with fish kills reported every year during heavy plankton blooms (Tokyo University of Fisheries 1976). However, the sublethal effect of red tide organisms on fish through direct toxicity of the organisms or their external metabolites is unknown. Certain red tide organisms, particularly *Gonyaulax* sp., cause paralytic shellfish poisoning among consumers of shellfish that have concentrated the toxin from these dinoflagellates. Incidents of shellfish toxicity have occurred on Atlantic and Pacific coasts of North America and in Europe. However, this type of red tide is not known to have an adverse effect on either the shellfish themselves, except when the toxin is present in very high concentrations, or on finfishes frequenting those waters.

Over-enrichment has had an adverse effect on oyster populations and possibly on other molluscan shellfish. Nutrients from duck farms in Great South Bay on Long Island, New York State, have been highly detrimental to oyster populations in the area through production of large blooms of undesirable algae. The native oyster, *Ostrea lurida*, on the Pacific coast of North America has been virtually wiped out by a combination of overfishing, pollution and other forms of destruction of habitat. At least one area on the Pacific coast of Canada with substantial production of this species in the early 1930s is now known to be highly eutrophic (Waldichuk 1969) and no longer supports any oysters. Pulpmill effluents have also led to declines in oyster populations, either through acute toxicity to larval stages of oysters or a reduction in the condition factor of adults.

Laboratory determinations of sublethal stress

The general stimulus–response relation of biological assays on the effect of pollutants on aquatic organisms is schematically represented in figure 1. It is seldom that there is a linear relation between dose and response under actual circumstances. With some pollutants there may be a real threshold of response, but at present there is no way of determining whether the threshold is real or whether our ability to measure response at very low doses is simply inadequate. There have been many laboratory techniques developed recently for measuring sublethal stress in aquatic organisms (table 1). Some of these have been borrowed from human clinical toxicology and adapted to aquatic toxicology. Many of the measurement tools developed for hospital laboratories have been only slightly modified for aquatic bioassays. The effect of a pollutant on marine organisms, and ultimately on populations and marine ecosystems, is illustrated in table 2.

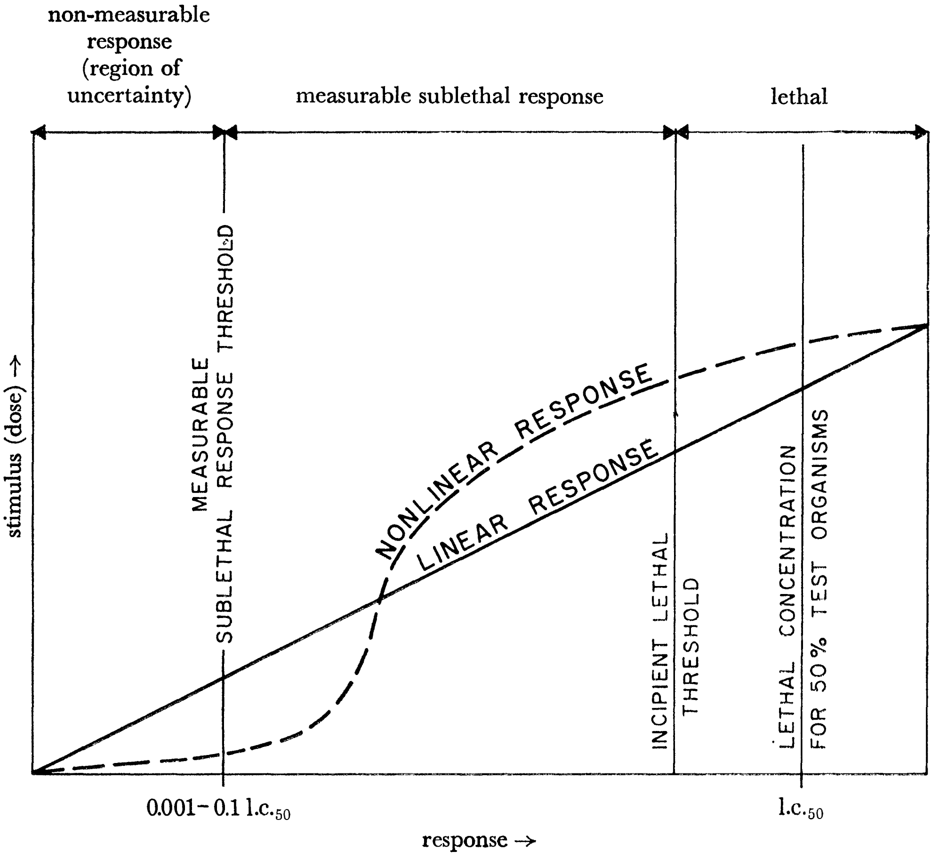

FIGURE 1. Hypothetical relation of concentration of pollutant to response of marine organisms, showing some significant points and regions on the curve.

The sublethal responses can be broadly subdivided into a number of categories, according to effect on the organism: (1) physiology; (2) biochemistry/cell structure; (3) behaviour; and (4) reproduction. It is worthwhile to examine each of these categories to note the long-term implications that they may have in survival of populations and propagation of the species. The use of biological assay for detection and measurement of water pollution has been discussed

Table 1. Sublethal responses of marine organisms to pollutants and some laboratory tests

response type	alteration in processes and other effects on organisms	laboratory tests
physiological	food intake (conversion efficiency) and growth rate (scope for growth)	feeding and growth measurements
	respiration and CO_2–O_2 exchange at gills	cough frequency; buccal pressure; ventilation volume (amount of water pumped per unit time across gills)
	blood circulation	arterial blood oxygen tension, CO_2, pH and haemoglobin
	heart rate	pulse rate recording through cannula to aorta
	metabolism	oxygen uptake and release of CO_2, NH_3 and other metabolic products in active and resting state
	swimming performance (scope for activity)	swimming speed measurement in tunnel respirometer
	mucus secretion	examination of gill filaments and intestine; measurement of mucus production
	osmoregulation	ionic concentration (e.g. Na^+, Mg^{2+}, Ca^{2+}) in blood, urine and test water, using waters of different salinities
biochemical	blood characteristics: haemoglobin, erythrocyte sedimentation rate; red and white blood cell count; pyruvate; lactate; glucose; leucocrit (number of leucocytes and thrombocytes); and serum proteins	established techniques for blood sampling and biochemical analyses; electrophoresis for serum proteins
histopathological	necrosis of tubules in kidney; decrease of glycogen and RNA and changes in the bile duct of the liver; decrease in RNA and changes in cell structure of the pancreas; cellular modifications in the spleen; changes in activity of goblet cells and necrosis of the epithelium in the intestine; necrosis of filaments and alteration in mucus secretion of the gills	histological and cytological examination with optical and electron scanning microscopes
behavioural	chemoreception (olfaction and taste), visual acuity and other orientation stimuli modification; central nervous system disruption	avoidance and preference ('choice') experiments; measurement of response to pollutant gradients; electrophysiology on exposed organisms
genetic	chromosome damage in egg cells; mutagenic and teratogenic effects on eggs and larvae; morphological deformities	egg and larval experiments with sublethal exposure to pollutants, by using advanced techniques of histology and cytology
reproduction	sterility of male and/or female; failure of gonads to develop fully; unsuccessful, or lack of, spawning	tests on viability of eggs and sperm; bioassays on full life cycle of test species under sublethal exposure

by Alderdice (1967). Sprague (1971) reviewed the various approaches to measurement of sublethal effects and evaluation of 'safe' concentrations.

1. *Physiology*

There is a host of physiological processes which can be measured in aquatic organisms for their response to sublethal stress on exposure to a low concentration of pollutant. Some of these processes with reference to salmonids have been reviewed by Davis (1976).

Table 2. Scheme showing how marine organisms might be affected by a pollutant at sublethal concentrations

pollutant
↓
sublethal exposure of marine organisms
↓
response:
biochemistry physiology morphology behaviour genetics reproduction
︸
impact on:
individual organisms
populations of species
communities
ecosystems

(*a*) *Growth*

One of the simplest measurements is the rate of growth of an aquatic organism exposed to different concentrations of a pollutant. Interpretations of results from growth experiments may also be the most difficult. Although there is usually a decreased growth at high concentrations, other factors sometime come into play at low concentrations. Anomalous results can be obtained, such as better growth rate at low concentrations of pollutant than in controls. This situation has been found with coho salmon and other species exposed to kraft pulpmill effluent by a number of investigators (Webb & Brett 1972; McLeay & Brown 1974; B. J. Mason & J. C. Davis, unpublished).

Perhaps more significant than actual growth rate is the food conversion efficiency or the *scope for growth* (s) (Warren & Davis 1967), represented by the energy difference between food consumed (E_c) and loss by excretion (E_e) and respiration (E_r), i.e.

$$s = E_c - E_e - E_r.$$

The growth efficiency (G) can then be expressed as:

$$G = (E_c - E_e - E_r)/E_c.$$

As pollutional stress reduces the numerator on the right of the above equation, the growth efficiency declines. Bayne (1975) used this approach to measure the effect of environmental stress in *Mytilus edulis*.

(*b*) *Swimming performance*

Fish can be exposed to different rates of flow in a tunnel-type respirometer to test their swimming performance and respiratory metabolism (Brett 1964). Dosed with given concentrations of pollutant, water in such a respirometer can be recirculated under controlled flow.

