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An Experimental Approach to the Determinants of Biological Water Quality [and Discussion]

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An experimental approach to the determinants of biological water quality

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A case is presented for the use of experimental bioassay techniques to detect and measure variations in water quality in the marine environment by exposing suitable organisms in the laboratory to water samples collected in the field. A technique is described which was developed for this purpose with the use of a clonal hydroid; preliminary results from Swansea Bay show that it is sensitive to the variations in water quality that occur there. Chemical techniques are being developed for use in conjunction with such bioassays to identify the kinds of contaminants responsible for a detected effect, and some preliminary experiments suggest that divalent metals and the volatile constituents of hydrocarbons can be removed selectively from sea water.

Introduction

Marine ecosystems can tolerate domestic and industrial effluents by reason of the rapid dilution that occurs, but also because of those biological processes which degrade, detoxify or sequester contaminants. While the capacity of biological systems to adapt to varying loads of such materials is considerable, it must be a guiding principle of environmental management that it is not exceeded, since small increases above the maximum tolerable load may lead to disproportionate effects on biological processes. To avoid overloading an ecosystem, precise and sensitive methods are needed to assess the biological quality of waters receiving effluents and to identify the variables that influence it. It is difficult in devising such methods to achieve results that have relevance to the environment because of the practical difficulties on the one hand of reproducing environmental conditions in the laboratory, and on the other of conducting controlled experiments in the environment. The latter approach has been taken in America (Reeve *et al.* 1976) and in Scotland (Gamble, Davies & Steele 1977; Davies & Gamble, this volume) in experiments where representative populations of the main trophic levels are isolated in enclosed bodies of water. An alternative approach to the problem is to use the responses of organisms as an index of biological water quality. This can be achieved by making physiological measurements on indigenous organisms recently removed from polluted waters (Bayne *et al.*, this volume). It is also possible to relate water quality to the responses of organisms kept in water samples brought back to the laboratory, and it is this approach that will be considered here.

Recently, laboratory experiments have become more environmentally realistic by dealing with more than one variable at a time; for example, temperature and salinity (Cain 1973; Lough & Gonor 1973; Lowthian 1974), or salinity, temperature and a toxicant (Vernberg & O'Hara 1972; Vernberg *et al.* 1973) or two toxicants (Gray & Ventilla 1973; Thurberg, Dawson & Collier 1973; Roales & Perlmutter 1974; Braek, Jensen & Mohus 1976). Although this is an important development, it remains difficult to construct from such work an understanding of the natural environment because of the many variables and their interactions. There is an increasing number of factors known to enhance or antagonize the effects of contaminants in sea water (Jernelöv 1972; Bryan 1971, 1976) in ways that are difficult to reproduce in the laboratory. A further important constraint is that the size of multivariate experiments makes this approach less practical.

By using suitable responses of organisms to the characteristics of water samples collected from the field, all these variables – known and unknown – are integrated to give an estimate of their combined effect on water quality. Such an integrated index may not necessarily advance understanding of interactions between toxicants, or of the factors that influence their effects, but it provides a means of measuring water quality in its totality, which may have more environmental relevance than many of the methods in use at present.

Such indices of biological water quality make it possible to describe fluctuations of water quality in space and time. The fluctuations may then be correlated with the occurrence of a known contaminant, although a causal relation cannot be established. However, if biologically important contaminants could be selectively removed from polluted water samples in such a way that the water is otherwise unaffected, disappearance of the biological response would establish with certainty that these effects were due to the contaminant.

In this paper the feasibility of using bioassay techniques to give an experimental index of water quality is examined in the light of published work and a new technique using the hydroid *Campanularia flexuosa*. In conjunction with this bioassay, chemical techniques have been adapted to remove contaminants from sea water selectively and some preliminary results are given.

Estimation of the biological quality of sea water

'Biological water quality' is usually understood to mean the capacity of sea water to sustain naturally occurring biological processes. Any more stringent definition must be expressed in terms of quantifiable biological processes, because the concept only has meaning in terms of what can be measured; it seems reasonable to assume that physico-chemical variables and their interactions are too numerous for non-biological methods to give a relevant measure of biological water quality. The responses of biological processes to waters of different quality can be studied at a number of levels: the community, the species, the physiological and cellular levels. It is relevant to discuss the relative merits of measurements at these different levels of organization. In a situation where inputs of toxic contaminants are increasing, changes at the cellular level may be expected to precede those at the physiological level, and the loss of homeostatic stability will precede the death of individuals. The closer the function measured is to the biochemical processes affected by a toxicant, the greater the apparent sensitivity of the index. Furthermore, at the cellular level of organization, the responses acquire generality because different organisms share similar ultrastructure and biochemical pathways. Consequently, the effects of stress induced by widely different factors can elicit the same response in organisms of different phyla (Allison 1969; Gabrielescu 1970; Bitensky *et al.* 1973; Moore & Stebbing 1976; Bayne *et al.*, this volume; Moore *et al.* 1978). However, at this level, environmental relevance is not always apparent, since the response has least to do with the survival of the whole organism; but where water quality is declining such techniques are important in that they provide earliest warning of undesirable effects.

