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The Need for Sublethal Studies [and Discussion]

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The need for sublethal studies

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In problems of waste management, the preoccupation of the would-be manager is the means whereby waste may be released to the environment without impairing the health of the biota inhabiting the receiving waters. In such a situation, measurements based upon acute poisoning are unhelpful since they tell nothing of the impact that the much lower concentrations found at some distance from the waste source have upon the ability of the affected organisms to undertake the responses necessary to ensure survival and more particularly to reproduce successfully. Such responses can only be investigated with organisms not at the point of death, i.e. in truly sublethal studies.

Introduction

It seems to be worth noting, at the outset, the essential reasons why we undertake toxicology and, when we do, the fundamental criteria which must be fulfilled if it is to have practical value. While we may talk in general terms of maintaining the environment as a whole in a healthy condition, despite the conflict of interests which is particularly evident in coastal waters, we are primarily concerned with the wellbeing of man and thus of the fish and shellfish of economic importance. Consequently, we should be examining not the mere survival of these important organisms, but the maintenance of truly viable populations. The primary criterion therefore must always be the effectiveness of reproduction in the population or populations exposed to the waste introductions. While all else is secondary to this consideration, successful reproduction does not of necessity imply the acceptable level of productivity in the fishery, which is vital to successful fishery management. By definition, then, studies which are preoccupied with the short-term alternatives of death or survival, i.e. lethal studies as in the case of median tolerance limit determinations, must of necessity be excluded from further consideration here. This exclusion is not arbitrary, for as Warner (1964) said 'Death is, by definition, the cessation of biological activities. Hence it is asymptomatic; no information relative to sublethal effects can be obtained from the response.' Clearly, in the short term this view is unarguable; in the longer term, however, this criterion may be acceptable, for as Perkins (1976) noted, the response is unambiguous, and when placed in a different framework may have a greater value than has perhaps been realized hitherto. Nevertheless, truly sublethal studies, not related to mortality, are required if the essential objectives of fishery management are to be fulfilled, but, from an examination of the literature, what is apparently much less clear is the nature of the sublethal studies required.

Although the aim is to prevent significant losses of production from our aquatic resources, it is reasonable to expect that the sums contributed for their preservation will be limited. This limitation will influence methodology to the extent that scope for very large, complex experiments outside the laboratory will be restricted; indeed it would be pointless to develop a protection 'industry' which would rival in scale that which it seeks to protect. Thus laboratory studies must be meaningful and effective; possibly the evident difficulty in relating laboratory results to events in the field is an indication of the extent to which our techniques must be improved.

DEFINITIONS EMPLOYED

There are three areas of research discussed below in relation to which it is essential to define the terms used.

The first relates to the terms 'sublethal', 'sublethal dose' of a toxicant and 'long-term sublethal study', each of which is the subject of much confusion in the literature. These terms are interrelated, though the third is controlled by the test organism chosen rather than the toxicant under examination. Alderdice (1967) defined sublethal effects of pollutants as debilitating and thus only bringing about death indirectly, but, as it stands, that definition while clear in meaning leaves too much scope for ill-conceived and badly executed experimentation. It is considered that a sublethal dose of a toxicant is one in which the direct mortality of the organisms exposed to the toxicant exceeds that in the controls by no more than 10% p.a., where the life span exceeds 1 year, or, when the life span is less than 1 year, does not exceed that in the controls by 10% at the median of the known life span. By accepting some direct excess death in the treated animals, this definition is apparently somewhat looser than that of Alderdice (1967). However, at this level of mortality, it would be difficult to distinguish between the direct and indirect effects of intoxication, and such a small difference would, by any criterion, represent a high degree of replication between tanks. Experiments which do not reach this standard should not be considered as sublethal but as chronic subacute, and whatever other parameter may be under investigation the terminal mortality should be recorded. The validity of this approach can be appreciated by reference to table 1, where each experiment lasted for only a minor proportion of the life span of this crab (see table 2), and thus could in no sense be described as sublethal, although in the natural environment so active an animal would remove itself rapidly from distasteful conditions.

It follows from the preceding paragraph that the description 'long-term study' would be inappropriate here, as it is in so much of the literature which includes experiment durations ranging from 240 h to *ca.* 1 year. Clearly, 240 h can be described as long-term if it relates to some organism which either divides every few hours or has a life span of not more than 2 weeks. Neither 240 h nor 1 year can be considered as long term when applied to some of the species recorded in table 2. It is considered that a long-term study is one defined as having a duration which is either at least as long as the whole life span in those organisms which live for up to one year, and in the case of the short-lived (i.e. life span not more than 20 days), pass through several generations, or in those organisms whose length of life exceeds one year, for at least as long as the time from birth or metamorphosis to first egg production. Experiments having a shorter duration should be defined in terms of the known life span (S_1). Thus experiments which have been conducted for 0.1, 0.5, 2 or 4 times a life span should be categorized as 0.1, 0.5, 2 and 4 S_1 respectively. By using a prefixed S_1, confusion with any kind of lethal dosage is avoided and an unambiguous expression of the duration and relative value of the experiment is given (see column 4 of table 1); equally a notional title of a paper could read 'The effects of sublethal exposure (0.5 S_1) of the crab, *A.b.*, to effluent constituents'. This approach permits a graphical expression of mortality against proportion of life span passed and this may be compared with expected mortality in untreated stocks; an approach which is both more explicit and informative than either the short period dose response curves of median tolerance limit ($\mathrm{t.l.}_\mathrm{m}$) against time or the use of $\mathrm{l.t.}_{50}$ in experiments of a greater duration. As O. J. Abbott, a coworker of mine, realized, it may enable actuarial techniques to be used to forecast the expected mortality and losses of production in a fishery, arising from waste disposal practices. This methodology would, however, require a very different scale of experimentation from that rightly considered to be asymptomatic by Warner (1964): because it is concerned with survival/mortality, it is excluded from further discussion.

Table 1. Mortality of the shore crab, *Carcinus maenas*, exposed to Siddick paper board mill effluent and its constituents

	experiment duration			toxicant concentration (%)		
		proportion of life span†		1.0	10.0	30.0
toxicant	days	%	proposed notation	terminal mortality (%)		
whole effluent	87	7	$0.07\,S_\mathrm{l}$	50	38	—
$Al_2(SO_4)_3$	33	3	$0.03\,S_\mathrm{l}$	20	0	—
wood fibre	186	15	$0.15\,S_\mathrm{l}$	—	—	33

† Taken to be 3.5 years.

Table 2. Life spans of some selected marine animals

species	life span/year	authority
Arenicola marina	2+	Newell (1948)
Crangon crangon	3–5	Tiews (1967)
Homarus spp.	*ca.* 10	Iversen (1968)
Carcinus maenas	3–4	Schmitt (1973)
Cancer pagurus	*ca.* 12	Schmitt (1973)
Littorina littorea	> 6	personal observation
Pecten maximus	18–22	Segerstråle (1960)
Mercenaria mercenaria	17, 40	Segerstråle (1960)
Tellina tenuis	7+	Segerstråle (1960)
Asterias rubens	5–6	Bull (1934)
Gadus morhua	6+	Wheeler (1969)
Pomatoschistus minutus	*ca.* 1	Wheeler (1969)
Pleuronectes platessa	♂ *ca.* 10–12 ♀ 20	Wheeler (1969)

See also Buchanan (1964), Chesher (1969), Dales (1950, 1951), Fretter & Graham (1962), Hancock & Urquhart (1965), Lagler, Bardach & Miller (1962), Lewis (1971), Tattersall & Tattersall (1951) and Zaika (1973).

