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MARINE ECOTOXICOLOGICAL TESTS WITH COELENTERATES

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ABSTRACT

The ecotoxicological tests available at present that employ marine coelenterates are reviewed. Those considered primarily involve hydroids and corals, although we also describe one test with a scyphozoan and some work with anemones. While hydroids are good experimental organisms and corals are best suited to in situ field studies, the primary problem is to adopt an approach that combines the rigour of the laboratory experiment with the environmental relevance of field studies, using an organism appropriate to one's criteria for water quality.

KEYWORDS

Marine ecotoxicology, Hazard assessment, Bioassays, Methods, Coelenterates, Review.

INTRODUCTION

Implicit in the title of this meeting "Ecotoxicological Testing for the Marine Environment" is a difficult problem. By "testing" we usually mean experimental procedures conducted in the laboratory which are controlled in a way that makes it possible to identify the consequences of varying a single factor at a time - typically levels of a toxicant. On the other hand, we are ultimately concerned with safeguarding communities of organisms in the environment. However, it is not possible to conduct experiments in the field with individual contaminants at controlled concentrations, making it impossible to determine concentration-response curves and threshold levels for different responses. Large enclosures (mesocosms) or bags of the CEPEX type (Davies and Gamble, 1979 ; Steele, 1979) provide a means of exposing organisms in the field to known levels of a toxicant where uncontrolled factors besides the toxicant may impinge upon organisms and influence their responses. We need therefore to maximise the environmental relevance of laboratory experiments so that extrapolations from the laboratory to the field can be made with confidence.

In recent years one approach has been to attempt to estimate the biological quality of environmental water samples in laboratory experiments using the responses of sensitive organisms to those samples (Woelke, 1968 ; Kobayashi et al., 1972 ; Stebbing, 1979 ; Stebbing et al., 1983 ; see review of some available and potential techniques by Stebbing et al., 1980). However, while this may provide some biotic index of water quality, there remains the difficulty of identifying the contaminants responsible for depressing it. The crux of the problem is to combine the rigour of the laboratory experiment with the ecological relevance of field observations in an approach that not only allows us to measure small variations in water quality, but also to identify their causes.

A review of the use of coelenterates for ecotoxicological testing needs first to consider the basic differences between the classes, because their major features also determine some advantages and disadvantages for experimental purposes. The coelenterates are now considered to include two phyla - the Cnidaria and the Ctenophora. The ctenophores are delicate pelagic organisms that are difficult to maintain in the laboratory and do not appear to have been used in toxicological work. They will not be considered further.

Within the Cnidaria there are three classes : the Hydrozoa, the Scyphozoa and the Anthozoa. The Hydrozoa (circa 3 000 species) are cnidarians whose existence as colonies of polyps predominates over their existence as medusae. They are most easily cultured as sessile colonies of hydroids and at least 40 species have been used in studies of growth and development since methods were developed for their cultivation using Artemia salina nauplii as food (see for example Crowell, 1957 ; Kinne, 1965 ; Braverman, 1974 ; Brock, 1975). the medusae of hydroids are more difficult to rear and, as planktonic organisms, are less convenient for experimental work.

Similarly in the Scyphozoa (circa 250 species) where the medusoid stage is dominant, it is the subordinate sessile polyp or scyphistoma stage which is more suitable. The medusae are delicate, often damaging themselves against the walls of tanks, and their large size makes them difficult to handle. The scyphistomae on the other hand have similar advantages to the hydroids and are sometimes used for experimental work ; in recent years particularly by Spangenberg (1968).

The Anthozoa, to which the anemones and corals belong, is the largest class of cnidarians with about 6 000 species. The anemones (order Actinaria) are polyps and unlike the hydrozoans the polyp are large and solitary, but like all other anthozoans they lack a medusoid stage. They are among the easiest marine organisms to maintain in laboratory culture, although little toxicological work has been done with them. The corals are another major anthozoan group and belong to the order Scleractinia. They are usually colonial and have a calcareous exoskeleton. A useful non-taxonomic distinction which needs to be made is between the hermatypic and ahermatypic corals. The hermatypic forms are the reef builders, they contain zooxanthellae and live in shallow tropical waters of 18.5°C or more. The ahermatypic corals which do not build reefs, do not possess zooxanthellae, live predominantly in temperate waters, but are found in all seas at all depths.

Corals are difficult to keep for long periods in the laboratory and so they are not good experimental subjects, but their size and sessile habit are advantages for in situ studies. In view of their great importance as reef builders, and the communities of organisms that depend directly or indirectly upon them, there is a requirement for suitable techniques to predict the susceptibility of corals to marine contaminants, so that appropriate measures can be taken to safeguard coral reef ecosystems.

The most obvious reason for choosing to use a coelenterate for toxicological tests is because the populations of organisms of concern belong to this group. Where this rationale does not apply, coelenterates may be chosen for practical reasons, perhaps because of the ease with which some coelenterates can be cultured in large numbers for indefinite periods in the laboratory, or because they reproduce asexually and can therefore be cloned. It may be important that the organisms are small or that the response is rapid, features that make a test more cost-effective.

There are considerable difficulties in attempting to extrapolate the results of experiments with organisms from one taxon to predict the sensitivity of those from another, difficulties for which satisfactory solutions have not been found within the mammals, let alone between invertebrate phyla. However, one may adopt the principle of the miner's canary where it is the greater sensitivity of the indicator organism that makes it possible to anticipate and take preventative actions to safeguard the organisms of concern, rather than their taxonomic affinity.

Our objective is to review the use of coelenterates in laboratory and field experiments on the effects of actually or potentially toxic marine contaminants. The title indicates that we are concerned with experimental procedures which have been developed as tests, implying that they could be learned and applied by others in a routine way with a predictable degree of sensitivity and precision. There are few such techniques with coelenterates that have been used by anyone other than their originator, and these will be considered in some detail. Nevertheless, there is a considerable body of literature on which we shall draw to indicate likely future developments and possibilities. The literature relates primarily to the hydroids and corals, there being very little toxicological work on jellyfish and anemones. Furthermore, there is a tendency for the hydroid work to be experimental as they are not only good experimental subjects, but they are relatively ephemeral in the environment. Corals, on the other hand, are poor experimental subjects, but are ideal for in situ studies because they are large, sessile and long-lived.