The ability of the fish to swim against a flow of water at different pollutant concentrations can be monitored by increasing the current until the fish collapses. The *scope for activity*, defined as the difference between the test animal's standard (animal at rest) and active metabolic rates, can be determined in a tunnel respirometer by using different concentrations of pollutant. Brett (1965) showed that salmon on a spawning migration may expend energy at a rate of 80 % of their maximum swimming speed. With a cannula inserted into the aorta of a fish being tested, it is possible to measure blood pressure, heart beat and to sample the blood for a variety of analyses. In a closed system, oxygen uptake and metabolic products, such as carbon dioxide and ammonia, can be measured. Because the system depends a great deal on having high quality fish that have been properly acclimated to the salinity and temperature under which the performance tests are being made, field testing of swimming performance presents certain technical problems (Davis, Shand & Mason 1976).

(*c*) *Respiration*

The ability of a fish to exchange carbon dioxide for oxygen across its gills is a valuable indicator of sublethal effects. With clinical equipment available to measure oxygen tension in small samples of blood, the technique can be readily applied in the laboratory where fish are exposed to different concentrations of a toxicant (Davis 1973).

A variation of a respiratory technique is in measurement of the coughing reaction of a fish attempting to clear its gills when exposed to a pollutant (Schaumberg, Howard & Walden 1967). By implantation of a tube in the buccal cavity of a fish and attachment to a transducer and pressure recorder, the coughing response can be measured. The rate of coughing usually increases with toxicant concentration, but the technique is not without its problems, especially in differentiating lower concentrations.

(*d*) *Circulation*

As in human medical diagnosis, a great deal can be learned about the health of an aquatic organism and its response to pollutant stress by monitoring the heart beat and blood pressure, especially when the animal is forced to undergo vigorous activity, e.g. subjected to a current in a swimming chamber. Circulation in sockeye salmon (*Oncorhynchus nerka*) exposed to bleached kraft mill effluent has been studied for sublethal effects (Davis 1973). However, careful examination is required to isolate the specific alterations that occur in the circulatory process when the test animal is exposed to different pollutant concentrations, and to differentiate these from various aberrations that may arise.

(*e*) *Others*

Other physiological processes, such as liver function, kidney performance, feeding, digestion, excretion and osmoregulation, can be monitored in test organisms exposed to different concentrations of pollutant. In filter-feeding organisms such as oysters and clams, a useful measurement is the rate of filtration. However, care has to be taken in making such tests, because some molluscs have the capability of stopping filtration when conditions in the water are unfavourable. Measurements of condition factor in oysters and mussels have provided a useful index of environmental conditions, including food availability. The choice of a suitable physiological process to measure has to be made on the basis of the most sensitive response at sublethal concentrations with reasonable linearity over the range of concentrations tested. Sometimes a remarkably simple sublethal test can be applied with rather basic laboratory equipment, as in the exposure of hydroids to metals (Karbe 1972; Stebbing 1976), which shed different parts of their body on exposure to increasing concentrations. Similarly, the reduction in growth rate of byssal threads in mussels exposed to sublethal concentrations of metals can be a useful index of environmental stress (Salazar 1974). A technique sometimes used to determine sublethal effects of pollutants is the exposure of organisms to different concentrations of a pollutant under environmental conditions, e.g. salinity, temperature and dissolved oxygen, near their limit of tolerance. By recording behavioural response, swimming performance, and even mortality at different pollutant concentrations under environmental stress, it is possible to obtain a measure of sublethal effects of the pollutant under normal environmental conditions. Long-term exposure (one month or more) of organisms to sublethal concentrations can also provide an indication of the effects of chronic exposure, especially with substances that are bioaccumulated.

In general, the harm in bioaccumulation of such substances as metals and organochlorines is usually associated with consumption of sea food by humans. Levels of mercury at 0.5 μg/g and higher in the tissues of organisms that bioaccumulate this metal have not been considered seriously harmful to the organisms themselves. However, some laboratory research demonstrates that this may not be true. When fish containing as little as 0.1 μg/g mercury in their muscle tissue are subjected to a torque in a rotating cylinder, they have greater difficulty in compensating for it than the control fish (Lindahl & Schwanbom 1971). Cadmium apparently affects calcium metabolism and this has adverse consequences on the otolith and the equilibrating mechanism of fish (Rosenthal & Alderdice 1976). How these factors actually affect fish in the field is uncertain, but one can assume that the young fish may have difficulty in avoiding predators and in seeking and capturing food.

2. *Biochemistry/cell structure*

A number of biochemical changes are known to occur when organisms are exposed to toxicants. These range from alterations in blood chemistry to enzyme changes.

(*a*) *Blood chemistry*

A variety of measurements can be made on the blood of aquatic organisms, some of which may show a relationship to level of pollutant exposure (Iwama, Greer & Larkin 1976). Plasma, haematocrit, glucose and chloride are some of the blood characteristics examined. Haematocrit is often measured, but only infrequently bears a relation to pollutant stress. Haemoglobin, erythrocyte sedimentation rates and red and white blood cell counts are usually better related to the general state of health of a test animal than to the short-term effect of exposure to a pollutant. Levels of haemoglobin, oxygen, carbon dioxide, pyruvate and lactate in venous blood have been used as a measure of effects of severe muscular activity in rainbow trout (*Salmo gairdneri*) by Black, Manning & Hayashi (1966). The lack of oxygen to active muscles leads to anaerobic metabolism of glycogen, which may become depleted while lactate accumulates. The amount of lactic acid in the blood is often related to the state of fatigue in fish. Burton, Jones & Cairns (1972) confirmed the hypothesis that death is related to tissue hypoxia when rainbow trout are exposed to acute zinc toxicity. Fish may recover from effects of fatigue and/or a pollutant, such as zinc, when returned to normal conditions. It has been found that lactate is oxidized to carbon dioxide by rainbow trout tissues (Bilinski & Jonas 1972).

Blood glucose has been found to be promising as a measure of stress in fish, an increase being noted in this constituent, for example, when fish are exposed to high concentrations of suspended solids (Noggle 1978). Serum protein has been measured by electrophoresis to provide a measure of stress on exposure to pollutants, particularly pulpmill effluent (Fujiya 1961), but there is some question of the consistency of the technique, when tested in different laboratories, for this purpose. More recently, blood chemistry techniques have been utilized in testing fish exposed in cages held in Stuart Channel at various distances from the pulpmill outfall at Crofton on the southeast coast of Vancouver Island (McLeay & Gordon 1977).

(*b*) *Enzyme activity*

The inhibition of enzyme activity was first recognized with the use of organophosphorus pesticides, when acetyl cholinesterase activity was found to be depressed (Weiss & Gakstatter 1964). Other enzymes, including δ-aminolaevulinate dehydrase, have been examined for effects of metals on *Fundulus heteroclitus* (Jachim 1974) and on other marine species (Brown 1976). Bayne, Livingstone, Moore & Widdows (1976) reported that lysosomal enzymes in *Mytilus* showed a good response to temperature and nutritive stressors. They also proposed the molar concentration ratio of taurine: glycine in the mussel's tissue as a sensitive biochemical index of stress. There is not always a clear-cut change in the activity of any one enzyme with changing metal concentrations, so that this technique does not offer a universal method of measuring sublethal effects with all pollutants.

(*c*) *Endocrinology*

Hormone function is considered to be a good potential technique for characterizing the effects of sublethal concentrations of toxicants. Hormonal imbalance undoubtedly occurs when an animal is exposed to pollutant stress. The plasma cortisone, cortisol and 'total' corticosteroid levels in sockeye salmon (*Oncorhynchus nerka*) were found to vary directly with exposure to different concentrations of copper ion (Donaldson & Dye 1975). Unfortunately, the relation is not linear with all metals, let alone with all pollutants. Too little work has been done in this important area to provide a full understanding of the rôle that endocrinology can play in sublethal studies of pollution, and possibly in demonstrating an effect on reproduction.

(*d*) *Histochemistry*

The change in tissue structure of vital organs can provide useful information to the skilled histologist/analyst on the effect of pollutant stress on the exposed organisms. This technique was compared with others in evaluating the effect of pulpmill pollution on fish (Fujiya 1965). However, insufficient work has been done on the technique to evaluate its sensitivity for pollution testing at the sublethal level. Like many of the specialized techniques for studying sublethal effects, it requires skills that can be acquired only with a great deal of practice, and fall into the category of an art rather than a science. The use of the electron scanning microscope extends the technique to the cellular level where cell changes can be noted, but again, good interpretation requires skills and experience.

(*e*) *Genetic effects*

Certain pollutants can lead to chromosomal and DNA changes in the cell. It is generally accepted that the greater proportion of genetic mutations is harmful and that they result in reduced fitness of a population. Mutations lead to a lessened capability of a population to cope with changes in the environment. The effects of radiation, of course, are quite well known in their ability to induce genetic mutations. How some of the non-radioactive pollutants behave in this respect is less well known. Nevertheless, the carcinogenic and mutagenic effects of certain substances are well recognized. Exposure of salmon eggs to selected metals has led to teratogenic effects (Servizi & Martens 1978) which can have an extremely important impact on the survival of the progeny. Mutation is now regarded by some geneticists as being involved in carcinogenesis, possibly as an initiating step. Therefore, the incidence of malignant tumours may be closely associated with mutagens.