At the community level, the field ecologist determining the abundance and distribution of individual species or assemblages operates at the most environmentally relevant level. However, natural cyclical fluctuations (Longhurst *et al.* 1972) or the effects of natural phenomena, such as severe weather conditions (Crisp *et al.* 1964), tend to obscure the possible effects of contaminants. Furthermore, since the ecologist's methods are most often observational rather than experimental, owing to practical constraints of working in the environment, he can do little more than correlate variables in the hunt for causal relations.

It becomes clear that the ideal method must incorporate both the environmental relevance of ecology and the rigour of experiment. The bioassay approach in which the responses of suitable organisms to water samples from the field are measured combines these features to a degree not hitherto achieved in other methods.

Review of some relevant work

It is intended here to consider briefly some work in which the responses of organisms have been used to give an experimental measurement of water quality. Organisms were first used in this way to study the natural differences in the quality of water characterized by *Sagitta* spp. (Russell 1939). Wilson (1951) used the development success of several invertebrate larvae as an index of water quality and attempted to identify the factors responsible for the differences in the development of these larvae in *S. elegans* and *S. setosa* water (Wilson & Armstrong 1961).

A similar method uses the occurrence of abnormalities in oyster larvae as an index of the quality of the water in which they are kept for 48 h after fertilization (Woelke 1967, 1972). It has been used to quantify the effects of effluents from paper mills at various concentrations and in water samples from estuaries and coastal waters (Woelke 1968). Kobayashi (1971) adapted the methods of Okubo & Okubo (1962) who used the success in fertilization and early development of sea urchin embryos and showed water from polluted sites to be of poor quality by their standards. Boiling these water samples sometimes improved water quality significantly, implying that the toxicants responsible might be volatile. This bioassay has since been used extensively in Japan by Kobayashi, Nogami & Doi (1972); they found marked differences in water quality between sampling sites and correlated poor water quality with 'metal refinery works' and the high metal levels that occurred in sediments nearby.

Morgan *et al.* (1973) reported experiments in which a number of physiological indexes of two fish species were significantly affected by 28 days' exposure in the laboratory to the polluted water of Baltimore Harbor. The appearance and activity of the fish were apparently unaffected, but the ratios of blood cell types and three enzyme systems were significantly affected.

Burrows (1971), using the macroalga *Laminaria saccharina* cultured in water samples from the sludge dumping grounds of Liverpool Bay, found that growth was typically depressed to 15% of that of controls grown in uncontaminated water. Johnston (1962) used a number of microalgal species to demonstrate geographical differences in the capacity of natural waters to support phytoplankton growth. He later showed (Johnston 1964) by the use of chelating agents that the supply and availability of trace metals are most important to the growth and reproduction of algae. Similar bioassays have been used to assess variations in the capacity of sea water to sustain algal growth (Aleem 1970; Smayda 1974), sometimes in the context of effluents (Skulberg 1970; Thomas, Seibert & Dodson 1974).

If the responses of any organisms are expected to show the effects of toxic contaminants, it is important to ensure that no other factors are limiting. In larval bioassays, non-feeding stages are used to avoid variations in response due to differences in nutrition, but the growth responses of autotrophs are typically related to the levels of available nutrients. To detect the effects of contaminants with algae it is therefore important to supplement water samples with nutrients so that they are not limiting (see, for example, Burrows 1971).

A number of biological systems have now been used to estimate the capacity of sea water to complex and detoxify metals. Lewis *et al.* (1972) found that various agents reduced the toxicity of copper to a calanoid copepod and their capacity to do so could be expressed in terms of equivalent concentrations of ethylenediaminetetra-acetic acid (EDTA). Whitfield & Lewis (1976) then used this technique to estimate the capacity of natural waters to complex metals. Other methods that are similar in principle have been developed by using the growth of diatom cultures (Davey *et al.* 1974) and [^{14}C]glucose uptake of a microbial system (Gillespie & Vaccaro 1978).

It is now clear that there are a number of experimental techniques which have the sensitivity to respond to variations in water quality, demonstrating that suitable organisms can be used in this way to give a direct experimental index of biological quality of polluted water.

A bioassay technique with the use of hydroids

A bioassay technique with the hydroid *Campanularia flexuosa* (figure 1) has been developed and described (Stebbing 1976; Moore & Stebbing 1976) for use in studying variations in the quality of sea water due to pollution. This hydroid has been found to react to levels of contaminants commonly found in coastal waters with a degree of precision that is due to the exclusion of genetic variability by the use of a single clone. All experiments are conducted in water collected offshore near the Eddystone Rock (*ca.* 13 km offshore) and in bioassays of contaminated water samples this water is used for the controls.

The reaction of hydroid colonies may be routinely assayed by using three responses, each varying characteristically in sensitivity and precision (figure 2). The first of these responses is the growth of colonies for which the numbers of colony members (hydranths, gonozooids and buds) are used as an index of biomass. Growth is expressed as the specific growth rate (K) which gives the rate of increase in biomass as a function of biomass. Mean rates for the seven replicate colonies over 11 days are expressed as percentages of the mean rate of the controls, so that only those changes in rate due to the experimental treatment are apparent (figure 2). The reason for considering the effects of exogenous variables in terms of the rate of growth, rather than the colony size, is because it is likely that the effects of these variables will be most marked and rapid on the growth process, rather than on its cumulative product. In experiments using a range of concentrations of a growth inhibitor, such as copper, the growth rate is similar to the controls up to 10 μg/l, but above this the growth rate declines sharply and linearly (figure 2).