SCYPHOZOA

Work by Spangenberg (1964, 1967, 1968) has led to what appears to be the only toxicological test system with a scyphozoan. The metamorphosis test system using Aurelia aurita, and some of the background work that preceded it, will therefore be considered in some detail. Spangenberg's objective was to provide a method for the rapid determination of the effects of pollutants on larval marine organisms undergoing metamorphosis (Spangenberg et al., 1980 ; Spangenberg, 1984). The system draws on expertise gathered over 20 years culturing Aurelia in the laboratory from polyps or scyphistomae to sexually mature medusae (Spangenberg, 1964). Of particular importance is the ability to initiate strobilation at will, by preconditioning at low temperature (one month at 19 °C for the Texas strain) and by induction with low concentrations of iodine after increasing the temperature (27 °C for the Texas strain). As Custance (1966) has demonstrated, European strains strobilate at much lower temperatures (< 8 °C) and he has shown that light of high intensity inhibits strobilation (Custance, 1964). Other factors reviewed by Spangenberg (1968) may also be important. As it appears that the techniques involved are not too difficult to learn and have been applied effectively by others (Coyne, 1973), it seems likely that the technique will become more widely used.

The test employs the polyp or scyphistoma stage which is induced by the appropriate preconditioning described above to segment at its oral end to produce ephyra larvae. By this asexual process of strobilation, the larvae develop and detach sequentially to join the plankton and grow into the characteristic medusoid form of the jellyfish. Tests are conducted with polyps that are isolated in 5 ml of artificial seawater together with the test compound. They are induced to metamorphose by the addition of 1×10^{-5}M iodine. Tests incorporate ten control organisms and ten replicates per treatment which are all maintained at 30 °C. The organisms are kept for 14 days and examined at 2 day intervals. The responses found to be sensitive to hydrocarbons are delays in the onset of metamorphosis, teratological and other abnormalities in the differentiation of the ephyrae, and their pulsing and swimming behaviour. These may be related to abnormalities in statolith differentiation.

Table I. Retardation of strobilation initiation by a petroleum oil (PO) and hydrocarbons at highest dosage of range tested (after Spangenberg, 1984)

Compounds	Dosage range	Number of organisms (Number of tests)	Time for 100 % of polyps to segment (days)					
			2	3	4	5	10	14
ASW Control			+					
PO	0.1 – 0.5 %	360 (3)						+
Aniline (24 h)	0.1 – 0.9 mM	420 (10)					+	
Anthracene	0.01 – 1 mM	120 (3)		+				
Benzathracene	0.01 – 1 mM	120 (3)			+			
Benzene	1.0 – 4.5 mM	180 (4)		+				
Benz-a-pyrene	0.04 – 0.2 mM	90 (4)		+				
Biphenyl	0.02 – 0.05 mM	360 (6)				+		
Cresol (24 h)	0.1 – 0.5 mM	550 (8)						+
Naphthalene	0.07 – 0.07 mM	180 (4)					+	
Perylene	0.05 – 1 mM	110 (3)		+				
Phenol (24 h)	0.3 – 0.7 mM	170 (2)						+
Pyrene	0.05 – 2 mM	180 (4)				+		
Toluene	1 – 2 mM	80 (3)				+		

In a recent set of experiments all 12 hydrocarbons and crude petroleum oil retarded the onset of strobilation at the highest concentration tested (Table I), and in the case of phenol and aniline there was also a reversal in development from strobila to polyp. This may be a generalised response to stress in that it can also be initiated by environmental factors such as temperature change.

Observations on the behaviour of ephyrae showed that those exposed to some hydrocarbons could either not swim, or pulsing was uncoordinated. More obvious were the teratological malformations which occurred with a frequency 2 to 4 times greater in the organisms exposed to hydrocarbon compounds than the controls. The most common defects were decreased numbers of arms or lappets, or shortened lappets. Reductions in statolith size and frequency were also common. Bizarre malformations were found in treated cultures that included multiple heads and stalks, fused parts, branched stalks, and in extreme cases a large clumped colonial appearance.

Spangenberg (1984). lists a number of advantages for her test system, some of which have already been mentioned, or are evident from the account we have given. Others include the fact that small organisms cultured in small volumes of water make it possible to keep costs low while permitting adequate replication. The fact that Aurelia can be cultured indefinitely in artificial seawater as a clone is another obvious advantage.

From the experiments conducted so far with the Aurelia metamorphosis test system, it appears that experiments involving a range of fixed concentrations are necessary to establish concentration - response curves and thresholds of sensitivity for individual toxicants. Only then can the sensitivity of the system be established by comparing threshold concentrations for test compounds with those for other systems. Furthermore, morphological and teratological changes need to be determined quantitatively as frequencies for a range of concentrations.

HYDROZOA

Hydrozoans have been used for experimental studies of growth and morphogenesis for many years and the first work on the effects of toxic agents on hydroids may have been that of Huxley and de Beer (1923). They studied the effect of potassium cyanide and mercuric chloride on the dedifferentiation and resorption of Obelia and Campanularia, and found the rates to be dose-dependent. Although techniques for culturing marine hydrozoa for long periods were developed some time ago (Rees and Russell, 1937), it was not until the introduction of Artemia salina nauplii as food that hydroids could become widely used for experimental work (Crowell, 1957). As a result it is only in the last 10 years that much toxicological work with hydroids has been published.

Dr. B. Werner in 1969 proposed that hydroids might be good test organisms for investigating marine contaminants, and at this suggestion Karbe (1972) developed a test with Eirene viridula in which he identified stages in the dedifferentiation and reorganisation of tissues - the same process described by Huxley and de Beer (1923). Threshold concentrations are given for a number of metals tested, both for morphological changes and tissue reorganisation, and another set of threshold for "acute" or short-term effects which are generally lower (Table II). Karbe's data are derived primarily from his identification of six stages in the degeneration of affected hydranths. At stage I the tentacle tips are swollen, while at stage II the tentacles are thicker and shorter. At stage III the tentacles have completely degenerated and the polyp itself begins to change shape. At stage IV the polyps have either fallen off or regressed completely and at stage VI even the stolon tissue is breaking up. Clearly the crucial change in determining threshold concentrations are changes in the form of tentacles, as they are always the first component to be affected.