Chromosome breaks may arise from changes resulting from mutation. Such rearrangements may lead to reduced fertility and/or fecundity in germ cells. Longwell (1977) has examined such genetic effects with respect to fish eggs exposed to oil. She noted that cells undergoing meiotic divisions which lead to the development of the female and male gametes in the gonads of fish are particularly susceptible to errors of chromosome separation and gene-level mutations. Chromosome errors are almost invariably lethal if they occur before the gastrula stage and, unlike physiological effects, there can be no recovery. Hence chromosome makeup abnormalities in the early embryo stage are perhaps the most sensitive practical indicators of the sublethal effects of marine pollutants on reproduction in fish.

3. *Behaviour/neurophysiology*

Pollutants even in low concentrations can elicit a behavioural response. Fish use their olfactory sense in part as a guide during migration. They are capable of detecting very low concentrations of certain substances, and no doubt anadromous species use this capability in homing in on their natal streams during their spawning migration. Species have been shown to exhibit an avoidance response to low concentrations of certain pollutants (Sprague & Drury 1969). Howell & Shelton (1970) suggested that the area of high turbidity on the southwest coast of England, receiving China clay wastes, was avoided by herring shoals. Wilson & Connor (1976) recorded evidence for such avoidance by whitebait in the same area. Normal schooling behaviour may be disrupted by a pollutant, and it has been suggested that fish show higher resistance to pollution when in a school than as individuals. In the Mediterranean Sea, it has been reported that the mining area of Sardinia and the marble industry near Trapani in Sicily have introduced much turbidity into adjacent waters and caused tuna to change their migration and avoid these areas (F.A.O. 1972).

Anderson (1971) demonstrated that concentration of DDT as low as 20 ng/g reduced a conditioned response (propellor tail reflex) in trout. His experiments showed that the learning ability of fish could be affected by very low concentrations of chlorinated hydrocarbons. This could have rather unfortunate consequences on the ability of fish to return to their home stream if they were exposed to even low concentrations of DDT during the imprinting period in their juvenile stage. Kleerekoper (1976) has indicated how behavioural responses can be influenced by multiple types of alterations in the aquatic environment with a gradient in metal concentration superimposed on a thermal gradient. Chemoreceptors can be affected in organisms by pollutants, and this could be of great significance to fish not only in homing to its parent stream on a spawning migration, but also in searching for food and possibly in avoiding predators. Equilibrium in fish can be affected by uptake of mercury (Lindahl & Schwanbom 1971) and cadmium (Rosenthal & Alderdice 1976).

It is desirable to carry out behaviour studies in the field to provide some measure of the real effect of pollutants on stocks of fish and shellfish. Saunders & Sprague (1967) investigated the effects of copper–zinc mining pollution on a spawning migration of Atlantic salmon. Birtwell (1977) examined the avoidance by fish of pulpmill effluent in a stratified inlet. In both studies certain significant avoidance reactions could be identified.

4. *Reproduction*

The sublethal effects of pollutants on reproduction of marine organisms might properly be placed under physiology, but it is considered to be the most important single function in the life cycle of an organism in connection with sublethal effects of pollution and therefore merits a section by itself. The real test of the long-term impact of a sublethal concentration of a pollutant on a fish population is whether it is capable of reproducing successfully.

TABLE 3. SOME OF THE SUBLETHAL EFFECTS OF A POLLUTANT ON VARIOUS LIFE STAGES OF A MARINE ORGANISM

life stage	vital life processes	critical effects of pollutants
egg	meiotic division of cells; fertilization; hatching enzyme activity; cleavage mitoses of fertilized egg; respiration	reduced fertility; gene damage; chromosome abnormalities; damage to egg's membrane; reduced hatching enzyme activity; direct toxicity to embryo from pollutant; impaired respiration
larva	metamorphosis; morphological development; feeding; growth; avoidance of predators, parasites and disease	toxicity from bioaccumulated poisons in yolk sac during early feeding; biochemical changes; physiological damage; deformities; behavioural alterations
juvenile	feeding; growth; development of immune systems, endocrine glands; avoidance of predators, parasites and disease	direct toxicity; reduced feeding and growth; altered predator–prey relations; impaired chemoreception; reduced resistance to parasites and disease
adult	feeding; growth; sexual maturation (development of gonads with male and female gametes)	direct toxicity; adverse alteration of environmental conditions, e.g. dissolved oxygen; physiological and biochemical changes; behavioural alterations
reproduction	spawning migration; spawning act (fertilization); successful completion of life cycle	avoidance reaction on spawning migration; destruction of spawning grounds; direct toxicity; reduced fertility

Reproductive failure can happen in a number of ways: (1) the fish may be unable to reach its spawning grounds because of unfavourable ecological conditions, or its own weak physical state, and goes unspawned; (2) the eggs may never be released by the female owing to some unsuitable physiological condition; (3) the eggs and alevins may die because of their unhealthy state or poor conditions on the spawning grounds; (4) the eggs and alevins may be poisoned by a substance bioaccumulated in the gonads of the parent, e.g. DDT; or (5) the eggs and alevins may be poisoned by toxic substances in the environment. Davis (1972) generally reviewed the effects of pollutants on reproduction of marine organisms. Rosenthal & Alderdice (1976) have discussed the sublethal effects of natural and polluted environmental conditions on marine fish eggs and larvae. Longwell (1977) specifically considered the effects of oil on fish eggs.

TABLE 4. SUBLETHAL EFFECTS OF SELECTED SUBSTANCES ON AQUATIC ORGANISMS

substance	effect	$10^9 \times$ concentration†	reference
cadmium	depressed O_2 consumption of winter flounder (*Pseudopleuronectes americanus*) under 60 days exposure	5–10	Calabrese *et al.* (1977)
	enzyme induction, lowered ligand sensitivity in the heart and antennal gland, and elevated oxygen consumption during 30-day exposure of American lobster (*Homarus americanus*)	3–6	Calabrese *et al.* (1977)
	successive hydranth reduction in *Eirene viridula*	300	Karbe (1972)
	herring larvae from eggs incubated in low-level cadmium concentration had difficulty in maintaining equilibrium	500–1000	Rosenthal & Sperling (1974)
copper	successive hydranth reduction in *Eirene viridula*	60	Karbe (1972)
mercury	elevated oxygen consumption in cunner (*Tautogolabrus adspersus*) during 30 days exposure	5	Karbe (1972)
	loss of equilibrium in roach (*Leuciscus rutilus* L.) when tested by the 'rotary-flow' technique after 5 days exposure	1 (as methyl mercury)	Lindahl & Schwambom (1971)
	successive hydranth reduction in *Eirene viridula*	6	Karbe (1972)
silver	depressed transaminase (heart), some loss of ligand sensitivity (antennal gland) and enzyme induction (gonad) in *Homarus americanus* with 30 days exposure	6	Calabrese *et al.* (1977)
	respiratory depression in *Tautogolabrus adspersus* during 96 h exposure	120	Thurberg & Collier (1977)
zinc	vertebral damage in *Phoxinus phoxinus* during 270 days exposure	200–300	Bengtsson (1974)
	avoidance reaction of salmonids (*Salmo gairdneri* and *S. salar*)	18 (0.032 of the lethal threshold concentration)	Sprague & Drury (1969)
chlorine	avoidance reaction (slight) of salmonids (*Salmo gairdneri* and *S. salar*)	1	Sprague & Drury (1969)
ABS detergent	threshold avoidance reaction of salmonids (*Salmo gairdneri* and *S. salar*)	370	Sprague & Drury (1969)
DDT	reduction in learning ability ('propellor tail reflex') and development of avoidance response	20	Anderson (1971)
kraft pulpmill effluent (including bleach-plant wastes)	various physiological effects on salmonids (*Salmo gairdneri* and *Oncorhynchus* sp.)	(0.05 of 96 h l.c._{50})	Walden (1976)
	physiological effects and tainting of salmonids	(0.02 of 96 h l.c._{50})	Davis (1976, 1977)
	avoidance reaction by salmonids (*Salmo gairdneri* and *S. salar*)	10000 (lethal at 56 %)	Sprague & Drury (1969)

Note: it is essential to specify characteristics (salinity, temperature, dissolved oxygen, etc.) of the water used, conditions under which tests were made and the form of the substance tested for a meaningful comparison of data from one laboratory to another. The reader is referred to the original literature for such information.

† Equivalent to 1 ng/g.

Failure of fish to reproduce in fresh water has been reported in connection with exposure to DDT (Burdick *et al.* 1964) and with acid precipitation (Schofield 1976). So far, no incidents have been reported of failure of marine fish to reproduce because of sublethal concentrations of pollutants. However, female seals in the Baltic Sea have exhibited pathological changes in the uterus, which have apparently led to a low reproductive rate. The seals showing these pathological changes had higher concentrations of DDT and polychlorinated biphenyl in their fat tissue than healthy animals (Helle, Olsson & Jensen 1976). Baltic Sea seal populations could seriously decline, if indeed the pathological effect is proven to be due to organochlorines, and these are not diminished in the marine environment.