The second response used is gonozooid production (figure 1), which has been found to increase under stress (figure 2) and at the expense of the growth of new hydranths (Stebbing, unpublished data). Numbers of gonozooids are expressed as a proportion of the number of colony members, and in experiments with copper, gonozooid frequency reaches a maximum at 0.1 μg/l before falling linearly with further increases in concentration.

The third response which provides a useful index is a feature of the habit of growth of *Campanularia* colonies (figure 3*a*). Typically, stolons grow radially from the explant, but when exposed to inhibitory agents, stolon growth becomes curved in an anticlockwise direction (figure 3*b*). The radius of the curvature decreases as the concentration of inhibitor increases, so that in extreme cases the stolons form circles (figure 3*c*). This characteristic is used here as an index of response by scoring the frequency of stolons which curve through 90° or more after 11 days (figure 2). In experiments with copper the frequency of curves increases markedly from 5 μg/l, reaching a maximum at 20 μg/l, before falling to zero at concentrations that are lethal.

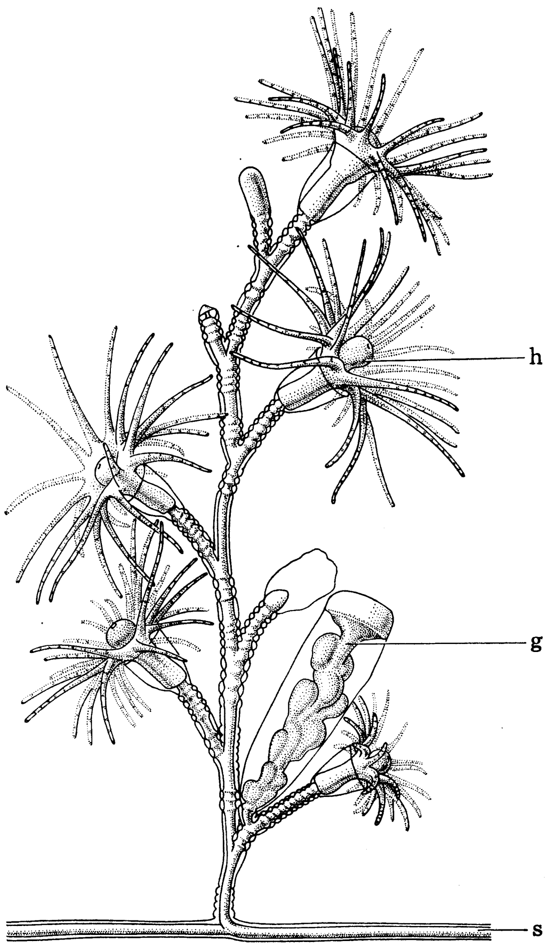

FIGURE 1. A single upright of a *Campanularia flexuosa* colony showing hydranths (h), a gonozooid (g) and the stolon (s). The upright is approximately 5 mm high.

Experiments with a range of toxicants (copper, cadmium, mercury and an organo-tin compound) and reduced salinity suggest that increased gonozooid production may be a generalized response to stress (Stebbing, unpublished). Similarly, the growth response of stolons appears general, although it has not been measured in response to as many toxicants.

THE BIOASSAY OF SEA WATER SAMPLES

Swansea Bay was chosen as a test locality for the field trials of this technique because there are significant inputs of ammonia, hydrocarbons and metals, particularly from the industrial complex and ore terminal at Port Talbot. Water samples (20 l) were untreated in early experiments, but membrane filtration (0.45 μm) immediately after collection was adopted as a means of stabilizing the levels of metals in solution (A. W. Morris, personal communication).

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However, it has since been found that this treatment removes pesticides (Kurtz 1977) and perhaps other contaminants from sea water. In some recent experiments unfiltered water samples have again been used. It might be better to freeze water samples on collection, but this is not yet practicable.

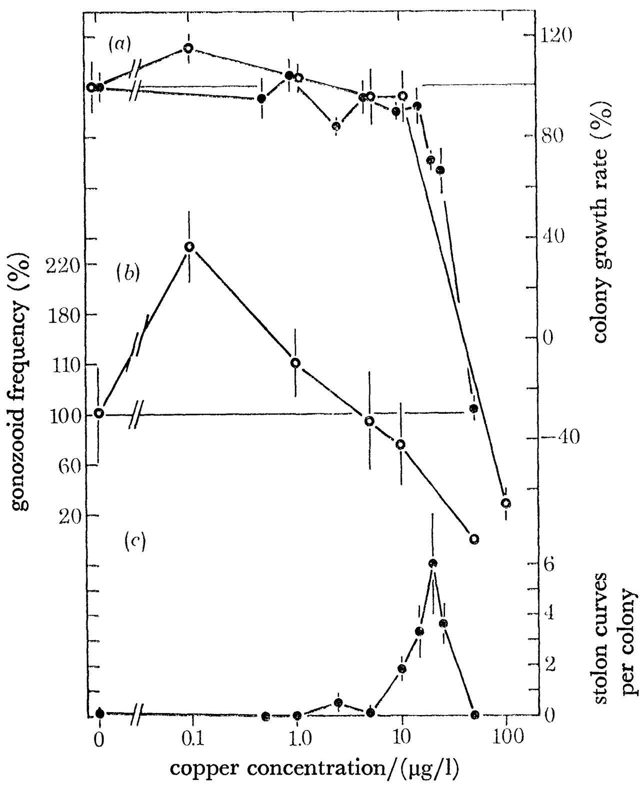

FIGURE 2. The responses of *Campanularia flexuosa* to the effects of copper: (*a*) specific growth rate for 11 days expressed as a percentage of the control; (*b*) the frequency of gonozooids as a proportion of the total colony members expressed as a percentage of the controls; (*c*) the mean number of stolon curves of 90° or more per colony. The results of two experiments are denoted by different symbols; each point is the mean of seven and the standard error of the mean is given.