Similar criteria for toxicological effects have been used by Fisher (1978) in his experiments on the effects of temperature and salinity on cadmium toxicity. He used Karbe's criteria as a sublethal index (Table II), which was marginally more sensitive than his so-called lethal response, i.e. the inability of polyps to catch and swallow Artemia nauplii.

Theede et al. (1979) investigated the effect of temperature and salinity changes on the toxicity of cadmium to Laomedea loveni. They found irreversible retraction of the hydranths to be a sensitive acute index of effect, and from their data derived a response surface which shows the influence of both temperature and salinity (Fig. 1). These data were also related to cadmium accumulation in the hydroid tissues. Their results demonstrate clearly that the susceptability of Laomedea loveni to cadmium is

Table II. Threshold concentration (ug.l^{-1}) for responses of different hydroid species to a range of toxicants

Toxicant	Species						
	Clava multicornis[1]	Eirene viridula[2]		Laomedea loveni	Laomedea (Campanularia) flexuosa		
	Morphological changes over 2 - 3 weeks	Morphological changes over 2 - 3 weeks	Acute effects	Irreversible retraction of 50 % of polyps	Specific growth rate	Cytochemical index	Increase in gonozooid frequency
Ammonia					17		
Cadmium	217 (10 °C/25 ‰) 218 (20 °C/10 ‰)	300 - 10 000	100 - 300	3 - 80[3] (see Fig. 1)	195[4]	57.5[5]	
Copper		60 - 1 000	30 - 60		14.3[4]	1.4[5]	0.05 - 0.1
Cyanide					22 (see Fig. 4)		5 - 10 (see Fig. 4)
Lead		1 000 - 10 000	1 000 - 3 000				
Mercury		3 - 100	1 - 3		1.6[4]	0.2[5]	0.01 - 0.1
Tributyltin					0.13		0.02 - 0.05
Zinc		3 000 - 10 000	1 500 - 3 000		740		500 - 1 000

Sources : 1 Fischer (1978), 2 Karbe (1972), 3 Theede et al. (1979), 4 Stebbing (1976), 5 Moore and Stebbing (1976).

increased by suboptimal temperature and salinity, and that organisms at the extremities of their normal distribution are likely to be more vulnerable to the effects of pollution.

A method has also been developed with Laomedea (Campanularia) flexuosa for ecotoxicological test purposes which involved scaling up previously used culture techniques, first to increase replication (typically 7 replicate colonies per treatment) in order to improve the precision of the response data, and second to increase the area available to each colony for growth to allow for larger colonies and longer experiments (Fig. 2) (Stebbing, 1976).

Initially some experiments with metals were conducted (Fig. 3) as a necessary step toward the development of a laboratory technique with which to estimate the biological quality of polluted samples using the responses of cultured hydroids. The occurrance of morphological abnormalities did not seem to be sufficiently objective or easily quantifiable, so initially effort was devoted to the use of colonial growth as an index of response. This led to a study of how growth is controlled an the counteraction of toxic inhibition by homeostatic mechanisms (Stebbing and Hiby, 1979 ; Stebbing, 1981a). Hormesis may result from the tendency of such control mechanisms to overcorrect in response to low levels of inhibitory load (Stebbing, 1981b, 1982).

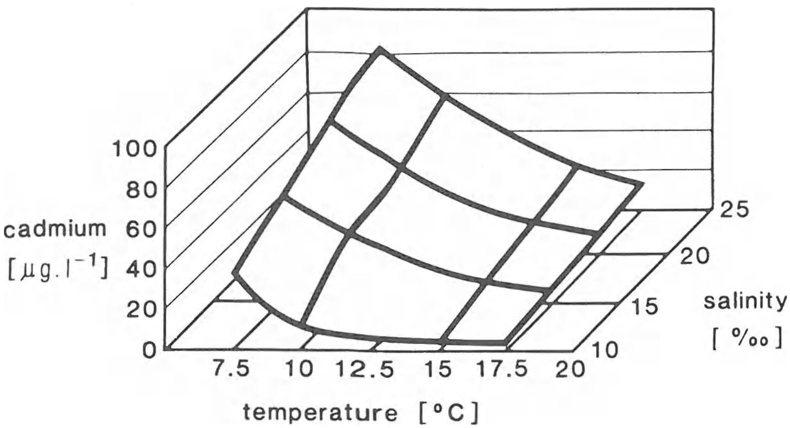

Fig. 1. Threshold concentrations of cadmium for the irreversible retraction of 50 % of the polyps of Laomedea loveni at different combinations of temperature and salinity (after Theede et al., 1979).

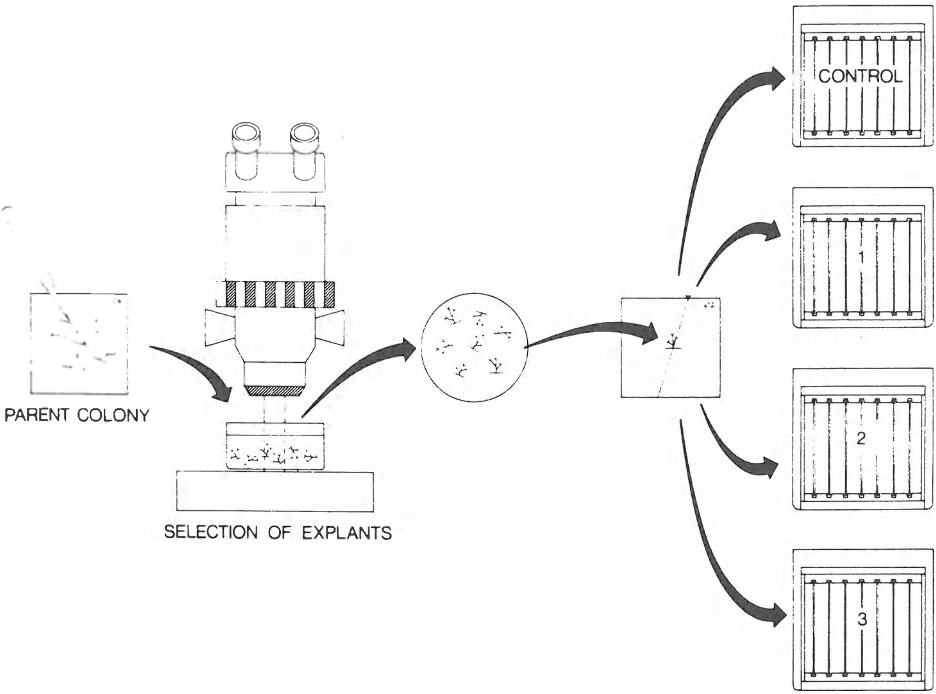

Fig. 2. Diagram summarizing the subculturing of a hydroid (Laomedea flexuosa) for an experiment. Established parent colony is peeled off its glass plate and healty uprights of three or more hydranths are selected for subculturing. These are attached to new plates by monofilament nylon threads until they are established.