The main adverse effects on reproduction reported for marine fish so far are in the destruction of preferred spawning grounds. In the North Sea, extraction of gravel threatens the habitat of sandeels (*Ammodytes* sp.) and the spawning grounds of North Sea herring (*Clupea harengus*). On the Pacific coast of Canada, elimination of eel grass (*Zostera* sp.) beds and other rooted aquatics along the seashore could have serious consequences for Pacific herring (*Clupea harengus pallasi*), which use these substrates for laying their eggs. It is believed that an oil spill in the vicinity of a Pacific herring spawning area could have serious consequences for this species, especially in late winter and early spring when they spawn (Canada Department of Fisheries and the Environment 1978). Gravel extraction could affect the Pacific cod (*Gadus macrocephalus*), which, like the North Sea herring, lays its eggs on the cobbly sea bottom along the outer coast of Vancouver Island. Sinking agents used for removal of oil from the sea surface and deposition on the bottom could also destroy Pacific cod eggs, and possibly adversely alter the spawning grounds, at least temporarily.

The sensitivity to a pollutant of each stage in the life cycle of a marine organism must be taken into account. Some of the effects of pollution at each stage are shown in table 3. Sublethal effects of selected substances on certain aquatic organisms are given in table 4.

LABORATORY AND FIELD SITUATIONS CONTRASTED: DIFFICULTIES OF EXTRAPOLATION

The relation of laboratory bioassay data to the field is central to the continuing debate between physiologists and ecologists. The physiologist maintains that, if laboratory tests are to be meaningful, they must be conducted under rigidly controlled conditions with test animals that have been cultured under ideal circumstances and properly acclimated to the environmental characteristics of the tests. An algal physiologist may even insist that his test algae come from axenic cultures and the invertebrate specialist may work with cloned specimens. The ecologist counters with the argument that neither the controlled conditions nor the specially cultured test organisms ever exist in nature. The laboratory tests have therefore little bearing on the real situation.

Both parties in the debate are right, at least in part. But the problem of bringing the two sides of the debate together does not get resolved if both continue their investigations in traditional ways without an effort to meet some of the demands of the other in providing reliable scientific information on dose–response, on the one hand, and meaningful application to cause–effect in the field situation on the other. The physiologist needs to take his laboratory facilities into the field, where he can conduct investigations on waters and contamination as it exists there, carefully recording all the environmental conditions. The ecologist must move toward observations on organisms in the vicinity of pollutant discharges over an extended period, systematically taking account of all environmental changes attributable to pollution. Ideally, the ecological data should be gathered on given sectors of the affected marine ecosystem in a way that produces as little interference as possible with the normal circulation and other physical characteristics. It may be necessary to introduce colonized substrates, prepared on artificial media, at different distances from an outfall to investigate the effects of pollution on benthic communities. Caged animals might be exposed to the condition *in situ* at different distances from a pollutant source; or they may be held in a vertically compartmented cage, where they can choose in a stratified system with vertical variation in pollution the preferred layer for swimming. Thus, the physiologist, ethologist and ecologist can come together in such field experiments, and produce information on effects of pollution that can have some application to real field situations.

We have endeavoured to conduct some of this kind of research on the Pacific coast of Canada. The research barge *L. Pacifica* at the Pacific Environment Institute has been fully equipped with recirculation systems and environmental control for bioassay experiments in the field. Similarly to previously reported laboratories (Smith *et al.* 1972), she can be anchored near an ocean outfall from industry, such as a pulp and paper mill, and bioassay experiments can be conducted with contaminated water taken *in situ*. Clean sea water from the recirculation system can be adjusted to the same temperature and salinity as the in-situ water and used as a control. Studies were conducted in Stuart Channel, adjacent to a kraft pulp mill at Crofton on the southeast coast of Vancouver Island in the summer of 1974 (Davis, Shand & Mason 1976; Davis 1977), in Neroutsos Inlet, near a sulphite pulp mill at Port Alice on the northwest coast of Vancouver Island, during 1973–7 (Davis, Shand, Christie & Kosakoski 1978), and in Howe Sound outside the kraft pulp mill in Port Mellon on the lower mainland coast of British Columbia in the summers of 1976 and 1977. Although time has had to be spent on refinement of techniques, and occasionally factors over which a scientist has no control, such as the labour strike at the Port Alice pulp mill in 1975, hamper experiments, progress is being made in bringing physiological and behavioural studies into the field. An interesting phenomenon noted in Stuart Channel was that tested fish exhibited the greatest cough response on the ebb tide when pulp-mill effluent from the outfall flowed in greatest volume past the barge laboratory (Davis 1977).

Ecological studies are being conducted under partly controlled conditions in a Controlled Ecosystem Pollution Experiment (Cepex) in Saanich Inlet under auspices of the International Decade of Ocean Exploration (I.D.O.E.) and funded by the United States National Science Foundation (N.S.F.). This experiment involves the use of large cylinders of water 10 m in diameter by 30 m deep, enclosed by clear polyethylene film, where water and pollutant replacement can be closely controlled and changes in plankton populations or introduced fish can be carefully monitored. A great deal of information on the sublethal effects of low concentrations of metals, petroleum hydrocarbons and other pollutants on an inlet ecosystem is being noted (N.S.F./I.D.O.E. 1973; Parsons 1977; Lee *et al.* 1977; Reeve *et al.* 1976). However, even such information cannot always be readily extrapolated to field conditions. The effects of wind, tide and runoff never allow truly uniform and constant conditions, even under steady-state discharge, to exist in space and time in nature.

How can laboratory bioassay data be best applied to field conditions? It should probably involve a series of steps for best results with our present technology. Bioassay experiments should ideally take into account the full range of environmental variables that exist in a site-specific situation. These include temperature, salinity and dissolved oxygen. Preferably, the bioassay tests should utilize the water from the area of concern for improved applicability of data. Response surfaces can be evaluated by the multi-factorial technique developed by Box & Wilson (1951) and extended to a variety of biological systems by Alderdice (1972). The ecological conditions in the system where the bioassay data are to be applied should be known for the full range of seasonal extremes. Ideally, a simple model of environmental conditions in the area of interest should be developed. Then by applying information from the response surface to the environmental model, a reasonable approximation can be obtained of the minimum concentration of pollutant that will elicit a response under a given set of environmental conditions. It should be noted that this is still only an approximation, and even under the best circumstances fields trials are necessary to test the predictions. With highly hazardous substances, such as radioactive wastes, discharge tests are normally conducted at considerably lower levels than the calculated allowable maximum. This was the scheme used, for example, in testing the predictions on radioactive waste disposal into the Irish Sea from the Windscale Works on the Cumberland coast of England (Dunster 1955).

There is a need to conduct field observations of a monitoring type on marine organisms in polluted and unpolluted marine environments. This would provide an inventory on the wellbeing of organisms in various geographical areas exposed to different degrees of pollutional stress. Some of the observations that would provide meaningful information on the health of stocks include gill damage, vertebral deformities, tumours, liver function, gonad state and general morphological characteristics. These measurements should be accompanied by water and tissue analyses for suspected contaminants; and in the case of benthic invertebrates, sediment analysis would be useful. In this way, an advance may be made to link information on effects of pollutants on marine organisms to residues in tissues and levels in water and sediments.

Every physiologist can probably offer his or her favourite test to establish the effect of a pollutant on marine organisms and ultimately on populations. Clearly, the test should have some bearing on reproductive success of the test organisms. In that respect, a gonad/somatic index should prove useful. Growth efficiency should be informative in that it could be a good indicator, at least in juvenile stages, of how rapidly organisms will grow and resist predation, disease, and parasitism under their particular state of wellbeing. A number of biochemical measurements have been suggested as diagnostic tests of viability of stocks of marine organisms: (*a*) steroid hormones, as a measure of effects of pollutants on steroid metabolism in detoxification; (*b*) lysosomal enzymes, as a quantitative index of lysosomal labilization due to pollutants; and (*c*) vitellogenin, which is a phosphoprotein in the blood serum of fish concerned with yolk formation, could provide a measure of vitellogenesis, and ultimately larval development, in a given stock. Research, including field-validation trials, is still required to establish the applicability of such tests to diagnose the health of marine stocks.

Marine observations on effects of pollutants on living resources would have the greatest promise of showing clear cause–effect relations in areas having discrete stocks of organisms with limited migrations. There should be a comparatively small exchange of waters with the open sea in such areas, so that physical and chemical characteristics of the waters undergo minimum fluctuations. The joint studies on sublethal effects of pollution on marine organisms in the Firth of Clyde, Oslo Fjord and the Wadden Sea (W.H.O. 1975) would appear to fulfil criteria for such regional investigations. The special field studies considered for these programmes merit noting: (*a*) investigation of biological conditions in direct relation to gradients of pollution; (*b*) study of special critical stages in the development of organisms; (*c*) analysis of changes in species diversity; (*d*) analysis of changes in genetic diversity within species; (*e*) observations on test organisms deliberately introduced into the field situation; (*f*) organized documentation of the occurrence and geographical distribution of certain pathological phenomena, such as tumours, lesions and deformities; (*g*) testing of the physical fitness of specimens taken from the field, utilizing physiological and biochemical techniques (e.g. by the 'rotary flow technique' applied to fishes and blood analysis), and (*h*) sampling and documentation of specimens in a collection for future reference and comparison.