Two further areas of definition need consideration and these are concerned with usage of the terms 'diversity' and 'fecundity'. In the following discussion accepted usage, i.e. both by biologists and in the Oxford Dictionary, is followed. Thus diversity refers to being diverse, unlikeness, different kind, variety, but diversity indices refer only to 'information content', a feature which is apparently not generally recognized. Thus Pielou (1966) noted that 'diversity in this connection means the degree of uncertainty attached to the specific identity of any randomly selected individual. The greater the number of species and the more nearly equal their proportions, the greater the uncertainty and hence the diversity'. Clearly the two definitions are different and the considerable potential for confusion is increased by the chaotic use of formulae: thus the popular Shannon & Weaver formula, indiscriminately referred to as the Shannon diversity index, the Shannon and Weaver formula and the Shannon–Weiner function, is variously quoted in at least nine different forms depending upon logarithmic base and use of notation; some are mathematically incorrect and some have no properly defined terms. Only two papers other than that of Pielou (1966) state that the calculation refers to uncertainty (Cameron 1972) or to information theory diversity (Watling 1975). In such a situation it is difficult either to accept the validity of such data or envisage their use in the solution of practical problems. Some of the confusion might be removed if it could be agreed that the two types of diversity do not mean the same thing and that the second should always be referred to as information theory diversity; even as *d* (diversity) and i.t.d. (information theory diversity) if that should be considered necessary; agreement regarding the logarithm base would be equally helpful. Given such improvements, this technique might be useful in ecological studies of sublethal effects.

Fecundity has always been taken to refer to egg production per female and this meaning is employed below. I respectfully suggest that the term fecundity as used by D. H. Cushing in this symposium conflicts with the normal usage though it would be perfectly satisfactory if it were only qualified in the manner proposed in relation to diversity.

RESPONSES AT THE SUBLETHAL LEVEL

The responses likely at the sublethal level may be considered under two broad categories.

1. *Effects upon reproductive potential*

Generally these might be considered to apply only to fecundity and successful fertilization of the egg. To many species, however, metamorphosis and settlement represents such a major transition that this would seem a more appropriate, if arbitrary, point of distinction.

Studies could be reasonably treated under four subheadings:

(*a*) direct effects upon fecundity and the successful fertilization of the egg;

(*b*) effects upon the fertilized egg and larvae to the point of metamorphosis;

(*c*) modifications in behaviour induced by the toxicant which may affect the settlement of planktonic larvae at the time of metamorphosis;

(*d*) studies of effects of a toxicant upon the course of the life history and successive generations.

2. *Effects upon the productivity of a fishery*

These may be influenced or examined by one or more of the following parameters:

(*a*) inhibition or enhancement of growth;

(*b*) interference in community structure and in particular in predator–prey relations through the agency of selective toxicity;

(*c*) modification in behaviour induced by the toxicant, including the avoidance of particular situations, and the motor functions related to swimming;

(*d*) effects upon biochemical and physiological mechanisms especially as they relate to respiration, osmoregulation, ionic regulation, the composition of the body fluids, effects upon enzyme systems, bioaccumulation and the development of tolerance;

(*e*) predisposition to disease, parasites and histo-pathological change.

This list of possibilities represents an ascending order of difficulty in obtaining and translating the information gained in the laboratory into some practical expression in terms of environmental management. Nevertheless, by preparing a list in this way one can, by using also table 2, perhaps see more clearly the manner in which particular aspects should be examined and the animals that can give the most effective results both in terms of laboratory resources and avoidance of intrinsically futile experiment. The acquisition of such information in the laboratory rather than in the field is emphasized here on grounds of cost and the need in a decision-making process for results within a relatively short time. Clearly, field studies cannot be excluded in some form from consideration since they represent the only means of verification of decisions made by extrapolating results from the relatively simple laboratory experiments to the complex environment of the sea.

Field studies

If we ignore cost, field studies of those situations which are inhabited by a given species or community in the presence of a waste or pollutant, by definition, offer the possibility of investigating tolerance, sensitivity, reproductive capacity and productivity under patently sublethal conditions. However, interpretation of the data obtained is difficult, not least with respect to establishing baselines where some form of succession is occurring (Lewis 1971). The theoretical expression of the relation between growth and production of a living system and the input of growth factors or pollutants by means of an oligodynamic dose response curve may be useful in environmental management (Perkins 1974), but it suffers from an inherent inadequacy, an examination of which gives a simple expression to the difficulties of relating laboratory and field data.

Essentially, the oligodynamic response curve as it refers to some population parameter (figure 1) must be considered as the line which envelopes the maximum response possible at any given concentration of pollutant. Moreover, in the laboratory, and particularly where the elements of competition are removed, this maximal response is normally evoked. In terms of natural reproduction, productivity and growth, however, there is no reason to suppose that the response is always maximal in either a temporal or a spatial sense. There is likewise no reason to believe that any given community necessarily includes all the species potentially capable of inhabiting that situation. Thus in a particular situation, apparently colonized fully, there may remain 'spaces' which, given the right opportunity, can be occupied by other species absent at the time of investigation. These 'spaces' may be filled only at times when there is a particularly successful larval survival which is not related directly to the conditions prevailing at the particular situation under review, but may well have a fundamental effect subsequently. This contention may be supported by the following observations:

(*a*) haphazard and intermittent recruitment of benthic invertebrates (see for example, McIntyre 1970; Moore 1978);

(*b*) the widely recorded temporal fluctuations in populations which attain possible maxima infrequently;

(*c*) brown algae, and associated epibionts, removed by human and other agency, may not recolonize rocky substrata readily, so that productivity and diversity are much reduced thereby (Fralick, Turgeon & Mathieson 1974; Lang & Mann 1976; Perkins 1976);

(*d*) Perkins (1977) noted the following succession mediated by biological interaction: *Halichondria* (1970/71) → *Balanus crenatus* (1973) → *Halichondria*/*Sabellaria* (1975 onwards) when *Halichondria* and *Sabellaria* colonized approximately equally but in different areas. Since 1975, the *Sabellaria* has received further recruitment and grown well; other sessile species, e.g. *Halichondria*, *B. crenatus* and the algae *Gigartina* and *Chondrus*, have been overwhelmed and eliminated to a distance far beyond the original area under scrutiny; vagile species, e.g. gammarid amphipods, *Carcinus* and *Pholis*, have been excluded by the loss of shelter. Since the *Sabellaria* colonies are far from mature the productivity and diversity of this shore is much reduced. A comparable overgrowth of *Crassostrea gigas*, in Hiroshima Bay, by the serpulid worm *Hydroides* caused production and other losses worth 3×10^9 yen (*ca.* £7 M) (Arakawa 1971).

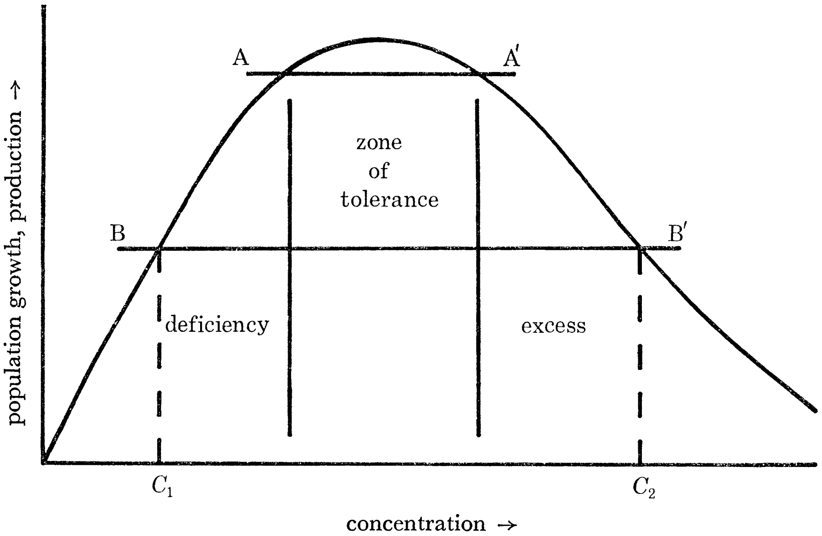

FIGURE 1. The oligodynamic dose relation between population parameters and concentration of metabolite or pollutant.