More important from the point of view of developing a water quality bioassay technique with hydroids was the need to find more sensitive indices than the inhibition of colonial growth, as levels of metals and other toxicants in seawater rarely exceeded the growth thresholds (Table II, Fig. 3). Attempts to use of cytochemical technique that measured increases in activity of a lysosomal enzyme were successful in that in experiments with metals, thresholds for enhanced enzyme activity were about an order of magnitude lower than thresholds for the inhibition of colonial growth rate (Table II) (Moore and Stebbing, 1976). However, the responses of hydroids to samples from contaminated waters were too difficult to interpret, and so we looked for other sensitive but generalised responses of Laomedea flexuosa to toxicological stress.

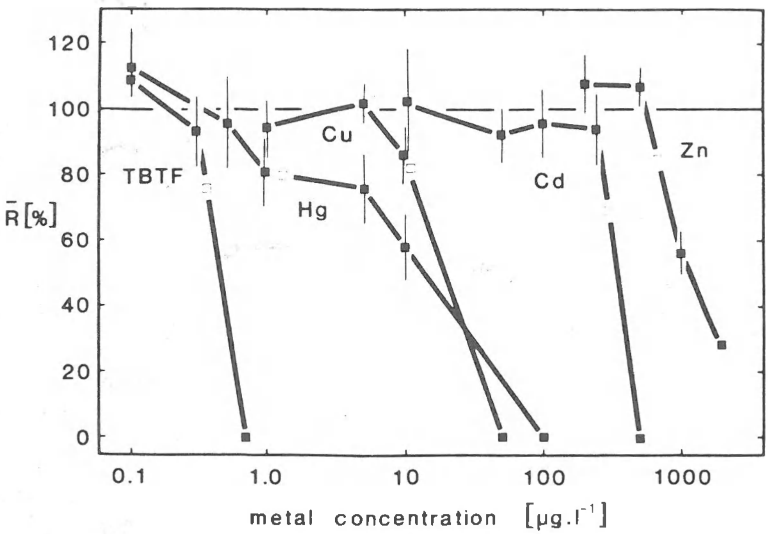

Fig. 3. Concentration-response curves for the effects of various metals on the mean specific colonial growth rates of Laomedea flexuosa expressed as percentages of those of the control colonies ($\bar{R}$ %) for 11 day experiments. Data points are means for seven colonies and are given as solid squares, and threshold concentrations as open squares. Vertical bars indicate two standard errors of the means.

This we found in their tendency to divert resources when stressed to produce gonozooids rather than hydranths (Stebbing, 1980, 1981b). Enhanced gonozooid production is apparently an adaptive response that increases the likelihood of the survival of the genotype when the parent colony is stressed by releasing a greater number of the planktonic phase. It may also be of significance that genetic heterogeneity is likely to be increased since this mode of reproduction is sexual and leads to meiosis. Here though we simply use gonozooid frequency as a sensitive response to stress which can be easily determined from living colonies (Fig. 4). Threshold levels in Table II are given as ranges rather than specific concentrations partly because the levels are lower in some cases than can at present be confirmed by chemical analysis.

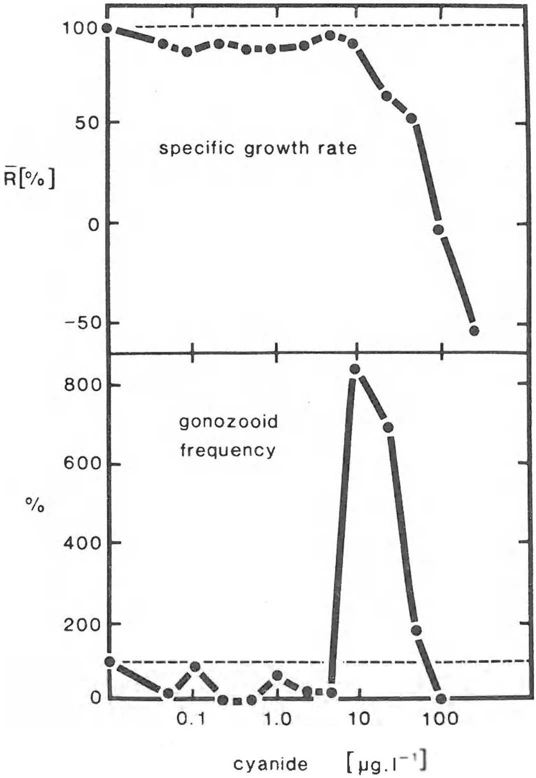

Fig. 4. Concentration-response curves for the effects of cyanide on the mean specific colonial growth rates as percentages ($\bar{R}$ %) and gonozooid frequency of Laomedea flexuosa. Gonozooid frequency is the number of gonozooids as a proportion of the total number of colony members expressed as a percentage of the frequency in control colonies.

We have used gonozooid frequency regularly for some years as a sensitive index of water quality in bioassay experiments (Stebbing, 1979 ; Stebbing et al., 1983, 1984). In recent experiments in the R. Tamar estuary in S.W. England, we have found an area of water that elicits the hydroid gonozooid response repeatedly in different experiments between stations 12 to 18 (Stebbing et al., 1983). We have since conducted experiments on samples from vertical profiles at the stations that elicit the more marked responses (Fig. 5). There is nothing to be gained from considering these data in detail, other than to say that the responses are clear and significant, they are sometimes repeated in different experiments and can on occasion by related to elevated levels of contaminants.