It was noted in discussions of the foregoing regional programmes that certain environmental contaminants have a known immunosuppressive action in marine vertebrates. Studies of the immunological response may be particularly relevant with respect to the locally increased incidence of tumours and parasites. It was recognized that applicability of many physiological and biochemical techniques, e.g. histological structure of the liver, blood haemoglobin, haematocrit values and blood glucose levels, suffers from lack of information about 'normal' values of these variables and about the way in which they are causally related with the pollutants in question.

Larger marine areas will require extremely careful studies over a long period to separate the effects of pollution from that of commercial fishery exploitation and natural environmental changes. Cole (1972) and Johnston (1976) showed that there was no apparent effect of pollution on North Sea stocks. In fact, there was a general increase in total catch of all species by all countries between 1950 and 1970. Johnston (1977) endeavoured to estimate the economic effect of North Sea oil on fisheries. He concluded that the effect would be small.

IMPORTANCE OF SUBLETHAL EFFECTS IN CONTROL OF POLLUTION

Studies on sublethal effects of pollutants have gained a great deal of impetus in the last decade, partly because of their practical importance and partly owing to academic interest. There are various ways of investigating sublethal effects, and each technique provides an insight into the physiology or behaviour of the organism in question. However, for the purpose of pollution control, it is essential to focus on a technique that provides the significant information. Much as the variety of data on diverse sublethal effects is of scientific interest, it may have little direct relevance for pollution control.

The most important sublethal effect of a pollutant that must be determined is the significance to reproduction. What impact does the pollutant have on the population of a given species or on a particular stock? This means that laboratory tests should be conducted on several generations of a species. Obviously, the choice of test organisms for convenience would involve those with a comparatively short generation time. In freshwater tests, *Daphnia* sp. have met with considerable success for simplicity. Amphipods, mysids or isopods may serve as useful test organisms with a short life cycle in the marine environment, but these are not necessarily the most sensitive organisms or the ones that it is most essential to protect. In the absence of bioassay experiments covering several generations of a test organism, the most sensitive life stages of the principal organism to be protected should be examined. These are usually the egg and early post-embryonic stages. The success of hatching of the eggs is an important criterion of water quality. The survival of the larvae to fry in the case of fish and to the first post-larval stages in the invertebrates is a major hurdle in the life cycle of most aquatic organisms. Certain sublethal effects, such as deformities and aberrant behaviour, can be recognized in these early life stages. Often an organism affected in this way during the early stages of its life will not survive for very long in nature.

Although it is difficult to achieve natural spawning of most organisms in captivity, it may be necessary to develop suitable techniques so that reproductive success can be measured at various sublethal concentrations of pollutants. So far, most of the conclusions on reproductive success have been inferred from changes in fish populations in freshwater systems. DDT and polychlorinated biphenyls have been implicated in the reproductive failure of Baltic Sea seals. Experiments are needed where direct observations can be made of reproductive success of marine species under exposures to different concentrations of pollutants. Such information is vital for the protection of valuable stocks of fish and shellfish. The haunting concern of conservationists is that a pollutant may affect an organism in a most unexpected way, as demonstrated with the Baltic seals.

The effect of sublethal stress on organisms may manifest itself in other ways than causing outright reproductive failure. It is known, for example, that some stocks of adult sockeye salmon migrating up the Fraser River must cover a distance of some 500 or 600 miles (800–960 km) to the spawning grounds in the Stuart Lake area near the middle of the province of British Columbia. These fish have a finite amount of energy stored in their system while they migrate (they do not feed at this stage), and it is known that during certain climatically unusual years, some of the migrating fish are unable to overcome natural obstacles on their route and fail to reach their spawning grounds. The question sometimes asked is: 'How many more sockeye would go unspawned if an additional pollutant stress were put in their path in the river or at its seaward approaches?' Ultimately, the sublethal stress has an impact on spawning success and the propagation of that particular stock of sockeye salmon.

A side effect that must be taken into account in sublethal stress of pollutants to marine organisms is the impact on the general vigour of the organisms and their ability to ward off predators, parasites and disease. It is known that sockeye smolts infected by parasites succumb to lower concentrations of metals than uninfected fish (N. P. J. Boyce, personal communication). It is also known that fish exposed to a pollutant stress either become infected by a disease more readily than unexposed fish, or may break out with a disease that previously existed only in a latent form. This is an extremely significant facet of pollution in intensive mariculture, where water quality can be an important factor in disease control.

A sublethal effect that can be hardly classed with the others, in that it may not actually be of any consequence to the organism itself, is tainting. This is a form of bioaccumulation that has its main impact on the consumer. The tainted animal could actually benefit by this effect in that predators, including man, would avoid it. However, petroleum hydrocarbons, many of which impart a taint to organisms exposed to them, could also have a harmful effect on the organisms.

So far, we have no scientific way of identifying properties of a substance which would be expected to impart a taint to aquatic organisms, although experience can often guide us to recognize groups of compounds that can cause a taint. Pulpmill effluents cause tainting in certain species of fish, especially those with a high lipid content. We have been largely unable to identify chemically the causative agent. It is hoped that once such identification can be made, we shall be able to treat the effluent to remove the offending substance. Chemical identification of tainting substances from petroleum hydrocarbons would put fish inspection services in a better position to control the quality of products for marketing. So far, only taste panels have been able to serve this purpose.

Pollution control agencies in the U.S.A. and Canada are beginning to utilize water quality criteria based on sublethal effects of pollutants (N.A.S. 1973) for development of guidelines and standards for effluent disposal into natural waters. To account for sublethal effects, it is still common practice to introduce an 'application factor' to $\mathrm{l.c.}_{50}$ values, ranging from 0.1 to 0.001, depending on the acute toxicity, bioaccumulation and long-term hazard to aquatic organisms and man. Data are now being acquired in both freshwater (Brown, Shurben & Shaw 1970) and marine environments (Davis 1976), which allow better estimation of the sublethal threshold of pollutants. The sublethal threshold for kraft pulpmill effluents, for example, has been given as 0.02 of the 96 h $\mathrm{l.c.}_{50}$ by Davis (1976, 1977), although Walden (1976) states that 0.05 of the 96 h $\mathrm{l.c.}_{50}$ would be more realistic. In any case, design of ocean outfalls for pulpmill effluent disposal on the Pacific Coast of Canada now takes into account the sublethal threshold concentration as a goal in achieving the required dilution and dispersion.

Concluding remarks

Bioassays integrate all the variables, known and unknown, that affect water quality. In mixtures of substances, such as pulpmill effluents (Walden 1976), chemical analyses cannot generally provide a measure of the toxic strength. Acute bioassays for 48 or 96 h are still the best means of toxicologically characterizing such materials and of detecting previously unsuspected contaminants. The effects of synergism and antagonism are also taken into account by bioassays. The choice of a test organism is often based on the ease of holding and culturing such organisms. Sometimes the most resistant stage, e.g. juvenile or adult, of fish is used for testing. A proper evaluation of both acute and sublethal effects of a pollutant should take into account the full life cycle of the aquatic organism to be protected, and repeated tests should utilize the most sensitive stage, usually larvae. In some cases, long-term sublethal effects can be identified in behaviour patterns over prolonged exposure of 21 days or more. However, more sophisticated measurements of a physiological, biochemical or behavioural nature may be required for a quantitative sublethal response.

There is now a wide variety of laboratory tests to measure sublethal effects of pollutants on marine organisms. The big question often posed is whether the responses measured have significance to the healthy survival of the test organism in the marine environment and to its successful reproduction. The ultimate test of the significance of a sublethal effect is whether it has an impact on the propagation of a species and on its population.

The stress of a pollutant measured on a laboratory test animal may be little more than the adaptation response exhibited by an organism in adjusting to normal environmental changes. Such acclimation capability may be essential for survival of the species through the various stressful conditions encountered in its life cycle. In fact, the practice is being increasingly adopted in fisheries enhancement activities of subjecting the early life stages of species to as many as possible of the kinds of stresses encountered in nature. Adaptive physiological response can be distinguished from harmful physiological response in the ultimate expressions of biological performance, which contribute to survival, growth and reproduction of the species.

There is clearly a big gap between the information acquired on sublethal effects in the laboratory under controlled conditions and its application to the polluted marine environment. Coastal waters are usually the most affected by pollution, but there is wide variability in such waters not only in pollutant concentrations but also in the natural environmental characteristics. A fish, for example, seldom encounters in nature the kind of uniform conditions established for tests in the laboratory. In nature, the peak concentrations of toxicant encountered by a fish for short periods, when it swims through a plume of pollutant, may be significant only to its immediate survival but may have little effect on its full life cycle. The exposure to the low, ambient, sublethal concentrations of the toxicant over a long period may have an impact on its long-term growth, survival and successful reproduction. It is the cumulative effect of some constituent, e.g. chlorinated hydrocarbon or metal, which ultimately may lead to reproductive failure through acute toxicity of high concentrations in the gonads to eggs and larvae, or a teratogenic effect on the progeny leading to early mortality of the larvae or the juvenile fish.

Some questions on sublethal effects and their measurement that must be answered are:

1. What responses to pollutant exposure can be reliably and simply measured in laboratory bioassays?

2. What meaning do the foregoing responses have in terms of healthy survival of the test species in the marine environment? Is the damage caused by such exposure cumulative and irreversible, or is recovery complete when the test species is returned to clean sea water? Is there a delayed toxicity effect? What is the mode of toxic action?