Marked differences between the responses of hydroid colonies grown in Swansea Bay water samples and the control colonies are common (figure 4), but some points should first be made about the interpretation of responses to water samples from the field. Owing to the shape of the response curve, any increase in gonozooid frequency can represent the effect of a level of stress equivalent to two distinct levels of copper (figure 2*b*), although the difference between them becomes less marked as gonozooid frequency increases. Furthermore, at levels greater than those which produce this increase, there may be no apparent effect at all, because the concentration–response curve must intercept the 100% control level. Interpretation of the stolon curving response is less difficult because the peak of increased frequency occurs over a narrow range of concentrations (figure 2*c*), and there can be no inhibition of this response relative to the controls because the stolons of unstressed colonies do not curve. In the January 1976 experiment the absence of an increase in gonozooid frequency (figure 4*a*) in water samples that gave a marked increase in stolon curving (figure 4*c*) is probably due to a level of stress above that which causes an increase in gonozooid production and below that which is inhibitory (figure 4*b*).

Numerous factors determine the distribution of an effluent upon its release: the direction and velocities of tidal currents in the receiving water, the rate at which the effluent is released, changes in the rate of entry with time, and the behaviour of the contaminant on release. For the same reasons the distribution of a detected biological effect is equally difficult to interpret, especially if the toxicants responsible are unknown. However, certain features may be mentioned

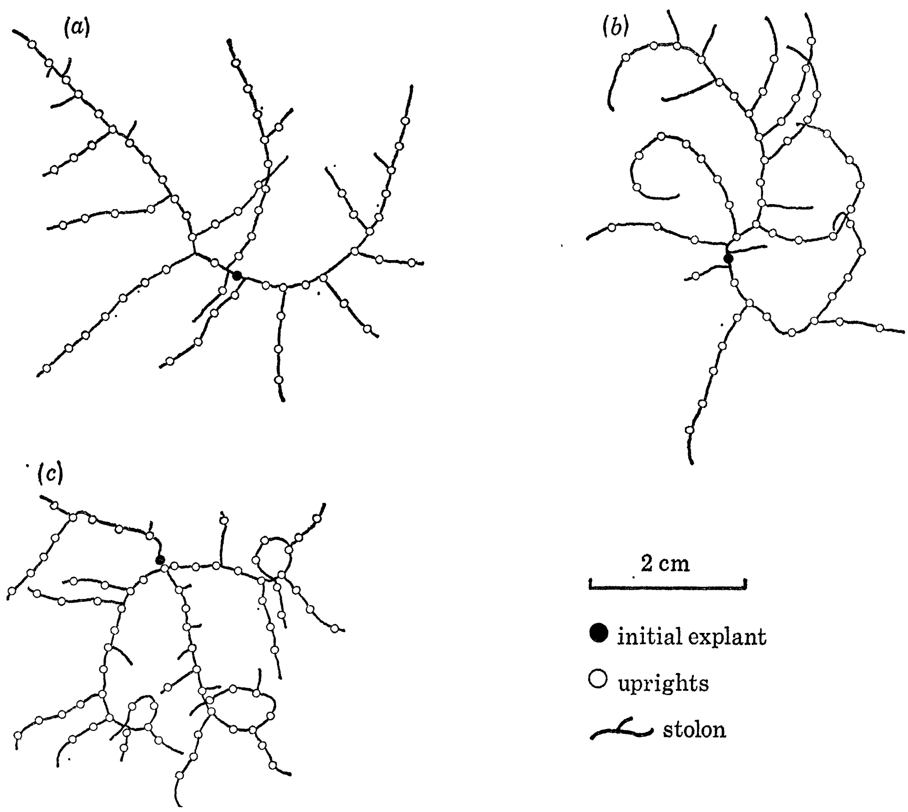

FIGURE 3. *Campanularia flexuosa* colonies at 14 days old drawn with the use of a camera lucida showing an unstressed control colony (*a*), a stressed (*b*) and a severely stressed colony (*c*). The position of the initial explant is shown as a solid circle and new uprights as open circles.

because they recur in nearly every experiment. Water of consistently poor biological quality is found off Port Talbot and this could be because of toxicants from several outfalls which discharge effluents offshore, the River Avon which flows through Port Talbot, or the harbour where bulk ore carriers are unloaded. One experiment suggested that the marked stolon growth response which occurred in a water sample collected off Port Talbot (figure 3*c*) was partly due to metals (table 5). Occasionally there were responses in water samples collected off Mumbles Head (figure 3*a*), where metalliferous effluents are released. Failure to observe a response may reflect the intermittent release of effluents from this outfall.

Although the distribution of contaminants must be continually changing, the distribution of responses can be correlated with the levels of metals, such as copper and lead, in the same samples. However, such correlations cannot establish causal relations, since any other toxicant sharing the same distribution could have had the same effect. Emphasis has therefore been given to the problem of developing methods for identifying significant toxicants with certainty.