Two corollaries follow concerning the relation between concentration and population: thus if we take line BB′ (figure 1), populations on this intercept only correspond to concentrations C_1, C_2 if it is known that this is the maximum possible population production. If it is not, then the line B corresponds with all those concentrations which range between C_1 and C_2; it is therefore only possible to relate these two parameters with certainty if the field measurements are so comprehensive that the relation between the mean value of C and population is unequivocal. Further, one can be certain that the zone of tolerance has been established only when the population parameter has exceeded the intercepts A,A′, which is not easy when the response is truncated.

Even in conditions which are apparently conducive to high productivity and diversity one cannot necessarily assume that this can be confirmed by a brief study. Thus, even when the problems related to the definition and usage of diversity indices are overcome, the foregoing considerations imply an inherent imprecision in application and relation to environmental problems, especially where the absence of experimental data permits no judgement of the maximum that might be expected. Possibly one might determine theoretical maxima for a given situation and relate observed changes to it rather than determine some statistical function for any given time and attempt to make projections from that. But are existing statistical techniques sufficiently sensitive to handle these problems? D. M. Reid, of this laboratory, has tried to relate the normal statistical expressions of soil grade to the changes of soil quality known to have occurred in a 10 year period at Siddick, Cumbria, and found them to be hopelessly inadequate, particularly where coarse or silt/clay fractions not more than 10 % have had pronounced mechanical effects. Here the need for a subjective element in the study is consistent with the view expressed by Norton & Rolfe (1978).

To summarize, field studies of population and production performed in the presence of waste discharges theoretically offer the possibility of effective studies of sublethal effects, but the inherent tendency for living populations to produce at levels below the theoretical maximum, coupled with the relative insensitivity of some of the existing statistical treatments, suggest that until the methods used to study normal unpolluted populations are refined, it will remain difficult to equate results obtained in the laboratory with those in the field. Equally, it suggests that because studies in the laboratory are conducted at the level of maximal response, they will reflect more clearly the likely outcome of a given waste introduction than the time-consuming, very costly short-term field study. Certainly where time is of the essence, judgement cannot wait upon field populations reaching the maximum value attainable. Furthermore, it is mandatory that those engaged in environmental management cannot permit a new waste release and observe its effect before setting working limits: for a new material these must depend totally upon a laboratory study confirmed by a subsequent field study, a view which does not differ from that expressed by Cole (1970).

Nevertheless, Cole (1970) noted that field studies of growth rate, behaviour and maturation of the gonads may be carried out where gradients of pollution are well defined, and it is interesting to note that it was by use of such a field situation combined with laboratory measurement that the polychaete worm, *Nereis diversicolor*, has been shown to develop a tolerance to heavy metal pollution (Bryan 1974).

‘BIG-BAGS’ AND LARGE TANKS

Of course, one of the principal arguments against the use of laboratory measurement is the essentially simplistic nature of such experiments and the difficulty in applying such data to the environment. An attempt to overcome at one move the essentially unmanageable nature of field studies and the simplistic nature of the laboratory study has been the development of the ‘big-bag’ introduced by McAllister, Parsons, Stephens & Strickland (1961) and very large tank experiments. In the former, the nylon-reinforced PVC and tough polyethylene bags of 100 m^3 upwards (Davies, Gamble & Steele 1975) used in recent developments do begin to approximate more nearly to the real environment than systems used hitherto. However, the very high cost of initiation, operation and evaluation of the substantial body of data obtained places such an approach beyond the reach of the smaller laboratories and probably also of many sponsors. Despite the increase in scale, interpretation may not be easy, for no one who has worked with small marine tanks, or even large freshwater tanks, can fail to have been impressed with the potential of each for the development of an individual and characteristic biota. Just as there is a disproportionate amount of surface present in the laboratory tank, this too must be a problem with the ‘big-bag’, and the contributions of Davies & Gamble and Steele, in this symposium, are clearly of great interest. One further point arises in connection with the ‘big-bag’, large tanks and the laboratory approach, namely the essentially static nature of the experiment which contrasts so much with the marked variations in pollutant concentration arising spasmodically from the interactions of wind and tide. Obviously, this particular problem could be overcome to some degree by use of analogue systems operating upon large tanks, but the capital outlay, working and interpretation costs would be of a formidable order, and at this level one must consider carefully whether (*a*) the gain in understanding is commensurate with the cost and effort expended, and (*b*) results of the same order could be obtained by careful, well constructed experimentation at a much lower level of organization.

Chemical problems associated with sublethal effects

By the manner of their initiation, studies of 'big-bags' have necessarily involved chemists. However, the general absence of chemists, or rather toxicologist and chemist associations, represents one of the largest, and possibly most worrying, deficiencies of contemporary toxicology. Thus the importance of chemical form or composition in the manner of uptake has long been appreciated with regard to radioactive nuclides (see, for example, Jones 1960) and in studies of excretion (see, for example, Kečkeš, Fowler & Small 1972; Fowler, La Rosa, Heyraud & Renfro 1976). In the practice of toxicology, however, a particular compound or effluent is merely added to sea water and the test organism immersed. While the original compound/mixture is known we may have no evidence either that it remains in this form or of what, in fact, takes place (or indeed any but the crudest knowledge of effluent chemistry). Thus there may be a knowledge of reactions in fresh water, but none which is applicable to the sea, as for example in the hydrolysis of tripolyphosphate (Blanchar & Reigo 1976). Similarly, the behaviour of chlorine in fresh water has long been understood, but remains virtually unknown with respect to sea water despite its widespread use to control the fouling of power station culverts. Again the organic material present in natural waters and sediments is known to control the availability of trace metals to the calanoid copepod *Euchaeta japonica* (Whitfield & Lewis 1976) and organic compounds are involved in the precipitation of gypsum from near-surface sea water in the tropics (Barcelona, Tosteson & Atwood 1976).

Viewed in another way, it is now well established that many multicellular marine organisms derive nutritive substances, namely carbohydrates, lipids, amino acids and vitamins, directly from the sea water and may, indeed, satisfy a substantial proportion of their requirements by these means: thus, for example, sea anemones (*Anemonia sulcata*) obtain L-amino acids and D-glucose (Schlichter 1975) and (*Calliactis parasitica*) lipids (Saliot 1976); brachiopods (*Terebratalia transversa*) glucose (McCammon & Reynolds 1976); crustaceans (*Tigriopus* and *Calanus*) monosaccharides and polysaccharides (Khailov & Erokhin 1971); molluscs (*Ostrea*) lipids (Saliot 1976), (*Crassostrea*) glucose (Swift, Conger, Exler & Lakshmanan 1976) and (*Mya arenaria*) amino acids (Stewart & Bamford 1975, 1976); echinoderms (*Paracentrotus lividus*) glucose and amino acids (West & Jeal 1973; Pavillon 1976; De Burgh, West & Jeal 1977; De Burgh 1978) and (*Arbacia lixula*) amino acids (Pavillon 1976); and algae (*Porphyra tenera*) amino acids (Imada & Saito 1971). Equally it is perhaps not irrelevant to note that the concentration of magnesium ions in sea water may influence the fertilization of sea urchin eggs (Sano & Mohri 1976).

By considering these two sets of observations two possibilities become evident:

(*a*) that the organic material present in sea water may modify the impact of a particular waste, or

(*b*) that a particular waste could, by combining with the organic matter present in sea water, influence the nutrition of its inhabitants.

Both possibilities may be subject to spatial and temporal variation (see, for example, Ohwada 1972; Ohwada & Taga 1972; North 1975). There is little evidence for either in the literature, although the following is suggestive. The Water Pollution Research Laboratory devised a method whereby the foam head which develops upon a standard detergent solution shaken under prescribed conditions can be related to the concentration. This relation seemed to hold with sea water, but, in the field, samples may yield significant foam heads when detergent is absent or no foam head when significant concentrations are present; apparently sea water contains substances which behave in one of three ways, i.e. as surfactants, foam suppressors or foam enhancers (Perkins 1978).