3. Are 'safe' concentrations, as defined in the laboratory, indeed the 'safe' (no effect) concentrations in the field?

4. How can the laboratory techniques be adapted to field studies of sublethal effects of pollution?

5. What are the diagnostic tests to establish the effect of a pollutant on reproduction of a species?

6. How would one establish the sublethal effect of a pollutant on marine communities of organisms and on the marine ecosystems?

7. What test or series of tests can be conducted to determine the impact that a pollutant present in sublethal concentrations will have on the population of a marine species?

8. How can one separate fluctuations in fisheries that are due to pollution from those due to fishing pressure, natural environmental factors or 'normal' production of poor year classes? In other words, for identifying effects of pollution, how does one separate the 'signal' from the 'noise'?

Field observations are essential to complete the picture of effects of pollutants on marine organisms. Benthic invertebrates integrate the effect of pollutants over a period of time, especially if they are sessile. Indigenous organisms, such as oysters and clams, may be analysed for condition factor or for bioaccumulated contaminants. Trays of invertebrates or cages of fish may be introduced for measurement of residues and effects *in situ*. More advanced techniques, such as use of tunnel respirometer or rotary-flow apparatus, might be used to test on-site resident populations of marine organisms. Various biochemical analyses can be applied to measure the health and vigour of local organisms, but these should be carefully field tested and significance of results ascertained beforehand. An effort should be made in any monitoring programme to link physiological and biochemical effects to residues of contaminants in tissues of exposed organisms and to levels found in water and sediments.

Sublethal effects of pollutants are now being recognized by regulatory agencies in establishing pollution controls. Rather than applying an arbitrary 'application factor', as a safety factor, to the l.c.$_{50}$ data obtained in acute toxicity bioassays, pollution control is now being developed by using the sublethal threshold level, derived in chronic-toxicity bioassays, as the limiting concentration for effluent disposal. Even in administering the International Convention for the Prevention of Marine Pollution by Dumping of Wastes and Other Matter (U.K. 1972), the term 'harmlessness' of a particular substance is being defined by application of data from sublethal toxicity studies, among others.

REFERENCES (Waldichuk)

Alderdice, D. F. 1967 The detection and measurement of water pollution – biological assays. *Can. Fish. Rep.* no. 9, pp. 33–39.

Alderdice, D. F. 1972 Factor combinations. In *Marine ecology* (ed. O. Kinne), vol. 1, pp. 1659–1722. New York: Wiley Interscience.

Anderson, J. M. 1971 Assessment of the effects of pollutants on physiology and behaviour. *Proc. R. Soc. Lond.* B **177**, 307–320.

Bayne, B. L. 1975 Aspects of physiological condition in *Mytilus edulis* (L.) with special reference to the effects of oxygen tension and salinity. In *Proc. 9th European Marine Biology Symposium* (ed. H. Barnes), pp. 213–238. Aberdeen University Press.

Bayne, B. L., Livingstone, D. R., Moore, M. N. & Widdows, J. 1976 A cytochemical and biochemical index of stress in *Mytilus edulis* L. *Mar. Pollut. Bull.* **7**, 221–224.

Beamish, R. J. 1974 Loss of fish populations from unexploited remote lakes in Ontario, Canada as a consequence of atmospheric fallout of acid. *Water Res.* **8**, 85–95.

Beamish, R. J. 1976 Acidification of lakes in Canada by acid precipitation and the resulting effects on fishes. *Water, Air, Soil Pollut.* **6**, 501–514.

Beamish, R. J. & Harvey, H. H. 1972 Acidification of the La Cloche Mountain lakes, Ontario and resulting fish mortalities. *J. Fish. Res. Bd Can.* **29**, 1131–1143.

Bengtsson, B. E. 1974 Vertebral damage to minnows *Phoxinus phoxinus* exposed to zinc. *Oikos* **25**, 135–139.

Bilinski, E. & Jonas, R. E. E. 1972 Oxidation of lactate to carbon dioxide by rainbow trout (*Salmo gairdneri*) tissues. *J. Fish. Res. Bd Can.* **29**, 1467–1471.

Birtwell, I. K. 1977 A field technique for studying the avoidance of fish to pollutants. In *Proc. 3rd Aquatic Toxicity Workshop, Halifax, N.S., Nov.* 2–3, 1976, pp. 69–86. (Environmental Protection Service Tech. Rep. no. EPS-5-AR-77-1.)

Box, G. E. P. & Wilson, K. B. 1951 On the experimental attainment of optimum conditions. *J. R. stat. Soc.* B **13**, 1–45.

Black, E. C., Manning, G. T. & Hayashi, K. 1966 Changes in levels of hemoglobin, oxygen, carbon dioxide, pyruvate, and lactate in venous blood of rainbow trout (*Salmo gairdneri*) during and following severe muscular activity. *J. Fish. Res. Bd Can.* **23**, 783–795.

Brett, J. R. 1964 The respiratory metabolism and swimming performance of young sockeye salmon. *J. Fish. Res. Bd Can.* **21**, 1183–1226.

Brett, J. R. 1965 The swimming energetics of salmon. *Scient. Am.* **213**, 80–85.

Brown Jr, G. W. 1976 Effects of polluting substances on enzymes of aquatic organisms. *J. Fish. Res. Bd Can.* **33**, 2018–2022.

Brown, V. M., Shurben, D. G. & Shaw, D. 1970 Studies on water quality and the absence of fish from polluted English rivers. *Water Res.* **4**, 363–382.

Burdick, G. E., Harris, E. J., Dean, H. J., Walker, T. M., Skea, J. & Colby, D. 1964 The accumulation of DDT in lake trout and the effect on reproduction. *Trans. Am. Fish. Soc.* **93**, 127–136.

Burton, D. T., Jones, A. H. & Cairns Jr, J. 1972 Acute zinc toxicity to rainbow trout (*Salmo gairdneri*): confirmation of the hypothesis that death is related to tissue hypoxia. *J. Fish. Res. Bd Can.* **29**, 1463–1466.

Calabrese, A., Thurberg, F. P. & Gould, E. 1977 Effects of cadmium, mercury, and silver on marine animals. *Mar. Fish. Rev.* **39**, 5–11.

Canada Department of Fisheries and the Environment 1978 *Potential Pacific coast oil ports: a comparative environmental risk analysis.* Working Group on West Coast deepwater oil ports, vol. 1 (98 pages). Vancouver, B.C.: Fisheries and Environment Canada.

Cole, H. A. 1972 North Sea pollution. In *Marine pollution and sea life* (ed. M. Ruivo), pp. 3–10. London: Fishing News (Books) Ltd.

Davis, C. C. 1972 The effects of pollutants on the reproduction of marine organisms. In *Marine pollution and sea life* (ed. M. Ruivo), pp. 305–311. London: Fishing News (Books) Ltd.

Davis, J. C. 1973 Sublethal effects of bleached kraft pulp mill effluent on respiration and circulation in sockeye salmon (*Oncorhynchus nerka*). *J. Fish. Res. Bd Can.* **30**, 369–377.

Davis, J. C. 1976 Progress in sublethal effect studies with kraft pulpmill effluent and salmonids. *J. Fish. Res. Bd Can.* **33**, 2031–2035.

Davis, J. C. 1977 Standardization and protocols of bioassays – their role and significance for monitoring, research and regulatory useage. In *Proc. 3rd Aquatic Toxicity Workshop, Halifax, N.S., Nov.* 2–3, 1976, pp. 1–14. (Environmental Protection Service, Tech. Rep. no. EPS-5-AR-77-1.)

Davis, J. C., Shand, I. G. & Mason, B. J. 1976 Biological and oceanographic studies at a kraft pulp and paper outfall at Crofton, B.C., 1974. *Fish. mar. Serv. tech. Rep.* no. 652, 67 pages.

Davis, J. C., Shand, I. G., Christie, G. & Kosakoski, G. 1978 Biological and oceanographic observations in the Neroutsos Inlet area with emphasis on the effect of sulfite pulp mill waste on Pacific salmon 1973–1977. *Fish. & Mar. Serv. MS Rep.* no. 1447, 88 pages.

Donaldson, E. M. & Dye, H. M. 1975 Corticosteroid concentrations in sockeye salmon (*Oncorhynchus nerka*) exposed to low concentrations of copper. *J. Fish. Res. Bd Can.* **32**, 533–539.

Dunster, H. J. 1955 The discharge of radioactive waste products into the Irish Sea. Part 2. The preliminary estimate of the safe daily discharge of radioactive effluent. *Proc. U.N. Int. Conf. Peaceful Uses of Atomic Energy, A/Conf.* 8/P/419, vol. 9.

F.A.O. 1972 The state of marine pollution in the Mediterranean and legislative controls. *Studies and reviews* no. 51, 68 pages. Rome: General Fisheries Council for the Mediterranean, Food and Agricultural Organization of the United Nations.

Fujiya, M. 1961 Effects of kraft pulp mill wastes on fish. *J. Wat. Pollut. Control Fedn* **33**, 968–977.

Fujiya, M. 1965 Physiological estimation of the effects of pollutants upon aquatic organisms. In *Advances in water pollution research* (Proc. 2nd Internat. Conf., Tokyo, 1964), vol. 3, pp. 315–331. Oxford: Pergamon Press.

Helle, E., Olsson, M. & Jensen, S. 1976 PCB levels correlated with pathological changes in seal uteri. *Ambio* **5**, 261–263.