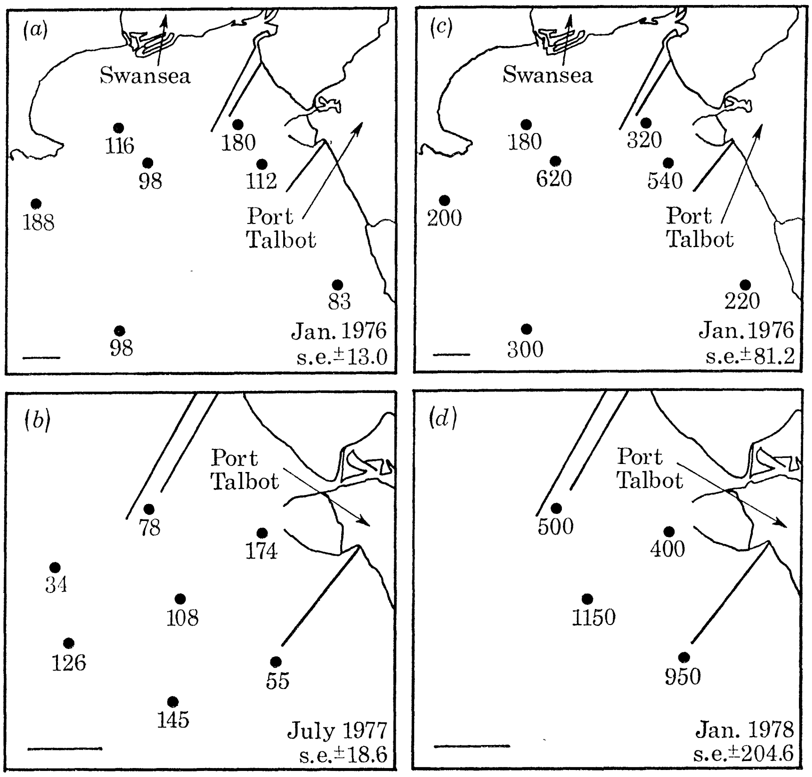

FIGURE 4. Charts showing the positions of stations at which water samples were taken in Swansea Bay (*a*, *c*) and off Port Talbot (*b*, *d*) for experiments with *Campanularia flexuosa*. The frequency of gonozooids as a proportion of colony members (*a*, *b*) and the frequency of stolon curves of 90° or more (*c*, *d*) are given for colonies grown for 11 days in the water samples. All responses are expressed as percentages of the responses of the control colonies. Figures given are means of seven colonies and pooled standard errors are given for each experiment. The positions of outfalls are marked and scale bars represent 1 nautical mile (*ca.* 1.85 km).

CHEMICAL MANIPULATION OF SEA WATER

If a response elicited by a contaminated water sample disappears upon removal of the contaminant, the agent responsible is identified unequivocally. Because sea water is chemically complex, the removal of specific contaminants does not yet appear feasible. However, some existing chemical techniques compatible with the bioassay can be used to remove whole groups of constituents. To be experimentally acceptable, the water sample should not change in any other biologically significant way.

The first technique tested was ultraviolet photooxidation of sea water (Armstrong *et al.* 1966) to find how dissolved organic compounds affected the complexation and detoxification of metals. Copper, for example, which is highly toxic in its ionic state (Steeman Nielsen & Wium Andersen 1970), has been found much less toxic when complexed or bound by organic compounds (Lewis *et al.* 1972); further, the proportion of copper in sea water that is bound can range from 5 to 70% (Williams 1969; Siegal 1971; Morris 1974). Photooxidation of organic matter in sea water destroys its capacity to complex metals and it has been demonstrated that the dissolved organic matter occurring in offshore waters can reduce copper toxicity. Thus the inhibitory effect of 20 μg/l of copper on colonial growth rate was 30% greater in offshore water in which the organic matter (1 mg dissolved organic carbon per litre; analysis by Dr F. Mantoura) was photooxidized by irradiating the water with ultraviolet light (table 1), although irradiation alone did not affect hydroid growth rate.

Swansea Bay receives metalliferous effluents from the industrial complex at Port Talbot, and it is known that high levels of dissolved metals (cadmium, copper, lead, nickel and zinc) are found in water samples from the bay (Abdullah & Royle 1974). It was necessary to find a way to remove metals from sea water to see whether they are wholly or partly responsible for the effects of Swansea Bay water on the hydroids (figure 4). Davey *et al.* (1970) have already used Chelex-100 ion exchange resin to prepare large volumes of metal-free sea water for algal culture; this indicated not only that the resin removed trace metals, but also that it did not release any biologically deleterious substances into the treated water. While it is possible that resin treatment of water removes essential micronutrients, it is assumed that any requirement by the hydroids for trace metals would be met by daily feeding to satiation with *Artemia salina* nauplii (Stebbing 1976), as the satisfactory growth of colonies in Chelex-treated water suggests (table 2).

TABLE 1. THE EFFECT OF PHOTOOXIDATION OF ORGANIC MATTER IN SEA WATER ON THE MEAN SPECIFIC GROWTH RATE (K) OF *CAMPANULARIA FLEXUOSA* COLONIES EXPOSED TO COPPER (20 μg/l) FOR 11 DAYS

(Rates are given both as values of K and as percentages of the control.)

	u.v. irradiation	
copper	0	+
0	0.292 (100 %)	0.295 (101 %)
+	0.246 (84 %)	0.154 (53 %)

Pooled s.e. = ±0.0146 (5.0 %).