Although the present outlook is not promising, toxicologists and chemists must clearly work much more closely in the future. It is just no use stating that we toxicologists have added so much of material X to a sea water test solution, it is imperative that the chemical form of X as it is released (in an effluent, say) and which it assumes in sea water be known. Furthermore, the nature of its interaction with the other materials present in the medium, and how much of it is really available to the treated organisms, must also be known. Failure to understand this aspect of the problem and the associated transformations by the living organisms themselves, with the obvious implications which relate to the mode of action of the toxin, presents a fundamental obstacle to the translation of laboratory studies into practical measures of effluent control, the understanding of the means of bioaccumulation and of the manner in which pollutants in general influence the physiological and biochemical processes of the organisms we seek to protect, including man.

Maintenance of experimental stocks

Evidently, any improvements made to further the degree of understanding of the chemical processes which occur in water must be matched by improvements in the technique of test organism handling and maintenance. In some respects little has changed since the early years of this decade when the means whereby the collection and use of field stocks might be improved (Perkins 1972*a*, *b*) or use of relatively limited laboratory stocks (Reish 1973) were discussed. In others, however, dramatic changes, occasioned to an overwhelming degree by the desire to farm marine organisms, have occurred. Thus May (1971) reviewed 68 attempts to rear the larvae of marine fish in the laboratory, and of these only 5 reported maintenance for not less than 100 days with a survival of not less than 50 %. Although some invertebrates were bred successfully in the laboratory before 1970 (e.g. crustaceans (Reeve 1969; Meixner 1969; Corkett 1967, 1970; Marshall 1973) and bivalve molluscs (Walne 1956, 1966; Loosanoff & Davies 1963)), this decade has been characterized by the marked and increasing ability to breed and rear marine animals under laboratory and farm conditions (see asterisked references which are not an exhaustive list). So that in contrast to the rather gloomy outcome of the review by May (1971), it is now apparent that, given the financial resources, large-scale recovery experiments and the prolonged exposure of a wide range of organisms of commercial importance to pollutants in large tank experiments is now perfectly possible, whatever the difficulties of inter-tank replication and extrapolation to field conditions. Such an approach is of particular value in studies of the rate at which pollutants are transferred to fish, e.g. in a phytoplankton–zooplankton–*Tellina tenuis*–O-group plaice (*Platessa*) food chain (Trevallion, Johnston, Finlayson & Nicoll 1973; Johnston 1973; Saward, Stirling & Topping 1972).

The wide range of organisms available in terms of size and systematic affinity ensures that responses can be studied effectively and according to the criteria stipulated above, but before examining these possibilities, it is apposite to consider first some critical problems of technique. The first of these relates to tank stocking density, for although breeding and raising of marine organisms can be conducted on a large scale, the requirements of toxicology on this scale may be different; moreover, work with small tanks will be predominant in the foreseeable future. Consequently, the effects of laboratory stress must be allowed for and the minimum requirements of each organism for healthy maintenance must be established. Thus Bayne (1976) reviews the impact of laboratory life upon the body condition of mussels, *Mytilus edulis*, while Bayne, Gabbott & Widdows (1975) and Helm, Holland & Stephenson (1973) demonstrated that parental stress adversely affected the survival of the larvae of *Mytilus edulis* and *Ostrea edulis*. The loss of intestinal parasites by *Gadus morhua*, *Zoarces viviparus*, *Myoxocephalus scorpius* and *Platichthys flesus* (Möller 1976) and the requirement of the individual sand goby, *Pomatoschistus minutus*, for 100 cm^2 of tank bottom, irrespective of tank size, for continued health and survival (Ross 1974) are indicative of the problems which await solution. Green (1977) showed that the alga *Acetabularia* grew well in artificial sea water, but natural sea water, especially that of neritic origin, supported faster growth and stimulated cap formation; it was concluded that the main advantage of the synthetic marine medium was its reproducibility and thus the removal of seasonal variations in quality. Such advantages may be real on a very small scale, but on a large scale cost is likely to be prohibitive; furthermore by excluding the important trace organics (see above) the laboratory becomes further removed from the real environment with all the interpretational problems that this implies.

METHODS OF TREATMENT

Because aquatic organisms obtain their essentials from and excrete to the same support medium, perhaps the greatest difficulty facing the toxicologist is that of relating effect and the nature of the dose or exposure received (Perkins 1976): thus the problem is normally discussed in terms of environmental concentration only (e.g. $l.c._{50}$). Here one is uncertain not only with respect to the relation between uptake, retention and excretion of a given material, but whether indeed this material is truly the effective agent of intoxication; indeed it is conceivable in both static and continuous flow experiments that the symptoms observed are due to some bacterial toxin the production of which has been stimulated by a substance itself innocuous to the test organism. Now, however, possibilities exist whereby some part, at least, of this problem may be overcome. Thus Chang, MacIntosh & Mason (1966) showed how microcapsules with a nylon–protein wall could be made to enclose an internal aqueous phase; Jones & Gabbott (1976) used such microcapsules (40 and 80 μm diameter) to study the dietary requirements of aquatic particulate feeders, namely *Artemia salina*, *Astacus astacus* and *Palaemon serratus*, and concluded that they could be used either for dietary purposes or to convey medicinal compounds for release in the digestive tract, rather than by the present inefficient and costly addition to the sea water. These conclusions indicate the means whereby direct dose–response relations may be investigated and for the first time the *effective dose* could be defined; furthermore the mode of uptake, the relative importance of the possible routes of uptake and the importance of chemical form, both in terms of intoxication and bioaccumulation, may be examined effectively. Moreover, the ability of bivalve molluscs to close their valves and exclude a distasteful external medium no longer seems an obstacle to investigation.

The lamellibranch molluscs offer yet other problems which arise from the enclosure of the body within two valves. Unfortunately, fenestration or removal of a valve to study the physiology or intermediary metabolism of the animal provokes healing responses and inevitably injures the animal more or less severely. Clearly, such events induce stress as defined by Bayne (1975): 'a measurable alteration of a physiological (or behavioural, biochemical or cytological) steady-state which is induced by an environmental change which renders the individual more vulnerable to further environmental change' and it is unreasonable to expect that animals so stressed can give results which have much meaning in terms of environmental management. However, this difficulty has been substantially overcome by the introduction of windows into the shells of oysters which remained healthy and attempted no repair of the shell during the two month period of the experiment (Veitch 1974). Evidently, this technique offers possibilities not only of metabolic studies and direct visual internal observations upon unstressed animals, and, since direct unstressed access to the mantle cavity may be so obtained, it becomes possible to perfuse the cavity with solutions for a known time without the problems raised by shell closure (see, for example, Davenport 1977). Moreover, a combination of this method with the use of microencapsulated food and food + toxin offers further possibilities in studies of mode of uptake, and comparison of internal action with that at the body surface.

Choice of experimental organism

The choice and use of a test organism is dependent upon the intended use of the information obtained. In terms of direct fishery interest, i.e. production and the consequences of changes in the balance of predatory effect, the larger, more abundant components must clearly form the principal objective of the study, but having a longer life span these organisms yield information relating to reproduction and the development of tolerance only very slowly. That both have an important bearing upon the application of laboratory data to real problems is illustrated by the instance of *Cristigera* spp. and *Capitella capitata* reviewed in the *South African Journal of Science* (Anon. 1976), where it was noted that a species may live in, i.e. be tolerant of, conditions that laboratory tests indicate to be inimical or may be absent from those situations where it might be expected simply because other conditions, e.g. unstable substratum, render the habitat unsuitable.

In the matter of reproduction, two questions must be answered, namely:

(*a*) Does a given toxin inhibit reproduction?

(*b*) If reproduction is inhibited to some degree, is tolerance to the pollutant developed during successive generations?