Hester, F. J. 1976 Economic aspects of the effects of pollution on the marine and anadromous fisheries of the western United States of America. *Food and Agricultural Organization of the United Nations Technical Paper* no. 162, 34 pages.

Howell, B. R. & Shelton, R. G. J. 1970 The effect of china clay on the bottom fauna of St Austell and Mevagissey Bays. *J. mar. biol. Ass. U.K.* **50**, 593–607.

Irukayama, K. 1967 The pollution of Minamata Bay and Minamata disease. Advances Water Pollution Research, In *Proc. 3rd Int. Conf., Munich*, 1966, vol. 3, pp. 153–183. Washington, D.C.: Wat. Poll. Control Fedn.

Iwama, G. K., Greer, G. L. & Larkin, P. A. 1976 Changes in some hematological characteristics of coho salmon (*Oncorhynchus kisutch*) in response to acute exposure to dehydroabietic acid (DHAA) at different exercise levels. *J. Fish. Res. Bd Can.* **33**, 285–289.

Jackim, E. 1974 Enzyme responses to metals in fish. In *Pollution and physiology of marine organisms* (ed. F. J. Vernberg & W. B. Vernberg), pp. 59–65. New York: Academic Press.

Johnston, R. 1976 Mechanisms and problems of marine pollution in relation to commercial fisheries. In *Marine Pollution* (ed. R. Johnston), pp. 3–156. London: Academic Press.

Johnston, R. 1977 What North Sea oil might cost fisheries. *Rapp. P.-v. Réun. Cons. perm. int. Explor. Mer* **171**, 212–223.

Karbe, L. 1972 Marine Hydroiden als Testorganismen zur Prüfung der Toxizität von Abwasserstoffen. Die Wirkung von Schwermetallen auf Kolonien von *Eirene viridula*. *Mar. Biol.* **12**, 316–328.

Ketchum, B. H. & Keen, J. 1948 Unusual phosphorus concentrations in the Florida 'red tide' sea water. *J. mar. Res.* **7**, 17–21.

Kleerekoper, H. 1976 Effects of sublethal concentrations of pollutants on the behaviour of fish. *J. Fish. Res. Bd Can.* **33**, 2036–2039.

Lee, R. F., Takahashi, M., Beers, J. R., Thomas, W. H., Seibert, D. L. R., Koeller, R. & Green, D. R. 1977 Controlled ecosystems: their use in the study of the effects of petroleum hydrocarbons on plankton. In *Physiological responses of marine biota to pollutants* (ed. F. J. Vernberg, A. Calabrese, F. P. Thurberg & W. B. Vernberg), pp. 323–342. New York: Academic Press.

Leivestad, H. & Muniz, I. P. 1976 Fish kill at low pH in a Norwegian river. *Nature, Lond.* **259**, 391–392.

Lindahl, P. E. & Schwanbom, E. 1971 Rotary-flow technique as a means of detecting sublethal poisoning in fish populations. *Oikos* **22**, 354–357.

Longwell, A. C. 1977 A genetic look at fish eggs and oil. *Oceanus* **20**, 46–58.

Mason, B. J. & Davis, J. C. Growth in underyearling coho salmon, *Oncorhynchus kisutch*, during chronic exposure to sublethal levels of neutralized bleached kraft pulp mill waste. Unpublished manuscript (18 pages). Pacific Environment Institute, West Vancouver, B.C.

McLeay, D. J. & Brown, D. A. 1974 Growth stimulation and biochemical changes in juvenile coho salmon (*Oncorhynchus kisutch*) exposed to bleached kraft pulp mill effluent for 200 days. *J. Fish. Res. Bd Can.* **31**, 1043–1049.

McLeay, D. J. & Gordon, M. R. 1977 Leucocrit: a simple hematological technique for measuring acute stress in salmonid fish, including stressful concentrations of pulpmill effluent. *J. Fish. Res. Bd Can.* **34**, 2164–2175.

Millbrink, G. & Johansson, N. 1975 *Some effects of acidification on roe of roach,* Rutilus rutilus *L, and perch,* Perca fluviatilis *L. – with special reference to the Åvaå Lake system in eastern Sweden.* Institute of Freshwater Research, Drottningholm, Sweden, Report no. 54, pp. 52–62.

N.A.S. 1973 *Water quality criteria 1972.* (594 pages.) Washington, D.C.: National Academy of Sciences.

Noggle, C. 1978 The behaviour and physiological effects of suspended sediment on juvenile salmonids. In *Proceedings 4th Aquatic Toxicity Workshop, Vancouver, B.C., November* 8–10, 1977, pp. 54–63.

N.S.F./I.D.O.E. 1973 *International Decade of Ocean Exploration. Program description.* Office of the International Decade of Ocean Exploration, Second Report. Washington, D.C.: National Science Foundation.

Oishi, K., Yamazaki, F. & Harada, T. 1976 Epidermal papillomas of flat-fish in the coastal waters of Hokkaido, Japan. *J. Fish. Res. Bd Can.* **33**, 2011–2017.

Parsons, T. R. 1977 Test tubes in the sea. *Unesco Courier,* January 1977, 2 pages.

Pearce, J. B. 1972 The effects of solid waste disposal on benthic communities in the New York Bight. In *Marine pollution and sea life* (ed. M. Ruivo), pp. 404–411. London: Fishing News (Books) Ltd.

Reeve, M. R., Grice, G. D., Gibson, V. R., Walter, M. A., Darcy, K. & Ikeda, T. 1976 A controlled environmental pollution experiment (CEPEX) and its usefulness in the study of larger marine zooplankton under toxic stress. In *Effects of pollutants on aquatic organisms* (ed. A. P. M. Lockwood), pp. 145–162. Cambridge University Press.

Rosenthal, H. & Alderdice, D. F. 1976 Sublethal effects of environmental stressors, natural and pollutional, on marine fish eggs and larvae. *J. Fish. Res. Bd Can.* **33**, 2047–2065.

Rosenthal, H. & Sperling, K.-R. 1974 Effects of cadmium on development and survival of herring eggs. In *The early life history of fish* (ed. J. H. S. Blaxter), pp. 383–396. Berlin: Springer-Verlag.

Saunders, R. L. & Sprague, J. B. 1967 Effects of copper–zinc mining pollution on a spawning migration of Atlantic salmon. *Water Res.* **1**, 419–432.

Salazar, M. H. 1974 Byssal thread production as a toxicity test. In *Proc. Conf. on Marine Biology in Environmental Protection, San Clemente Island, Calif. 13–15 Nov. 1973.*

Schaumberg, F. D., Howard, T. E. & Walden, C. C. 1967 A method to evaluate the effects of water pollutants on fish respiration. *Water Res.* **1**, 731–737.

Schofield, C. L. 1965 Water quality in relation to survival of brook trout, *Salvelinus fontinalis* (Mitchell). *Trans. Am. Fish. Soc.* **94**, 227–235.

Schofield, C. L. 1976 Acid precipitation: effects on fish. *Ambio* **5**, 228–230.

Servizi, J. A. & Martens, D. W. 1978 Effects of selected heavy metals on early life of sockeye and pink salmon. International Pacific Salmon Fisheries Commission, New Westminister, Progress Report no. 39 (26 pages).

Smith, L. S., Cardwell, R. D., Mears, A. J., Newcomb, T. W. & Watters Jr, K. W. 1972 Physiological changes experienced by Pacific salmon migrating through a polluted urban estuary. In *Marine pollution and sea life* (ed. M. Ruivo), pp. 322–325. London: Fishing News (Books) Ltd.

Sprague, J. B. 1971 Measurement of pollutant toxicity to fish. III. Sublethal effects and 'safe' concentrations. *Water Res.* **5**, 245–256.

Sprague, J. B. & Drury, D. E. 1969 Avoidance reactions of salmonid fish to representative pollutants. In *Advances in water pollution research* (Proc. 4th Internat. Conf., Prague, 1969.), vol. 1, pp. 169–179. Oxford: Pergamon Press.

Stich, H. F., Acton, A. B. & Forrester, C. R. 1976 Fish tumors and sublethal effects of pollutants. *J. Fish. Res. Bd Can.* **33**, 1993–2001.

Stich, H. F., Acton, A. B., Oishi, K., Yamazaki, F., Harada, T., Hibino, T. & Moser, H. G. 1977 Systematic collaborative studies on neoplasms in marine animals as related to the environment. *Ann. N.Y. Acad. Sci.* **298**, 374–388.

Stebbing, A. R. D. 1976 The effects of low metal levels on a clonal hydroid. *J. mar. biol. Ass. U.K.* **56**, 977–994.

Thurberg, F. P. & Collier, R. S. 1977 Respiratory response of cunners to silver. *Mar. Pollut. Bull.* **8**, 40–41.

Tokyo University of Fisheries 1976 *Assessment of the effects of pollution on fisheries and aquaculture in Japan.* Food and Agricultural Organization of the United Nations Technical Paper no. 163, 105 pages.

Walden, C. C. 1976 The toxicity of pulp and paper mill effluents and corresponding measurement procedures. *Water Res.* **10**, 639–664.

Waldichuk, M. 1958 Some oceanographic characteristics of a polluted inlet in British Columbia. *J. mar. Res.* **17** (suppl.), 536–551.