TABLE 2. THE EFFECT OF REMOVING INHIBITORY CONCENTRATIONS OF COPPER (20 μg/l) FROM SEA WATER WITH THE USE OF CHELEX-100 ION EXCHANGE RESIN ON THE MEAN SPECIFIC GROWTH RATE (K) OF *CAMPANULARIA FLEXUOSA* COLONIES OVER 11 DAYS

(Rates are given both as values of K and as percentages of the control.)

	resin treatment	
copper	0	+
0	0.306 (100 %)	0.340 (111 %)
+	0.142 (46 %)	0.312 (102 %)

Pooled s.e. = ±0.0212 (6.9 %).

The use of ion exchange resin to remove metals from sea water was tested by passing water with copper through the resin. It was found that the copper could be removed and the growth rate of colonies restored to that of the controls (table 2). The amount of copper accumulated in hydroid tissues during the 11 day experiment reflects the concentrations in the water, in that tissue levels were much higher in water to which copper had been added and that this level was reduced to less than that of the controls by resin treatment. The growth rate and copper content of the hydroids in resin-treated water also confirms that they did not become copper deficient as a result of the resin treatment.

Another technique has been used to investigate the toxicity of oil at environmentally realistic levels. A first step in the identification of oil as a biologically significant contaminant in water samples from the field could be to determine whether an effect is due to a volatile constituent of the water, because it is well known that the most toxic fractions of oil are volatile.

Table 3. The effect of bubbling sea water with oil (26 μg/l) on gonozooid production by *Campanularia flexuosa* colonies over 11 days

(Numbers are the frequency of gonozooids as a proportion of the total colony members, and are also expressed as percentages of the control.)

	bubble treatment	
oil	0	+
0	0.894 (100 %)	1.017 (114 %)
+	3.593 (402 %)	2.903 (324 %)

Pooled s.e. = ± 0.912 (102 %).

Table 4. The effect of bubbling sea water with oil (26 μg/l) on the frequency of stolon curves (90° or more) in colonies of *Campanularia flexuosa* after 11 days

(The frequencies are also given as percentages of the control.)

	bubble treatment	
oil	0	+
0	2.0 (100 %)	1.0 (50 %)
+	4.143 (207 %)	2.429 (121 %)

Pooled s.e. = ± 0.462 (23 %).

A preliminary experiment (in collaboration with P. Donkin) to test this idea was conducted with water 'extracts' of North Sea oil diluted in a recirculated laboratory sea water system. Water for bioassay was then removed from the system, at which time the concentration was about 20–30 μg hydrocarbon/l. Oiled water was passed slowly through columns against a stream of fine bubbles to drive off the volatile fractions of the oil. The efficiency of the bubbled columns in extracting the oil was greater than 80 %. Water with and without oil was also passed through similar columns which were not bubbled.

Results from this preliminary experiment are not as clear-cut as the others, but the data nevertheless serve to illustrate another approach. The overall improvement in water quality after bubbling was probably due to the removal of ammonia which had built up in the sea water system. However, changes in the frequency of gonozooids (table 3) and stolon curves (table 4) suggest greater improvements in the quality of oiled water after bubbling, and it appears that some of the volatile constituents of oil that are toxic have been removed by bubbling.

Manipulation of polluted water samples

The waters of Swansea Bay contain many contaminants and any may be present at different times and places at biologically detectable levels. It is therefore most unlikely that the removal of any single group of contaminants would restore a response to normal. Off Port Talbot, Abdullah & Royle (1974) and Morris (personal communication) have found levels of copper, for example, to be within the range detectable by the hydroid bioassay; so the ion exchange technique has been used on water collected off Port Talbot to see if metals caused the effects on the hydroids. Preliminary results (table 5) indicated that the stolon curving response detected off Port Talbot (figure 4*c*) may in part be attributable to metals, because the response decreased after passing the water through Chelex-100 ion exchange resin.

Table 5. The effect of passing water collected off Port Talbot through Chelex-100 ion exchange resin on the frequency of stolon curves (90° or more) in colonies of *Campanularia flexuosa* after 11 days

(The frequencies are also given as percentages of the control.)

	resin treatment 0	resin treatment +
Eddystone water	0.714 (100%)	0.429 (60%)
Port Talbot water	3.857 (540%)	1.571 (220%)

Pooled s.e. = ±0.494 (69%).

To develop this approach it is necessary to improve the selectivity of the manipulative techniques, so that one may determine not just the broad chemical group to which a biologically active contaminant belongs, but may identify it more specifically. The problems in doing so are not only to develop techniques to remove specific contaminants without otherwise changing biological water quality, but also to ensure that other contaminants of unrelated groups are not unintentionally removed at the same time.