Clearly, if a suitable situation is available then the problem may be approached by a combination of field and laboratory studies like those performed upon *Nereis diversicolor* by Bryan (1974). However, in most field situations the input of waste, being derived from many sources, is normally complex and the results are usually ambiguous. Because so many animals can now be maintained wholly in the laboratory both problems are soluble. Thus the short-lived protozoans, e.g. *Cristigera*, polychaetes, e.g. *Ophriotrocha*, and copepods, e.g. *Tisbe* and *Pseudocalanus*, have been maintained in the laboratory for many generations. Their small size and short generation time means that the investigation of reproductive and tolerance effects requires relatively small facilities and confers the possibility that some meaningful answers can be obtained within a reasonable time and at acceptable cost, particularly by further work with the methods developed by Hoppenheit (1975*a*, *b*, 1976, 1977) and Hoppenheit & Sperling (1977) in relation to cadmium toxicity. Similarly, diatom community development could be studied readily by an appropriate adaptation of the methods developed in fresh water by Evans & Marcan (1976). Nevertheless, the problem of scale remains both in terms of size of experimental organism and the relatively large proportion of surface to solute in each experiment. Thus such experiments can probably never be taken on their own, but must serve as an early warning of effects which must be sought and confirmed elsewhere, a fate likely to be shared also by attempts to examine the effects of intoxication by using tissue culture techniques (see, for example, Li & Traxler 1972); nevertheless these methods have an obvious importance in relation to the study of carcinogenesis.

It would seem reasonable to advocate that animals likely to reach maturity in 1–2 years, e.g. the lugworm, *Arenicola cristata*, prawns and shrimps, and the shore fish, *Pomatoschistus minutus*, should be exposed to a toxicant either for the whole period from metamorphosis to maturity or for a substantial portion of this period and that the reproductive ability should be measured in terms of fecundity, successful fertilization of eggs and the ability of the larvae to develop normally. Most molluscs and fish of commercial importance are characterized by a greater longevity and a longer time to first maturity; nevertheless reproductive performance in the face of a protracted exposure is possible in many cases. Even though some information will be derived from reproductive studies, these longeval species must assume a much greater importance in relation to studies of production, especially efficiency of food conversion, condition and growth. Though it may not be easy to correct for tank-to-tank and individual ageing effects, useful information could be provided within a reasonable time by parallel experimentation upon fish of different ages in large tanks for periods of one year. This approach may have least value with respect to bioaccumulation since this may be a function of life span as much as of diet (Hamilton 1973; Boyden 1977; Bryan & Hummerstone 1978). It is therefore essential to consider the influence of chemical form in relation to the amount of toxin absorbed and the proportion accumulated as a body burden (i.e. tissue residues), and the effect of both upon the bodily functions in the laboratory. These may not be easy to relate, as workers in the radioactive field have found (e.g. Hill 1965, 1966; Fowler *et al.* 1976); moreover, the time scales involved suggest that the field will remain by far the most important source of information regarding the tissue residues accumulated by normal populations. Indeed, the time scale, i.e. in relation to life span, possibly renders these studies among the most difficult in aquatic toxicology. Nevertheless a 90 day study of fluoride uptake by the blue crab, *Callinectes sapidus* showed that 20 μg/g F decreased growth by 4.5 % per moult and that a 52 % reduction in the final average size could be expected; fluoride accumulation was significant from concentrations of not less than 8 μg/g, but the tissue content remained unchanged in a concentration of 2 μg/g and fell in 0.5 μg/g. The accumulated F was lost once the animal had been removed from the higher concentrations (Moore 1971). Such studies are informative, unequivocal and relate to commercially important species, as indeed do the physiological measurements made on field populations of *Mya arenaria* exposed to a severe oil spill (Gilfillan *et al.* 1976). Moreover, the physiological stress index proposed by Widdows (1978) may prove valuable in the future. Less certain, however, is the extent and manner to which biochemical functions, e.g. the induction of enzymes (Gruger, Wekell, Numoto & Craddock 1977), and physiological functions, namely respiration, osmoregulation, ionic regulation and the composition of body fluids, can be utilized in environmental management. Clearly they must be unequivocally linked to a stress index (Widdows 1978) or stress profile (Eisler 1970), itself directly related to actual or expected field conditions; otherwise they must remain as interesting information, but essentially unhelpful to the environmental manager.

INDUCTION OF DISEASE

Polluting sources, especially sewage, have often been blamed for inducing disease, a connection which is entirely logical. The few laboratory studies which have been carried out do demonstrate carcinogenesis (Ishio, Yano & Nakagama 1971; Korotkova & Tokin 1968; Fries & Tripp 1976), structural changes (Couch & Nimmo 1974; Maharty & Fineran 1976), induction of virus disease (Friend & Trainer 1970) and potentiation of epidermal ulcers (Pippy & Hare 1969; Rødsaether *et al.* 1975). In the field, however, the situation is confused: thus Schlotfeldt (1972) reported an association between detergent concentration and black necrosis in North Sea shrimps. Here, however, the maximum incidence of 8.9% was only slightly greater than the 8% minimum (maximum 50%) reported in the unpolluted Solway Firth (Abbott & Perkins 1977). Indeed, Abbott (1978) considered that an association between pollution and black necrosis might be particularly difficult to demonstrate in practice, and that far from indicating pollution, black necrosis may in fact indicate a high water quality where intense fishing and a greater consequent exposure to mechanical damage is likely. He further contends that we have no knowledge of the possible antibiotic effect of heavy metals or of the selective action of predators in removing sickly animals before a disease becomes overt. Sprague (1971) regarded evidence on this topic as equivocal and this seems to be a very fair view. Given the conclusions of Abbott (1978), it seems very clear that only effective laboratory studies can resolve this problem, but that once so resolved the environmental syndrome and its mechanism may be interpreted correctly. This seems to be a very good example of the principle enunciated by Cole (1970), namely, 'a different approach may be preferable by which the effects of a potentially toxic pollutant on the processes of growth, physiology, behaviour and breeding are established in controlled laboratory experiments and then looked for in the field'.

CONCLUSION

The demands of environmental management, particularly that of fisheries, are such that it is essential to produce unambiguous information regarding the effects of introduced wastes. This information must be produced in a reasonable time and at a reasonable cost. Short-term studies of mortality/survival, which employ imperfectly known chemical systems, are of limited use to individuals whose concern is the maintenance of a viable, productive fishery. Although field studies would appear to be direct in their application, the problems of information collection and interpretation at present are such that the norm is uncertain. It therefore follows that sublethal studies carried on at concentrations which are debilitating, but not directly lethal, must be developed in the laboratory with the use of entities whose chemistry in sea water is fully understood. Perhaps the clearest example of the need for such sublethal studies relates to pollution and the induction of disease, where the field situation is so complex that the issue can only be resolved satisfactorily by careful work in the laboratory.

REFERENCES (Perkins)

(References with an asterisk refer to methods of cultivation.)