Such bioassay responses have the advantage that the organism integrates the effects of all contaminants that have an influence upon it. However, it is obviously necessary - if one expects to such an approach in a regulatory context - to identify the contaminants that occur at biologically significant levels or in biologically active forms. In principle this can be done by chemically manipulating the water samples before bioassay. Thus selective removal of the divalent metals using an ion exchange resin and the subsequant removal or reduction of the hydroid stress response positively demonstrates the importance of metals in the original sample (Stebbing, 1979). While this approach shows some promise it has not been developed far yet.

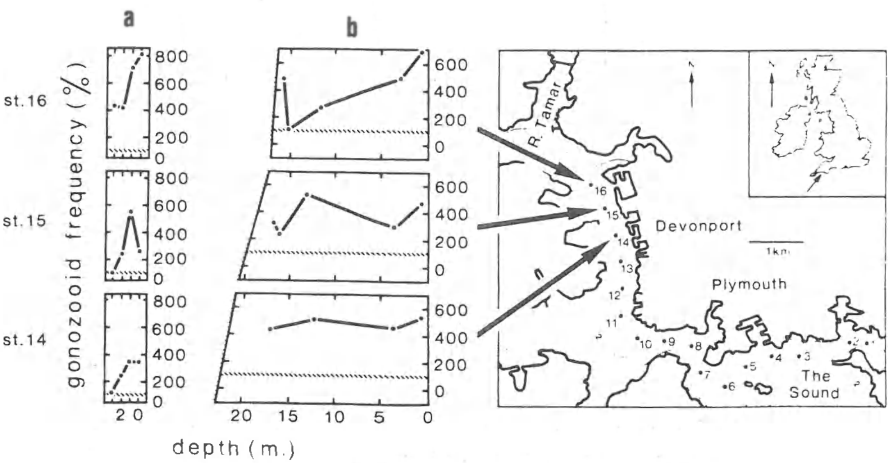

Fig. 5. The results of experiments in which the biological water quality of samples from an estuary are estimated by culturing colonies of Laomedea flexuosa in the samples. Samples were taken on profiles at three stations from which near-surface water samples had been found previously to stimulate gonozooid production. Results from two experiments in September 1982 (a) and April 1982 (b) are given.

Since hydroids as such are not of particular ecological or economic importance, their major advantage is the ease with which they can be cultured as a clone. Their sensitivity and the fact that they can be cultured on a small scale is an economic advantage, but experiments and their culture tend to be labour-intensive. For these kinds of reasons they were not recommended for inclusion in marine pollution monitoring programmes (Stebbing et al., 1980), although hydroid tests have been recommended for general use in the Federal Republic of Germany (Karbe, pers. commun.).

Laomedea (Campanularia) flexuosa has been used by Wermuth and coworkers on the effects of radiation on various colonial growth functions (Wermuth and Barnes, 1975). They have established dose-response curves which show how growth - whatever way it is assessed - decreases with dose, in a way that it is also shown to be temperature-dependent (Fig. 6). It is also demonstrated that hydranth longevity increases with dose (Wermuth, 1980).

ANTHOZOA

As the bulk of the work carried out on anthozoans has been centred on hermatypic corals, this section of the review will deal (a) with scleractinian corals, and (b) other anthozoa (incorporating the limited literature on anemones and octocorals).

SCLERACTINIAN CORALS

A wide variety of tests have been carried out on scleractinian corals exposed to pollutants in the laboratory. Parameters quantitatively measured include skeleton growth rate (Jokiel and Coles, 1977 ; Neff and Anderson, 1981 ; Dodge 1982), metabolism (Coles and Jokiel, 1977 ; Krone and Biggs, 1980 ; Szmant-Froelich et al., 1982), loss of zooxanthellae (Howard et al., in press), sediment shedding (Bak and Elgershuizen, 1976 ; Thompson and Bright, 1980 ; Thompson, in Dodge and Szmant-Froelich, in press), reproductive biology (Rinkevich and Loya, 1979), and survivorship and settlement of coral larvae or planulae (Rinkevich and Loya, 1977). Many other responses such as mesenterial filament extrusion, mucus production, polyp retraction, and histopathological effects have been described by workers during laboratory experiments but these effects have not been quantified and therefore will not be considered further in the present review.

Skeleton growth of corals may be monitored in a variety of ways. These include X-radiography of seasonal growth bands deposited by massive corals, reference marking by alizarin red S stain and subsequent measurement of growth beyond the marker line, measurement of an increase in skeletal weight, and finally recording ^{45}Ca deposition rates in the skeleton.

Using an increase in weight of skeleton produced over time, Jokiel and Coles (1977) demonstrated a reduction of growth in a selection of Hawaiian corals exposed to a thermal increase of approximately 4°C. Measurement of skeletal extension beyond an alizarin reference band in the Caribbean coral Montastrea annularis exposed to 100 ppm drilling mud revealed a significant decrease in skeletal extension compared with control specimens (Dodge, 1982), while rather variable results were obtained for ^{45}Ca incorporation into skeletons of selected Caribbean corals exposed to water soluble fractions of fuel oil and Louisiana crude oil (Neff and Anderson, 1981).

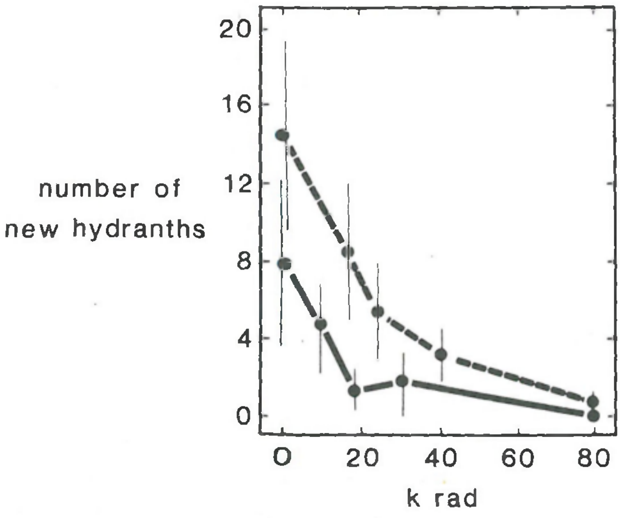

Fig. 6. Dose-response curves for irradiated colonies of Campanularia flexuosa (= Laomedea flexuosa). The average number of hydranths added to the colonies in the 8 days following irradiation on day 11 are plotted against the gamma radiation dose. The broken line indicates colonies kept at 9 °C and the continuous line those kept at 18 °C, (after Wermuth and Barnes, 1975).