Concluding remarks

At present the most satisfactory method of assessing the quality of sea water is by the chemical analysis of contaminants considered biologically deleterious. However, this approach alone is inadequate, largely because it is not yet possible to translate data on the chemical constituents of sea water into biological terms. Woelke (1968) demonstrated that the Pearl–Benson Index, a chemical measure of water quality commonly applied to pulp and paper wastes, did not reflect the relative toxicity of different wastes. Kobayashi *et al.* (1972) found that the chemical oxygen demand (c.o.d.) of polluted waters did not agree with a biological estimate of water quality obtained from their larval bioassay. Furthermore, chemical data may not reflect the biological availability of contaminants. For example, analyses of the total levels of a metal present may give little indication of its toxicity, because a significant and variable proportion of that total may be bound to organic matter and biologically unavailable. Consequently, the

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toxicity of copper is most accurately reflected by its ionic activity, rather than the total amount of the metal present (Sunda & Guillard 1976). It is also known that the combined effects of toxicants may be more or less than their additive effects predicted from experiments with single toxicants (Sprague 1970). These and other factors may alter the toxicity of marine contaminants in ways that cannot yet be predicted by using chemical techniques alone.

In the context of this meeting, the criteria for quality of sea water must be biological, since pollutants that have no such effects are unimportant, and it follows that the most relevant approach is to attempt to assess water quality in biological terms. However, biological methods are generally less precise, sensitive and rigorous than chemical and physical measurements, with the result that water quality is usually assessed in chemical and physical terms. Recent developments reviewed here show that it is becoming possible to assess water quality directly, by using as indices some generalized biological responses to stress of suitable organisms. Such methods offer not only a means of detecting and assessing water quality in time and space, but also the possibility of identifying the kinds of contaminants responsible.

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Discussion

H. A. Cole (*Forde House, Moor Lane, Hardington Mandeville, Yeovil BA22 9NW, U.K.*). It is interesting that the initial response of *Campanularia flexuosa* to pollutants is to divert energy from growth to the production of additional gonozooids. This is a response which has survival value and one wonders how common such responses may be and whether they could have practical value, e.g. in culture work.

The word 'stress' is used very frequently by pollution workers but is rarely defined. Presumably natural variations of, say, temperature and salinity above and below the optimum must be described as stresses. Is stimulation of growth by a pollutant correctly described as a stress?

A. R. D. Stebbing. It might be considered a natural progression that those studying the biological consequences of marine pollution should initially consider higher concentrations and their toxic effects, before proceeding to lower levels and the adaptive responses they elicit. Implicit in this approach is that perhaps we should attempt to find the highest levels of contaminants that organisms can adapt to and tolerate indefinitely, rather than the lowest levels that have no apparent deleterious effect.

'Stress' is a term used here to denote the force imposed by any exogenous variable which tends to disturb physiological processes. Since homeostatic mechanisms work to neutralize the effects of disturbing influences, the effects of stress may not necessarily be apparent. However, one effect of stress will be to render the organism more susceptible to further change, because the capacity to counteract disturbance is limited and must have a metabolic cost. The term was first used in this way by Selye (1950) to describe those stimuli which elicit the 'general adaptation syndrome'. The term is important since it allows one to refer collectively to variables of different kinds, which may have a common effect on a physiological process, or may elicit the same responses. For example, the increase in gonozooid production in hydroids is a response elicited by toxic metals, reduced salinity or water of poor quality. It is of adaptive significance because it must increase the likelihood of survival of the genes by their distribution in the plankton. It is helpful to refer to the common stimulus for this response as stress, and the variables that elicit the response as stressors.

Every inhibitory variable which we have studied so far also stimulates growth of hydroid colonies within a narrow range of subinhibitory concentrations. The phenomenon is known as the Arndt–Schulz Effect or hormesis and our work on the dynamics of growth control suggests that it is due to transient overcorrections by the control mechanisms to inhibitory challenges well within its capacity to counteract (Stebbing, unpublished).

Reference

Selye, H. 1950 *Stress*. Montreal: Acta Inc.

P. A. Driver (*Lancashire and Western Sea Fisheries Joint Committee, University of Lancaster, Bailrigg, Lancaster LA*1 4*XY, U.K.*). I was very interested in the correlation between stolon-turning frequency and concentration of pollutant. In Morecambe Bay we occasionally have rolling spherical colonies of the hydroids *Sarsia tubulosa* and *Tubularia larynx* washed ashore. It has been suggested (Clare, Jones & O'Sullivan 1971) that this phenomenon might be caused by a chemical pollutant in the seawater. In Dr Stebbing's experiments, have high concentrations of pollutants ever led to such increased stolon-turning frequencies that spherical colonies are formed?

Reference

Clare, J., Jones, D. & O'Sullivan, A. J. 1971 On the occurrence of detached spherical colonies of the hydroids *Sarsia tubulosa* and *Tubularia larynx* in Morecambe Bay. *J. mar. biol. Ass. U.K.* **51**, 495–503.

A. R. D. Stebbing. In our experiments the increase in stolon-turning frequency does not involve any detachment of the stolon from the glass plates on which they are grown, but that does not imply that the response you speak of is not due to contaminants. In fact, one might envisage the formation of mobile spherical colonies as an adaptive response by which a sessile organism is able to leave an unfavourable area, carried by tidal currents.

R. C. Newell (*Zoology Department, University of Cape Town, South Africa*). We have heard a good deal of the use of 'indicator organisms' to assess the effects of pollutants on marine organisms. Equally, one strategy for the survival of marine organisms in a variable environment is the maintenance of a high genetic diversity. Dr Stebbing has used a clone in his work. Should other authors use 'clones' of their indicator organisms, and to what extent does one clone represent the potential tolerance of the species?