Abbott, O. J. 1978 Ph.D. thesis, University of Strathclyde.
Abbott, O. J. & Perkins, E. J. 1977 *Cumbria Sea Fisheries Committee, Scientific Report* 77/4.
* Åkesson, B. 1970 *Helgoländer wiss. Meeresunters.* **20**, 293–303.
* Åkesson, B. 1975 In *Sublethal effects of toxic chemicals on aquatic animals* (ed. J. H. Koeman & J. J. T. W. A. Strik), pp. 121–135. Amsterdam: Elsevier.
Alderdice, D. F. 1967 *Can. Fish. Rep.* **9**, 33–39.
Anon. 1976 *S. Afr. J. Sci.* **72**, 35–36.
Arakawa, K. Y. 1971 *Venus* **30**, 75–82.
* Ayres, P. A. & Wood, P. C. 1977 *Lab. Leafl., M.A.F.F. Direct. Fish. Res., Lowestoft*, no. 37, 9 pages.
Barcelona, M. J., Tosteson, T. R. & Atwood, D. K. 1976 *Mar. Chem.* **4**, 89–92.
* Bardach, J. E., Ryther, J. H. & McLarney, W. O. 1972 *Aquaculture*. London and New York: Wiley Interscience.
Bayne, B. L. 1975 In *Proc. 9th eur. mar. biol. Symp.* (ed. H. Barnes), pp. 213–238. Aberdeen University Press.
* Bayne, B. L. (ed.) 1976 *Marine mussels: their ecology and physiology*. Cambridge University Press.
* Bayne, B. L., Gabbott, P. A. & Widdows, J. 1975 *J. mar. biol. Ass. U.K.* **55**, 675–689.
Blanchar, R. W. & Reigo, D. C. 1976 *Soil Sci. Soc. Am. J.* **40**, 225–229.
* Boletzky, S. v. 1974 *Vie Milieu* **24**, 309–340.
* Boletzky, S. v. 1975 *Bull. Soc. zool. Fr.* **100**, 361–367.
Boyden, C. R. 1977 *J. mar. biol. Ass. U.K.* **57**, 675–714.
Bryan, G. W. 1974 In *Pollution and physiology of marine organisms* (ed. F. J. Vernberg and W. M. Vernberg), pp. 123–135. New York, San Francisco and London: Academic Press.
Bryan, G. W. & Hummerstone, L. G. 1978 *J. mar. biol. Ass. U.K.* **58**, 401–420.
Buchanan, J. B. 1964 *J. mar. biol. Ass. U.K.* **44**, 565–576.
Bull, H. O. 1934 *Rep. Dove mar. Lab.*, ser. 3, no. 2, pp. 60–63.
Cameron, G. N. 1972 *Ecology* **53**, 58–72.
Chang, T. M. S., MacIntosh, F. C. & Mason, S. G. 1966 *Can. J. Physiol. Pharmac.* **44**, 115.
Chesher, R. H. 1969 *Bull. mar. Sci.* **19**, 72–110.
Cole, H. A. 1970 In *F.A.O. Technical Conference on marine pollution and its effects on living resources and fishing*. F.I.R.: MP/70/R20. Rome, 9–18 December 1970.
* Corkett, C. J. 1967 *Nature, Lond.* **216**, 58–59.
Corkett, C. J. 1970 *Helgoländer wiss. Meeresunters.* **20**, 318–324.
* Corkett, C. J. & McLaren, I. A. 1969 *J. exp. mar. Biol. Ecol.* **3**, 90–105.
* Corkett, C. J. & McLaren, I. A. 1970 *J. mar. biol. Ass. U.K.* **50**, 161–168.
Couch, J. A. & Nimmo, D. W. R. 1974 *Bull. Soc. Pharmac. and Environ. Pathol.* **2**(2), 17–20.
Dales, R. P. 1950 *J. mar. biol. Ass. U.K.* **29**, 321–360.
Dales, R. P. 1951 *Biol. Bull. mar. biol. Lab. Woods Hole* **101**, 131–137.
* D'Asaro, C. N. & Chen, H. C. K. 1976 *Florida Sea Grant Program.*, rep. no. 16, 114 pages.
Davenport, J. 1977 *J. mar. biol. Ass. U.K.* **57**, 63–74.
Davies, J. M., Gamble, J. S. & Steele, J. H. 1975 In *Estuar. Res.* **1**, 251–264.
De Burgh, M. E. 1978 *J. mar. biol. Ass. U.K.* **58**, 425–440.
De Burgh, M. E., West, A. B. & Jeal, F. 1977 *J. mar. biol. Ass. U.K.* **57**, 1031–1045.
* Dugan, C. C., Hagood, R. W. & Frakes, T. A. 1975 *Florida Mar. Res. Pub.* no. 12, 28 pages. Florida Department of Natural Resources.
Eisler, R. 1970 In *F.A.O. Technical Conference on marine pollution and its effects on living resources and fishing*. F.I.R.: MP/70/E-12. Rome, 9–18 December 1970.
* Escritor, G. L. 1972 In *Coastal aquaculture in the Indo-Pacific region* (ed. T. V. R. Pillay), pp. 355–365. London: Fishing News (Books) Ltd.
Evans, G. H. & Marcan, E. 1976 *Environ. Pollut.* **10**, 115–126.
* Forster, J. R. M. & Beard, T. W. 1973 *Fishery Invest., Lond.* (2) **27**(7), 16 pages.
* Forster, J. R. M. & Wickins, J. F. 1972 *Lab. Leafl., M.A.F.F. Direct. Fish. Res., Lowestoft*, no. 27, 32 pages.
Fowler, S. W., La Rosa, J., Heyraud, M. & Renfro, W. C. 1976 *Mar. Biol.* **30**, 297–304.
Fralick, R. A., Turgeon, K. W. & Mathieson, A. C. 1974 *Nautilus* **88**, 112–114.
Fretter, V. & Graham, A. 1962 *British prosobranch molluscs*. London: The Ray Society.
Friend, M. & Trainer, D. O. 1970 *Science, N.Y.* **170**, 1314–1316.
Fries, C. & Tripp, M. R. 1976 *Mar. Fish. Rev.* **38**, 10–11.
* George, J. D. 1975 *Proc. 10th Eur. mar. biol. Symp.*, pp. 143–159.
Gilfillan, E. S., Mayo, D., Hanson, S., Donovan, D. & Jiang, L. C. 1976 *Mar. Biol.* **37**, 115–123.
* Grave, H. 1975 *Ber. dt. wiss. Kommn Meeresforsch.* **24**, 209–211.

Green, B. R. 1977 *Phycologia* **16**, 87–94.

Gruger, E. H., Wekell, M. M., Numoto, P. T. & Craddock, D. R. 1977 *Bull. environ. Contam. Toxicol.* **17**, 512–520.

Hamilton, D. Y. 1973 B.Sc. Honours thesis, Department of Biology, University of Stratchclyde.

Hancock, D. A. & Urquhart, A. E. 1965 *Fish. Invest. Lond.* (2) **24**(2), 40 pages.

* Harris, R. P. 1977 *Chesapeake Sci.* **18**, 245–252.

* Harris, R. P. & Paffenhöfer, G.-A. 1976*a* *J. mar. biol. Ass. U.K.* **56**, 675–690.

* Harris, R. P. & Paffenhöfer, G.-A. 1976*b* *J. mar. biol. Ass. U.K.* **56**, 875–888.

* Helm, M. M., Holland, D. L. & Stephenson, R. R. 1973 *J. mar. biol. Ass. U.K.* **53**, 673–684.

Hill, C. R. 1965 *Nature, Lond.* **208,** 423–428.

Hill, C. R. 1966 In *Radioecological concentration processes,* (ed. B. Aberg & F. P. Hungate), pp. 297–302. Oxford and New York: Pergamon Press.

* Hoppenheit, M. 1975*a* *Helgoländer wiss. Meeresunters.* **27**, 235–253.

* Hoppenheit, M. 1975*b* *Helgoländer wiss. Meeresunters.* **27**, 377–395.

* Hoppenheit, M. 1976 *Helgoländer wiss. Meeresunters.* **28**, 109–137.

* Hoppenheit, M. 1977 *Helgoländer wiss. Meeresunters.* **29**, 503–523.

* Hoppenheit, M. & Sperling, K.-R. 1977 *Helgoländer wiss. Meeresunters.* **29**, 328–336.

Imada, O. & Saito, Y. 1971 *Bull. Jap. Soc. Sci. Fish.* **37**, 1125–1133.

Ishio, S., Yano, T. & Nakagama, R. 1971 *Bull. Jap. Soc. Sci. Fish.* **37**, 105–110.

* Iversen, E. S. 1968 *Farming the edge of the sea*. London: Fishing News (Books) Ltd.

Jones, D. A. & Gabbott, P. A. 1976 In *Microencapsulation* (ed. J. R. Nixon), pp. 77–91. New York: Marcel Dekker.