Waldichuk, M. 1966 Effects of sulfite wastes in a partially enclosed marine system in British Columbia. *J. Wat. Pollut. Control Fedn* **38**, 1484–1505.

Waldichuk, M. 1969 Eutrophication studies in a shallow inlet on Vancouver Island. *J. Wat. Pollut. Control Fedn* **41**, 745–764.

Warren, C. E. & Davis, G. E. 1967 Laboratory studies on the feeding, bio-energetics and growth of fish. In *The biological basis of freshwater fish production* (ed. S. D. Gerkin), pp. 175–214. Oxford: Blackwell Scientific Publ.

Webb, P. W. & Brett, J. R. 1972 The effects of sublethal concentrations of whole bleached kraft mill effluent on the growth and food conversion efficiency of underyearling sockeye salmon (*Oncorhynchus nerka*). *J. Fish. Res. Bd Can.* **29**, 1555–1563.

W.H.O. 1975 *Ecological aspects of water pollution in specific geographical areas: study of sublethal effects on marine organisms in the Firth of Clyde, the Oslo Fjord and the Wadden Sea.* (38 pages.) Long-Term Programme in Environmental Pollution Control in Europe. Report on a Working Group convened by the Regional Office for Europe of the World Health Organization, Wageningen, Netherlands, 2–4 December 1974. Copenhagen, Denmark: Regional Office for Europe, World Health Organization.

Weiss, C. M. & Gakstatter, R. H. 1964 Detection of pesticides in water by biochemical assay. *J. Wat. Pollut. Control Fedn* **36**, 240–253.

Wilson, K. W. & Connor, P. M. 1976 The effect of china clay on the fish of St Austell and Mevagissey Bays. *J. mar. biol. Ass. U.K.* **56**, 769–780.

Young, D. R., Young, C. S. & Hlauka, G. E. 1973 Sources of trace metals from highly urbanized southern California to the adjacent marine ecosystem. In *Cycling and control of metals* (ed. M. G. Curry & G. M. Gigliotti). Cincinnati, Ohio: Natural Environment Research Center.

U.K. 1972 *Final Act on the inter-governmental conference on the convention on the dumping of wastes at sea (with convention, technical memorandum and resolution).* Cmnd 5169 (18 pages). London: Her Majesty's Stationery Office.

Discussion

D. J. Crisp, F.R.S. (*Marine Science Laboratories, Menai Bridge, Gwynedd, U.K.*). A figure of 0.02–0.05 of the l.c.$_{50}$ was quoted as the limiting sublethal effect level. Such generalizations could be very useful if the limiting sublethal level were interpretable as a safe level. Would Dr Waldichuk please comment on the relations between sublethal limits and safe limits?

M. Waldichuk. In general, the level of 0.02–0.05 of the l.c.$_{50}$ for full-bleach kraft mill effluent is the threshold of biochemical techniques. It is considered now as the 'no effect' or 'safe' level, because we are at the limit of our capability for measurement of sublethal response with this type of material on the species (mainly salmonids) tested. If our capability improves with greater sensitivity in measuring sublethal responses, or if we use life stages of organisms which are more sensitive, then we might find it necessary to lower the concentration of kraft mill effluent to one considered to be safe for protection of our living resources.

R. J. Pentreath (*Fisheries Radiobiological Laboratory, Hamilton Dock, Lowestoft, Suffolk NR32 1DA, U.K.*). Dr Waldichuk has given an adept paper on a 'review of the problems' without once mentioning concentrations of chemicals; and yet surely one of the 'problems' is the difference – even for sublethal effects – between the concentrations required to induce effects and the concentrations of the majority of chemicals found in contaminated environments. In view of his varied experience in Canada would he care to comment on the magnitude of this difference with regard to different classes of pollutants?

M. Waldichuk. I should like to refer to the concentration of kraft pulpmill effluent at which sublethal effects have been measured and relate this to concentrations found in the marine environment. The sublethal threshold has been given as 0.02 (Davis 1977) or 0.05 (Walden 1976) of the 96 h l.c.$_{50}$, depending on how one arrives at it. Sublethal thresholds of this waste are given in terms of the l.c.$_{50}$, because there is no one toxic constituent that can be measured chemically and related linearly to the toxicity of kraft pulpmill effluent. Work done *in situ* near the Crofton pulp and paper mill on the British Columbia coast during the summer of 1974 (Davis, Shand & Mason 1976; Davis 1977) showed that concentrations of between 0.05 and 0.25 of the 96 h l.c.$_{50}$ existed in Stuart Channel at times during the study, and induced a coughing response in the test fish. The studies were done within about 1 km of the outfalls, which terminate in diffusers set at about 20 m below lower low tide.

It is noteworthy that we are now requiring that outfalls for pulp and paper mills are so designed that they provide an immediate dilution that will achieve concentrations below the sublethal threshold.

I am afraid that I cannot say much about other classes of pollutants except that such metals as copper and zinc have been found to be high enough in water of a New Brunswick river to affect migration of Atlantic salmon.

H. Williams (*The Open University in Wales, Cardiff CF1 3PH, U.K.*). In response to Dr Waldichuk's reference to salmon I should like to mention that about 40 species of parasites have been recorded from European salmon and about 100 from Pacific salmon species. Some of this parasitological knowledge has already been used as an aid in the solution of various biological problems concerning salmon. I should be very surprised if none of the parasites of the salmon is suitable as a model or indicator for studying the sublethal effects of pollutants especially since there is a considerable amount of information on the life cycles, physiology and biochemistry of the groups of parasites involved.

The same might be said of the parasites and associated fauna of seals. Dr Waldichuk's reference to the Baltic Sea seal is interesting. It is a final host for the nematode known as *Anisakis*. As many as 90% of the herring in the Baltic Sea are infected and yet Baltic Sea seals are rare and there are no crustaceans or cetaceans present. The problem continues to attract the attention of Polish parasitologists who seem to be among the first to have published useful information on parasites as indicators of the state of the environment. (see Wiadomosci, *Parazyt* **20**, 775–786 (1974)). I should like to return to details of this work later in the discussion.

M. Waldichuk. Certainly, I can only agree that parasites and parasitic infection might give some clues on the state of pollution in coastal waters. I should like to refer to some work done in Babine Lake, British Columbia, in which sockeye salmon smolts spend a year before proceeding to sea through the Skeena River system. This is the site of a major salmonid enhancement project where artificial spawning channels were installed in streams tributary to Babine Lake during the 1960s to increase salmon production. In 1973, there was a major output of sockeye smolts (90 million compared with an average of 40 million), but this failed to produce the expected return of adults in 1974, 1975 and 1976. Various possible causes for low returns are being examined. It has been found that approximately 30% of the sockeye smolt population is infected by the tapeworm *Eubothrium salvelini*, which affects the stamina, growth, vitality and survival of sockeye salmon in their first year. A copper mine in Babine Lake releases tailings into a lagoon formed among the islands, which have been connected by causeways to seal the lagoon from the lake. Some of the tailings seep out carrying with them copper and zinc, among other metals, in low concentrations. Smolts infected with *E. salvelini* have been found to be less resistant to zinc pollution, and probably copper as well, than uninfected smolts. No doubt other stresses could seriously affect the infected smolts during their migration to sea. Various strategies are now being attempted to reduce the incidence of infection by this cestode.

K. W. Wilson (*North-West Water Authority* (*Rivers Division*), *Buttermarket St.*, *Warrington*, *Cheshire*, *U.K.*). Could Dr Waldichuk say why he has been able to find only limited documented evidence of sublethal effects in the marine environment? Is it because of the use of inappropriate detection methods to date, or is it that sublethal effects are not there to be found?

M. Waldichuk. The main problem is in selection of suitable techniques for measurement of sublethal effects on marine organisms in the field. We have been able to observe certain behaviour manifestations with some species in the presence of pollutants. For example, Saunders & Sprague (1967) found that about 25% of the Altantic salmon encountering sublethal levels of copper and zinc in a migration upriver in the Canadian Maritimes returned to sea unspawned. Howell & Shelton (1970) suggested that the turbid area on the southwest coast of England receiving china clay wastes was avoided by herring shoals, and Wilson & Connor (1976) actually recorded avoidance by whitebait in the same area. There are many problems, of course, in unequivocaly relating avoidance, or other behavioural aberrations, to a particular pollutant. We have had difficulty in the laboratory and in the field in documenting reproducible avoidance reactions of salmonids to kraft pulpmill effluent.

Communities of benthic organisms can be altered by sublethal concentrations of pollutants, but it is difficult to quantify such changes. Species diversity does not appear to provide the answer because normal environmental changes may have a large impact on diversity. Physiological and biochemical effects of sublethal concentrations of pollutants on particular species in the field cannot be monitored too easily because there are no reliable routine techniques available so far. A report of a Sub-Group on the Feasibility of Effects Monitoring of the I.C.E.S. Working Group on Pollution Baseline and Monitoring Studies in the Oslo Commission and I.C.N.A.F. Areas (I.C.E.S. 1978) has reviewed potentially useful techniques. Perhaps Dr Bayne in his paper will discuss some of these, such as latency of lysosomal enzymes in *Mytilus edulis*, and their present state of development for routine monitoring.

Reference

I.C.E.S. 1978 *On the feasibility of effects monitoring.* Council for the Exploration of the Sea, Charlottenlund Slot, Denmark, Cooperative Research Report no. 75, 42 pages.