Although no attempts have been made to standardise methods employed in monitoring coral growth, it would seem that this is a very useful parameter in assessing the effects of pollutants on corals, since it not only integrates a variety of physiological processes but it may be applied in both the field and laboratory to branching and massive corals. Indeed, several workers have assessed the impact of environmental disturbances on corals in the field by using controlled growth rate experiments. Examples include the establishment of experimental enclosures (Rogers, 1979), the monitoring of experiments quadrats (Bak and Criens, 1982, and transplantation of corals between different locations (Hudson and Robbin, 1980 ; Hudson, 1982). Since massive corals act as chronometers of environmental change, producing high-density skeletal deposits or 'stress-bands' (Fig. 7) during periods of rapid chilling and mixing of shallow inshore waters (Hudson et al., 1976 ; Hudon 1977, 1981), they offer potential in recording effects of pollutants in the field. X-radiography may be used on such specimens to produce a growth history of the coral (Fig. 8). Such measurements on Montastrea annularis in the Florida Keys (Hudson, 1981) suggest a decline in coral growth from 1953 - 1968 and a slight improvement in growth from 1973 - 1981. The author speculates that these effects may coincide with increased dredge and fill operations in the area between 1953-1968, and the ban on these operations from 1973 onwards.

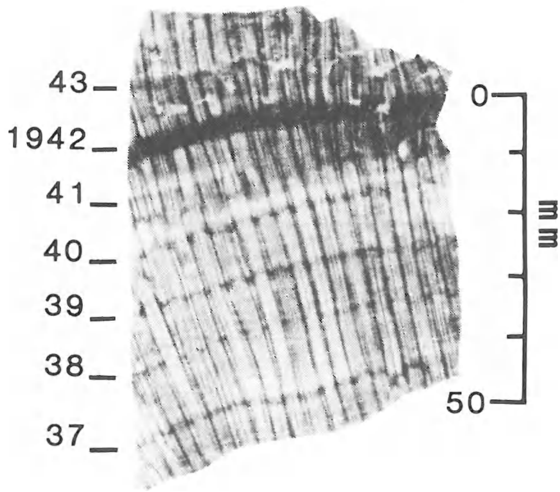

Fig. 7. X-radiagraph of a section of Montastraea annularis showing the annual winter growth check bands from which growth data can be derived (see Fig. 8). Note particularly the dark stress band laid down in the winter of 1941-2 (after Hudson, 1981).

Laboratory measurements of various metabolic parameters in corals exposed to pollutants include the monitoring of calcification rate, respiration rate, photosynthesis rate and ammonium and nitrate uptake. Disadvantages of these experiments could be described as the relatively high levels of pollutant required to produce an effect (often unrealistic with respect to environmental concentrations), the variability in response of individual heads of the same species, and the possible insensitivity of the assay methods used. For instance, in experiments where all five parameters cited above were measured, Szmant-Froelich et al. (1982) demonstrated considerable mortality and partial mortality in colonies of Montastrea annularis after 6 weeks exposure to 100 ppm drilling mud, but significant changes in metabolic parameters could only be monitored after a period of 4 weeks exposure.

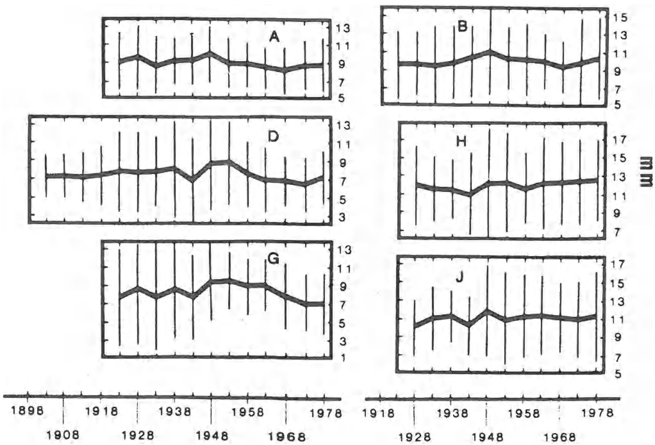

Fig. 8. Growth history graphs of Montastraea annularis derived from annual bands (see Fig. 7). Mean growth rates are plotted at 5-year intervals with maximum-minimum growth rates at the 95 % confidence level indicated. Graphs A, D and G are derived from corals living in an inshore reef environment, while B, H and J are from corals living in a shallow midshore environment. (after Hudson, 1981).

Not surprisingly, data from other workers (Kendall et al., 1983) using more toxic drilling muds, exposures in field enclosures, and a branching coral Acropora cervicornis, revealed rather different results. In these experiments, of the three parameters measured (soluble tissue-protein, concentration, total ninhydrin-positive substance (NPS) concentration, and calcification rate), calcification rate was the most sensitive, the dose-response curve showing a significant reduction in calcification rate at 25 ppm drilling mud, the lowest concentration used (Fig. 9). Fig. 12 illustrates the relative sensitivity of the parameters used in this study. Few researchers working with corals have attempted to produce dose-response curves for pollutants studied or rank the relative sensitivity of tests used in assessing toxicity. Studies such as those of Szmant-Froelich et al. (1982) and Kendall et al. (1983) highlight some of the problems of toxicological testing, namely the variability in toxicity of the pollution source, the relative sensitivity of the species used and the test applied. Nevertheless coral reefs are composed of many species of coral which can be broadly divided into branching and massive variaties, and there is a growing amount of evidence (see Brown and Howard, 1984, for review) to suggest that branching corals may be more susceptible to certain pollutants than massive species.

Metabolic studies may also have considerable potential in in situ measurements of the energy requirements of reef corals in the future (Spencer-Davies, pers. commun.). By constructing energy budgets for corals by in situ monitoring of respiration and photosynthesis, it may ultimately be possible to determine the overall "health" of corals in polluted environments.