Jones, R. F. 1960 *Limnol. Oceanogr.* **5**, 312–325.

Johnston, R. 1973 In *North Sea science* (ed. E. D. Goldberg), pp. 293–307. London: M.I.T. Press.

Kečkeš, S., Fowler, S. W. & Small, L. F. 1972 *Mar. Biol.* **13**, 94–99.

Khailov, K. M. & Erokhin, V. E. 1971 *Okeanologiya* **11**, 117–126.

Korotkova, G. P. & Tokin, B. P. 1968 *Acta biol. hung.* **19**, 465–475.

Lagler, K. F., Bardach, J. E. & Miller, R. R. 1962 *Ichthyology*. London and New York: John Wiley.

Lang, C. & Mann, K. H. 1976 *Mar. Biol.* **36**, 321–326.

* Leighton, D. L. 1972 *Fish. Bull.* **70**, 373–381.

Lewis, J. R. 1971 In *Marine pollution and sea life* (ed. M. Ruivo), pp. 401–404. London: Fishing News (Books) Ltd.

Li, M. F. & Traxler, G. S. 1972 *J. Fish. Res. Bd Can.* **29**, 501–505.

* Liao, I. C. & Huang, T. L. 1972 In *Coastal aquaculture in the Indo-Pacific region* (ed. T. V. R. Pillay), pp. 328–354. London: Fishing News (Books) Ltd.

* Liao, I. C., Lu, Y. J., Huang, T. L. & Lin, N. C. 1972 In *Coastal aquaculture in the Indo-Pacific region* (ed. T. V. R. Pillay), pp. 213–243. London: Fishing News (Books) Ltd.

Loosanoff, V. L. & Davis, H. C. 1963 *Adv. mar. Biol.* **1**, 1–136.

McAllister, C. D., Parsons, T. R., Stephens, K. & Strickland, J. D. H. 1961 *Limnol. Oceanogr.* **6**, 237–258.

McCammon, H. M. & Reynolds, W. A. 1976 *Mar. Biol.* **34**, 41–51.

McIntyre, A. D. 1970 *J. mar. biol. Ass. U.K.* **50**, 561–575.

Maharty, H. K. & Fineran, B. A. 1976 *N.Z.J. Bot.* **14**, 13–18.

Marshall, S. M. 1973 *Adv. mar. Biol.* **11**, 57–120.

May, R. C. 1971 *N.O.A.A. Tech. Rept. N.M.F.S., S.S.R.F.*-632. (24 pages.) Washington, D.C.: U.S. Department of Commerce.

Meixner, R. 1969 *Ber. dt. wiss. Kommn Meeresforsch.* **20**, 93–111.

Möller, H. 1976 *J. mar. biol. Ass. U.K.* **56**, 781–785.

Moore, D. J. 1971 *Chesapeake Sci.* **12**, 1–13.

Moore R. J. 1978 *Nature, Lond.* **271**, 56–57.

Newell, G. E. 1948 *J. mar. biol. Ass. U.K.* **27**, 554–580.

North, B. B. 1975 *Limnol. Oceanogr.* **20**, 20–27.

Norton, M. G. & Rolfe, M. S. 1978 *Fish. Res. Tech. Rep.* no. 45, 9 pages. Lowestoft: M.A.F.F. Direct. Fish. Res.

Ohwada, K. 1972 *Mar. Biol.* **14**, 10–17.

Ohwada, K. & Taga, N. 1972 *Mar. Chem.* **1**, 61–73.

* O'Sullivan, B. W. & Wilson, J. H. 1976 *Fish Farming int.* **3**(3), 12–15.

* Paffenhöfer, G.-A. 1970 *Helgoländer wiss. Meeresunters.* **20**, 346–359.

* Paffenhöfer, G.-A. 1971 *Mar. Biol.* **11**, 286–298.

* Paffenhöfer, G.-A. 1976 *Limnol. Oceanogr.* **21**, 39–50.

* Paffenhöfer, G.-A. & Harris, R. P. 1976 *J. mar. biol. Ass. U.K.* **56**, 327–344.

Pavillon, J.-F. 1976 *Mar. Biol.* **34**, 67–75.

* Perez, C. A. & Ramon-Cabello, G. 1973 *Publ. téc. Div. gen. Pesca. marit.* (*Spain*) no. 10, 297–314.

* Perez, R. 1972 *Science et pêche* (*Bull. Inst. Pêches marit.* no. 216). (12 pages.)

Perkins, E. J. 1972*a* *Proc. Soc. analyt. Chem.* **9**, 105–114.

Perkins, E. J. 1972*b* *Mar. Pollut. Bull.* **3**, 13–15.

Perkins, E. J. 1974 *Biology of estuaries and coastal waters.* London and New York: Academic Press.

Perkins, E. J. 1976 In *Marine pollution* (ed. R. Johnston), pp. 505–585. London, New York and San Francisco: Academic Press.

Perkins, E. J. 1977 In *Environment and man*, vol. 5 (ed. J. Lenihan & W. W. Fletcher), pp. 70–101. Glasgow and London: Blackie.

Perkins, E. J. 1978 *The behaviour of surfactants in sea water.* Cumbria Sea Fisheries Committee, Scientific Report 78/7.

Pielou, E. C. 1966 *J. theor. Biol.* **13**, 131–144.

Pippy, J. H. C. & Hare, G. M. 1969 *Trans. Am. Fish. Soc.* **98**, 685–690.

* Purdom, G. E. 1977 *Lab. Leafl., M.A.F.F. Direct. Fish. Res., Lowestoft*, no. 35, 28 pages.

Reeve, M. R. 1969 *Fish. Invest., Lond.* (2) **26**(1).

Reish, D. J. 1973 *Mar. Pollut. Bull.* **4**, 46–47.

Rødsaether, M. C., Olafsen, J., Raa, J., Myrhe, K. & Steen, J. B. 1975 *J. Fish. Biol.* **10**, 17–21.

Ross, O. H. 1974 M.Sc. thesis, University of Strathclyde.

* Rothbard, S. 1976 *Bamidgeh* **28**(4), 80–105.

Sano, K. & Mohri, H. 1976 *Science, N.Y.* **192**, 1339–1340.

Saliot, A. 1976 Ph.D. thesis, University Pierre et Marie Curie, Paris. (167 pages.)

Saward, D., Stirling, E. A. & Topping, G. 1972 *I.C.E.S. Fisheries Improvement Committee C.M.*1972/E: 24.

Schlichter, D. 1975 *Proc. 9th eur. mar. biol. Symp.* (ed. H. Barnes), pp. 395–405. Aberdeen University Press.

Schlodfeldt, H.-J. 1972 *Ber. dt. Kommn Meeresforsch.* **22**, 397–399.

Schmitt, W. L. 1973 *Crustaceans.* Newton Abbott: David & Charles.

Segerstråle, S. G. 1960 *Commentat. biol.* **23**, 1–72.

Sprague, J. B. 1971 *Wat. Res.* **5**, 245–266.

* Stebbing, A. R. D. 1976 *J. mar. biol. Ass. U.K.* **56**, 977–994.

Stewart, M. & Bamford, D. R. 1975 *Comp. Biochem. Physiol.* 1975, **52**A, 67–74.

Stewart, M. & Bamford, D. R. 1976 *J. exp. mar. Biol. Ecol.* **24**, 205–212.

* von Stosch, H. A. 1969 *Proc. Int. Seaweed Symp.* **6**, 389–399.

Swift, M. L., Conger, K., Exler, J. & Lakshmanan, S. 1976 *Life Sci.* **17**, 1679–1684.

Tattersall, W. M. & Tattersall, O. S. 1951 *The British Mysidacea.* London: The Ray Society.

Tiews, K. 1967 *F.A.O. Fisheries Reports* no. 57, vol. 4, (ed. M. N. Mistakidis), pp. 1167–1224. Rome: F.A.O.