A. R. D. Stebbing. One of the problems with sensitive bioassay techniques is that their responses to toxicants are rather variable. In the *Campanularia* bioassay, and others using *Hydra* and *Lemna*, we have attempted to minimize the variability by using a single clone to exclude genetic variability. To what extent these clones are representative of the populations from which they were selected I do not know, but it would not be difficult to ensure that they were. Precision must determine reproducibility and limits of sensitivity of any bioassay, and to these ends the use of a clone is helpful.

M. Waldichuk (*Pacific Environment Institute, West Vancouver, B.C., Canada*). Can the use of cultured cloned organisms in the laboratory have any relation to the field situation where no such genetic uniformity exists?

A. R. D. Stebbing. Yes, I believe that it is feasible to use a clone to predict what might occur to populations in the field, by simply ensuring that the clone is representative of that population. Alternatively, it might be helpful to use a clone that is more sensitive than the norm of a population one wishes to conserve.

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H. Williams (*The Open University in Wales, Pearl Assurance House, Greyfriars Road, Cardiff, CF*1 3*PH, U.K.*). I should like to congratulate Dr Stebbing on his approach and use of a coelenterate as a sensitive indicator organism. In fact I am persuaded by his comments and those made by Dr Burton and Dr Bengtsson to mention once again the possible value of some fish parasites as indicator organisms for the following and other reasons. Some parasites attack hosts that are under stress, but in mentioning this word I am conscious that its use has already been questioned during this discussion. Other parasites, for instance viruses, are thought to attack healthy fish only. It is possible that some parasites will desert unhealthy fish or those in a polluted environment. One of the classical ways of collecting some ectoparasites from fish is immersion in very dilute formaldehyde. It has been emphasized during the discussion that the time in which an animal becomes reproductive is far more important than longevity. Many parasites reach the reproductive phase in a very short time and having done this the entire animal consists mainly of reproductive organs producing large numbers of eggs. Nothing could be more sensitive to the environment than the delicate ciliated free-living larvae which emerge from these eggs. The eggs of many species can now be maintained in the laboratory and hatching induced experimentally. For these reasons I can think of about 12 species of helminths which might be good indicator organisms.

A tapeworm of the pike has already been used for this purpose by Alicia Guttowa in Poland who carried out experiments on the effects of the pesticide Vapam on its larva. A lethal dose was as high as 0.8 parts/10^6. The respiration rate was reduced at sublethal doses and the rate of infection to copepods reduced by one-third. Larvae subjected to Vapam survived in the gut of a copepod but did not enter the body cavity as they normally do.

In the light of this information on the possible use of parasites as indicators of pollution does Dr Stebbing know of similar work of this nature?

A. R. D. Stebbing. No, I am not aware of any similar work.

K. W. Wilson (*North-West Water Authority* (*Rivers Division*) *Buttermarket St, Warrington, Cheshire, U.K.*). Dr Stebbing's figures showed that there were very marked spatial variations in the water quality in Swansea Bay as measured by the various hydroid indices. Has Dr Stebbing been able to show corresponding variations in the existing biota, planktonic and benthic, in the area?

A. R. D. Stebbing. No, we have not looked at the plankton and benthos in the area, although it would be a good idea to do so. I believe that work on the benthos in this area, with the effects of industrial pollution in mind, is being done at the Department of Zoology, University College of Swansea.

A. D. McIntyre (*Marine Laboratory, P.O. Box* 101, *Victoria Road, Aberdeen AB*9 8*DB, U.K.*). Dr Stebbing found the circular growth described to be a reaction of hydroids when exposed experimentally to polluted water from certain sites. Has he tried to validate these conclusions in terms of field effects by looking at specimens of hydroids collected from these sites to see if the same type of growth occurs in nature?

A. R. D. Stebbing. No, I have not tried to find this growth form in the field and it certainly would be a good idea to look. It is interesting when comparing the responses of different clones that not all of them responded in this way, although for those that did it appeared to be a generalized response.

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G. J. Smith (*Imperial Cancer Research Fund, Lincoln's Inn Fields, London W.C.2, U.K.*). Research into carcinogenic chemicals has indicated that many carcinogens are not harmful without prior activation by microsomal enzyme systems. In view of the presumed difference in metabolic competence between, for example, fish and hydroids how will Dr Stebbing's bioassay prevent false negative results which are due to failure of hydroid cells to activate harmful chemicals?

A. R. D. Stebbing. Dr Smith's question assumes that the intention is to extrapolate results from hydroids to fish, but our objective initially was merely to find a biological response that was sensitive to differences in water samples in polluted areas. I appreciate that the problems associated with predicting the sensitivity of one species from the responses of another are considerable; at the present stage one must depend on the fact that species such as this are more sensitive than those that one would wish to protect.

J. D. Burton (*Department of Oceanography, The University, Southampton SO9 5NH, U.K.*). Photooxidation of sea water produces changes in pH which could lead to changes in the inorganic speciation of a metal, for example in the proportion of free ions. Did the design of the experiments described take account of such possible effects?

A. R. D. Stebbing. Yes, the experimental design did take this into account by incorporating the necessary controls. The growth rate of the hydroids cultured in control water did not differ significantly from those grown in photooxidized water (table 1) showing that even if photooxidation does cause the changes suggested, they did not affect the organism's growth rate.

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