Another recent development in metabolism studies involves the measurement of productivity and calcification of an entire reef zone such as the reef flat. Work by Barnes (1983), in which he floated a buoy equipped with pH and oxygen electrodes plus a sensitive thermistor across a reef flat to obtain values for reef productivity and calcification, could be applied to polluted reef environments. Barnes cites Kinsey (1979) who suggests that reef flats operate within narrow metabolic limits, any departures from these limits possibly reflecting perturbation. Once the respiratory and metabolic characteristics of reef communities are better understood methods such as those described above may have an established place in pollution studies on reefs.

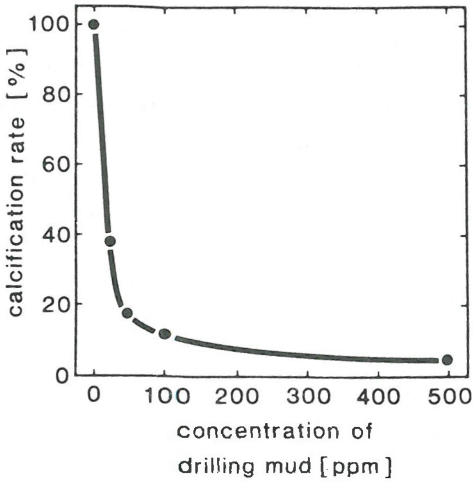

Fig. 9. Dose-response curve showing the effect of drilling mud at different concentrations on the calcification rate of *Acropora cervicornis* tips during 24 h exposure (after Kendall *et al.*, 1983).

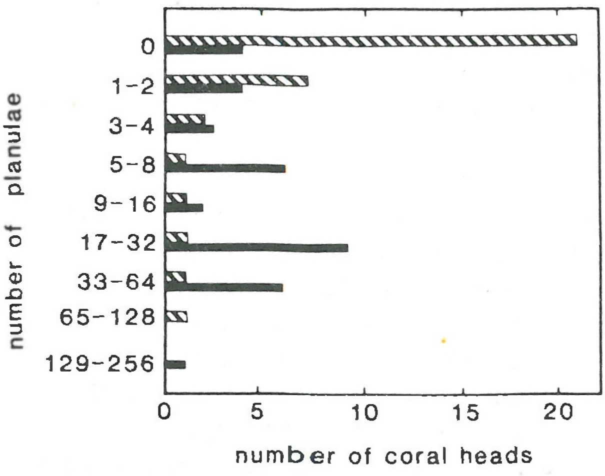

Fig. 10. Differences in the release of planulae by *Stylophora pistillata* on reefs near an oil port compared with those released by a control population. Data are given as the number of planulae released per coral head (after Rinkevich and Loya, 1977). The solid bars indicate the control population and the striped bars the experimental population from the oil port reef.

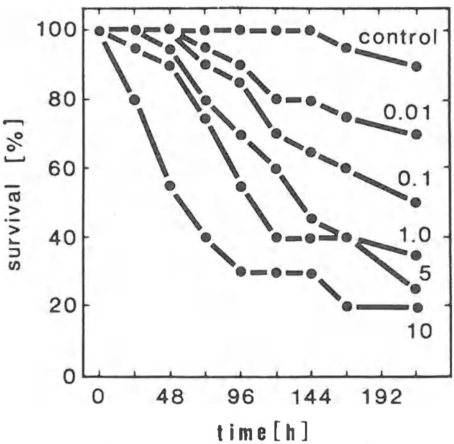

Fig. 11. Survivorship curves for the planulae of Stylophora pistillata in different concentrations of crude oil as ml crude oil/litre of seawater (after Rinkevich and Loya, 1977).

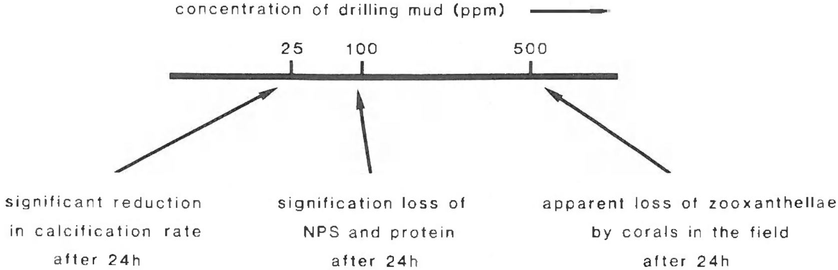

Fig. 12. Response of Acropora cervicornis to various concentrations of drilling mud (after Kendall et al., 1983).

Table III. Settlement of *Stylophora pistillata* colonies on petri plates in the oil port and control area (after Rinkevich and Loya, 1977)

Locality	Number of petri plates	Plates with colonies	% Plates with colonies	Total number of colonies	Plates with 1 colony	Plates with 2 colonies	Plates with 3 colonies	Plates with 4 colonies
Oil port	50	1	2.0	1	1	0	0	0
Control	14	8	57.1	15	4	2	1	1

A technique of some potential, though limited in its use to date, is the experimental manipulation of coral planulae in toxicological work. Loya (1976) was the first to recognise that oil was potentially damaging, not only to the reproductive system of sexually mature corals but also to the survivorship and settlement of their planulae. After observing poor coral recruitment on oil polluted reefs near Eilat he carried out field and laboratory experiments on the planulae of Stylophora pistillata, one of the most abundant coral species in the shallow waters of the Gulf of Eilat.