Trevallion, A., Johnston, R., Finlayson, D. M. & Nicoll, N. Y. 1973 *J. exp. mar. Biol. Ecol.* **41**, 189–206.

* Varikul, V., Phumiphol, S. & Hongpromyart, K. 1972 In *Coastal aquaculture in the Indo-Pacific region* (ed. T. V. R. Pillay), pp. 366–374. London: Fishing News (Books) Ltd.

Veitch, F. P. 1974 *Mar. Chem.* **2**, 65–68.

Walne, P. R. 1956 *Fishery Invest., Lond.* (2) **20**(9).

Walne, P. R. 1966 *Fishery Invest., Lond.* (2) **25**(4).

* Walne, P. R. 1976 *Aquaculture* **8**, 371–381.

* Walne, P. R. 1977 *Lab. Leafl., M.A.F.F. Direct. Fish. Res., Lowestoft*, no. 40, 15 pages.

Warner, C. E. 1964 Ph.D. Thesis, University of California, Berkeley.

Watling, L. 1975 *J. exp. mar. Biol. Ecol.* **19**, 275–313.

West, A. B. & Jeal, F. 1973 *Proc. Challenger Soc.* **4**, 121–123.

Wheeler, A. 1969 *The fishes of the British Isles and north-west Europe.* London, Melbourne and Toronto: Macmillan.

Whitfield, P. H. & Lewis, A. G. 1976 *Estuar. coast. mar. Sci.* **4**, 255–266.

* Whyte, J. N. C. & Englar, J. R. 1976 *Fisheries and Marine Service Tech. Rep.* no. 623, 34 pages.

* Wickins, J. F. 1972*a* *Fishery Invest., Lond.* (2) **27**(4), 23 pages.

* Wickins, J. F. 1972*b* *Fishery Invest., Lond.* (2) **27**(5), 23 pages.

Widdows, J. 1978 *J. mar. biol. Ass. U.K.* **58**, 125–142.

Zaika, V. E. 1973 *Specific production of aquatic invertebrates* (transl. A. Mercado). New York and Toronto: Wiley.

Discussion

H. A. COLE (*Forde House, Moor Lane, Hardington Mandeville, Yeovil BA*22 9*NW, U.K.*). The question of the right choice of organisms for laboratory bioassays has been examined and re-examined many times without coming to any very useful conclusions. If, as has often been suggested, we select organisms of short generation time which can readily be reared in the laboratory, we are likely, as Professor Gray mentioned, to be using hardy resistant species, the 'weeds' of the sea. However, it is generally agreed that pollution effects are most serious in estuaries and immediate coastal waters, rather than in the open sea. Estuaries are sometimes described as containing 'sensitive' ecosystems but to my mind estuaries are inhabited by tough and adaptable organisms capable of surviving substantial and often rapid fluctuations in environmental conditions. If they were not able to do so they would not maintain their hold on the estuary, and therefore we are back again, if we use common estuarine organisms in bioassays, to something very like Professor Gray's weeds. The result is, as Dr Perkins notes, that most people simply rely upon what is available.

J. S. Gray (*University of Oslo*). Many of the organisms used in bioassays are in fact classical '*r*' selected opportunists which by definition are robust and often highly tolerant. Furthermore in the normal unpolluted environment such species are rare and unimportant. I feel that there is a need to concentrate more on the rarer '*K*' selected species since they may be more sensitive and are ecologically important.

E. J. Perkins. I fully agree that the inhabitants of estuaries are tough, adaptable organisms which are capable of withstanding environmental conditions unlikely to be tolerable to sensitive ecosystems. Given that pollution effects are likely to be most serious in these situations, there seems to be little point in worrying about whether we have or have not secured the most sensitive species for bioassay. Equally, there seems to be little point in suggesting that the species which live abundantly in estuaries can be regarded as 'weeds'; moreover as every informed gardener knows the presence of a given species of 'weed' can often affect positively the production of a desired crop. Since we do not have the resources to examine more than a fairly narrow range of species, it seems reasonable to me to concentrate upon those species which are of commercial importance either directly in a fishery or indirectly as the prey or predators of these species. The first two are likely to be very abundant, the last rather less so, but none could be defined as ecologically unimportant, and work with all three does at least have the merit of answering directly questions which relate to management of the fisheries. By assuming this approach, I cannot believe, indeed the literature indicates the lack of reason for the belief, that an insufficient range of sensitivity to toxins is available in this group. Moreover, it embraces a considerable variation of life span and so many can now be maintained experimentally throughout their life cycle.

It seems to me that this direct approach has merit in that it avoids both the polemics relating to whether we have or have not secured the most sensitive organism possible and the equally wasteful, futile tests based on fresh water animals stressed by acclimation to low salinity, for example rainbow trout in 25% sea water, and which in both chemical and biological terms cannot be considered to yield information applicable to the major productive areas of estuaries.

A. V. Holden (*D.A.F.S., Pitlochry, U.K.*). Are all sublethal effects to be considered as undesirable? It would seem possible to have some effects which would, for example, increase productivity, or increase the proportion of a commercially desirable species. We should be careful not to assume that *every* detectable effect is necessarily unacceptable, or that *every* increase in residue concentration must imply some consequent effect on the species concerned.

E. J. Perkins. This has emphasized the point which I have tried to make repeatedly since 1972, by noting the importance of the oligodynamic dose–response curve. Clearly, not all sublethal effects are undesirable, and if we consider trace metals and vitamins as examples then such sublethal doses may be entirely necessary to the well being of both plant and animal, however poisonous they may be when taken in excess. What we do not know, is difficult to approach, and yet must be defined for each type of waste eventually, is the point at which sublethal effects cease to be beneficial or harmless and begin to act adversely either directly or (perhaps) by potentiation of disease. It is my belief that we would do well to stop thinking in terms of pollutants, but rather of wastes for disposal, since well managed waste disposal does not necessarily lead to pollution; in many cases, for example animal excrement, waste must be returned to the environment for the maintenance of a healthy biosphere.

P. A. Driver (*Lancashire and Western Sea Fisheries Joint Committee*, *University of Lancaster*, *U.K.*). Surely, a problem in using the reduction of life span as a measure of the sublethal effect of a pollutant is the fact that within any species life span is very variable. I would also have thought that the natural variation in life span would be far greater than the variation due to the presence of a low concentration of pollutant.

E. J. Perkins. Of course, marine organisms show a great variation in life span, but so too do human beings. The latter difficulty seems not to worry actuaries too much, and, since the methodology exists, it would seem to be worthwhile considering its use in relation to the sublethal effects of pollutants. If nothing else, studies related to life span would have the effect of improving experimental technique. If, in addition, production losses could be related to changes in effective life span, that is of productive rather than senile individuals, the effects of pollution could be discussed on a precise quantified basis rather than by description.

D. J. Crisp, F.R.S. (*Marine Science Laboratories*, *Menai Bridge*, *Anglesey*, *U.K.*). Dr Perkins's suggestion that in order to safeguard a species one should investigate the influence of ambient concentrations of toxicants on longevity is an interesting and valuable one. However, one *caveat* needs entering, namely that the ultimate safeguard should take account of 'fitness' rather than longevity alone. Sometimes greater longevity operates against fitness – for instance the removal of reproductively effete individuals in a resource limiting situation is actually beneficial. Conversely, any toxicant which delays the onset of reproduction will reduce fitness though not necessarily affecting longevity.

E. J. Perkins. I would not quarrel with the statement that reproduction and fitness are of overriding importance, indeed I made this point at the beginning of my paper. Equally, no one who is conscious of the effects of under-fishing would suggest that there is any point in concerning themselves with senile individuals. However, stunted individuals who can and do reproduce are not likely to be of great interest to managers who wish to maximize the return from a fishery. The further point which was being made here was that the appelation long-term is much abused and this abuse could be overcome by relating the duration of the experiment to a function of the organism's life rather than the patience or skill of the experimenter.