Table IV. Settlement of Stylophora pistillata planulae on petri plates in different concentrations of crude oil (after Rinkevich and Loya, 1977)

Factor measured	Oil concentration ($ml.l^{-1}$)					
	10	5	1	0.1	0.01	control
Number of planulae	0	0	4	7	7	11
Percent settlement	0	0	20	35	35	55

In the field 60 plastic petri plates were attached to rocks at two stations, the oil port and the control area. After one year 50 plates remained on the oil polluted reef but only 14 at the control site, the remainder having been removed by vandals. The results, however, (Table III), clearly indicated that the colonisation rate of new colonies was higher at the control than at the polluted site. Rinkevich and Loya (1977) suggested that the low settlement could be the result of a low number of potential planulae (Fig. 10) and/or decreased planulae viability. Survivorship of planulae in varying concentrations of crude oil indicated that after 144 h more than 50 % of the planulae in 1.5 and 10 ppb oil concentrations had died (Fig. 11). In addition settlement of planulae in laboratory experiments (Table IV) indicated a significant lower rate of settlement over 120 h in 1.5 and 10 ppb oil concentrations than in the controls. Subsequently Rinkevich and Loya (1979) carried out long-term (2 to 6 months duration) laboratory measurements on the effect of crude oil on the reproductive biology of Stylophora pistillata. By splitting each coral colony into two parts and studying each half in polluted and clean tanks, Rinkevich and Loya were able to test effects on the same colony, thus avoiding the variability in response discussed earlier. Exposure to 3 ppb oil for 24 h once a week resulted in a significant decrease in the number of female gonads produced by 75 % of the colonies in the polluted tanks. Poor colonisation at the oil port could therefore be explained by a smaller number of breeding colonies, a decrease in the number of eggs per polyp, a smaller number of planulae produced per coral head and a lower settlement rate of planulae.

Since 1979 no other workers have followed the example of Rinkevich and Loya, but these methods applied to other pollutants would give valuable information. Not only are planulae a sensitive stage in the life history of the coral but they are small and available in numbers which make them extremely suitable for laboratory experiments. In addition the relevance of laboratory observations may be tested in the field since parameters such as planulae settlement and growth on artificial substrates may be monitored in situ, with the condition that control and polluted sites are subject to similar natural environmental influences. One disadvantage of the use of planulae is that they cannot be obtained from all corals since many species produce sperm and eggs which are broadcast into the sea, there being no brooding of planulae within the body cavity. Nevertheless further work on planulae settlement would prove a valuable tool in future pollution research on many reef corals.

OTHER ANTHOZOA

References to the use of Anthozoa, apart from Scleractinia, in toxicological work are very limited. They include tests with the sea anemone Actinia equina (Ormond and Caldwell, 1982) and the alcyonarian Heteroxemia fuscescens (Cohen et al., 1977) to establish the effects of oil pollutants on these species, and the suggested use of the Caribbean gorgonid Gorgonia ventalina as an indicator of environmental stress (Morse et al., 1977).

In the former studies similar responses were elicited on exposure to oil, these were the expulsion of premature and mature planulae at concentrations of 10 ppb crude oil after 72 h (for the alcyonarian), and 2.5 ppb crude oil after 1 to 7 weeks (for the see anemone). In addition Ormond and Caldwell (1982) noted a reduction in the size of ova and the eliciting of a feeding response in anemones exposed to oil, while Cohen et al. (1977) observed a reduction in pulsation rate of the soft coral in oil concentrations of 1 ppb to less than 50 % of the rate of untreated colonies.

Morse et al. (1981) describe tumour-like growths in the gorgonic Gorgonia ventalina from Bonaire and infer that these may be formed in response to high levels of chronic or intermittent hydrocarbon pollution from nearby petroleum tanker lanes and loading depots. However no cause-effect relationship has been established to explain the evidence of tumours in this species and the authors' conclusions must remain tentative.

CONCLUDING TRENDS

There are relatively few ecotoxicological tests using coelenterates. Tests with corals are needed because of the importance of coral species to reef ecosystems and some techniques, such as backplotting growth curves from annual growth lines, or the survival and settlement of planula larvae of corals, are good ways of assessing contaminant effects. Hydroids are better experimental organisms as they are less difficult to culture, but as a group they are not ecologically important. Nevertheless, where there are no taxonomic constraints, they have many practical features that are ideal. They can be easily cultured on a small scale for indefinite periods in genetically homogenous populations, and have responses that are as sensitive as any to some toxic contaminants (Table II). Their sensitivity makes them ideal for the kind of bioassay experiments where organisms are exposed in the laboratory to water samples from the field. Using this approach it is possible to detect and define areas of poor biological water quality that cannot be shown by other methods, particularly when the contaminants involved are unknown.

Our review highlights a number of features of existing tests that look promising, and others that might best be avoided for ecotoxicological tests. It is obvious that tests should be sensitive enough to detect the levels of toxic contaminants that occur in polluted waters. The choice of response or effect is important in achieving sensitivity, as the thresholds for a range of responses in a coral (Fig. 12) and a hydroid (Fig. 13) indicate. However, in choosing such a response, it is important that it can be readily quantified. Qualitative changes such as the occurrance of morphological abnormalities or teratological effects are difficult to use in tests unless they can be measured, or occur commonly enough to handle as frequencies. The use of stages in dedifferentiation under toxicological stress require experience in interpretation that is not easy to convey to others who might wish to learn the test by way of the printed word. Furthermore, any test must incorporate adequate replication which can be used to assess its own precision by demonstrating satisfactory "signal to noise ratios".

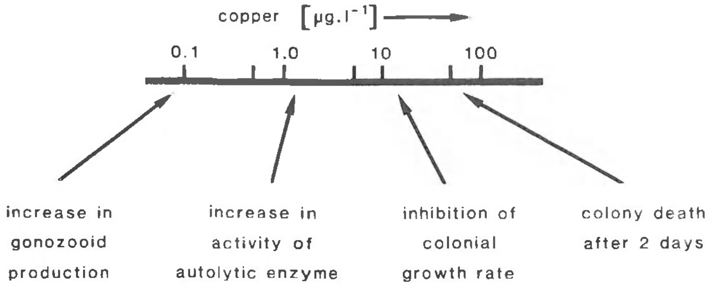

Fig. 13. Response of Laomedea flexuosa to various concentrations of copper (after Stebbing, 1981c).

We can identify some tests that satisfy the usual criteria of the kind considered above, while at the same time having a direct bearing on the survival of populations in the field. Growth is an obvious response that, as an integration of numerous physiological processes, is a good index. In experimental studies fast growing species are required if the test is not to be too long, but backplotting growth curves of corals is clearly a good way of detecting the effects of pollution events retrospectively. Tests based on adaptive responses to toxicological stress, such as enhanced gonozooid production in hydroids, make it possible to detect a change in biological water quality before it becomes harmful to the biota. Similarly, much more could be done with planula and ephyra larval stages, which are more sensitive to toxic contaminants than their adults.

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