Vol. 46: 235-243, 1988 | MARINE ECOLOGY - PROGRESS SERIES Mar. Ecol. Prog. Ser. | Published June 30

An overview of the GEEP Workshop

B. L. Bayne[1], R. F. Addison[2], J. M. Capuzzo[3], K. R. Clarke[1], J. S. Gray[4], M. N. Moore[1], R. M. Warwick[1]

[1] Plymouth Marine Laboratory (West Hoe), Prospect Place, The Hoe, Plymouth PL1 3DH, United Kingdom
[2] Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, Nova Scotia, Canada B2Y 4A2
[3] Department of Biology, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, USA
[4] Department of Marine Zoology and Marine Chemistry, Biology Institute, University of Oslo, PO Box 1064, N-0316 Blindern, Oslo 3, Norway

ABSTRACT: This paper reviews the main highlights of the GEEP Workshop, both its successes and failures, draws conclusions from these and from lessons learnt in convening and participating in the workshop, and suggests a way forward for research and application of biological effects techniques. We point to the importance of maintaining close links between the research effort and the evaluation of procedures that emerge, in the context of a wide spectrum of climatic and environmental regimes. The utility of measures of biological responses to pollution will depend on the extent to which a suite of such measurements can be put together so as to link, in a cause/effect manner, the results of chemical analyses and responses at the biochemical, cellular, physiological, population and community levels. Some hopeful trends and some basic difficulties are indicated. We suggest that further practical workshops on the same model as the GEEP Workshop, but held in different climatic regions and with different faunas, will play an essential part in furthering the development of techniques for measuring the biological effects of pollutants.

INTRODUCTION

The GEEP Workshop was the first attempt to undertake a practical inter-calibration and evaluation of techniques for measuring the biological effects of pollutants in the sea. The techniques tested during the 3 wk of the workshop included biochemical, cytochemical, histological and physiological procedures, and procedures to measure features of benthic community structure. By applying careful statistical design and thorough chemical analysis to accompany the biological measurements, the workshop provided a unique opportunity for an evaluation of the different techniques, set within the context of a particular fauna (a shallow-water, fjordic, boreal community) subjected to a complex mixture of contaminants (aromatic hydrocarbons, polychlorinated biphenyls and trace metals in the case of the field samples; water-accommodated diesel oil and copper in the case of the mesocosm experiment). It was not our intention to conclude by ranking the techniques evaluated (as poor, better, best, for example), nor to imply that the techniques tested were uniquely capable of detecting biological responses to pollution, or that these techniques would necessarily be appropriate under all possible circumstances of pollutant impact on a marine fauna.

Our reasons for stating these caveats here are 3-fold. Firstly, attempts to understand and to measure the impact of contaminants on biological processes are very much the subject of continuing research. In no single case could it be argued that further investigation into an appropriate biological response is no longer required, so that a technique may be 'fixed' for application in monitoring or in toxicological assessment. Indeed, the insights to be gained from further research into the processes represented at the workshop are considerable, and the increase in information thus obtained will be of value in meeting the need to measure and to predict the biological effects of pollutants in the field.

Secondly, it was clear to all involved in the workshop that there is no single biological measurement that will

0171-8630/88/0046/0235/$ 03.00

serve to indicate the effects of pollution. This simple point may seem obvious enough not to need saying, but there are still those who, with a poor appreciation of the true nature of biological complexity, continue to expect of the biologist a single all-embracing measurement (preferably simple, cheap and easy to apply!) that will encapsulate all the important features of biological response. Such a requirement is not only unrealistic, but fails to appreciate the different types of information that can be gained from an appropriate suite of 'effects measures', from the sensitivity and specificity of some biochemical determinations to the integrated appreciation of ecological damage that can be gleaned from measures of community structure.

Thirdly, the GEEP Workshop was held in an area already well studied with regard to pollutant inputs, and it involved species (a bottom-living flat-fish, mussels, crabs) which are the objects of considerable toxicological research. These criteria were necessary for this first attempt at inter-calibration. However, the real value of the procedures tested at the workshop, and other techniques still to be evaluated in the same way, will be measured by their relevance to the effects of pollutants over a much wider spectrum of climatic province (polar, tropical, sub-tropical), environmental regime (different sediment types, coral reefs, mangroves) and taxonomic coverage.

Within the constraints as originally perceived, the GEEP Workshop was a considerable success. In this Overview we draw attention briefly to some of the main highlights and lessons learnt, and consider where the study of the biological effects of pollutants now stands.

SUMMARY OF THE MAIN HIGHLIGHTS

An essential element in the design of the GEEP Workshop concerns the fact that samples were analysed 'blind', i.e. samples were coded and the coding (and the results of the chemical analyses) only made known to the participants after their analyses had been completed. Inevitably, contamination at the field sites was complex, as evidenced by the results of the chemical analyses; nevertheless, it is possible to rank the 4 stations at which flounders, crabs, mussels and periwinkles were sampled in order of increasing contamination from Sites 1 to 4 (Klungsøyr et al. 1988). These samples, together with the samples for community analysis, provided the main focus of the workshop, since our primary intention was to evaluate the procedures under conditions of typical spatial complexity (with regard to contaminating inputs, hydrography, microhabitats, etc.).

Conditions within the mesocosm experiment at Solbergstrand were necessarily more artificial. No mesocosm can fully replicate natural conditions in all respects, and it was not our intention to attempt this. Specifically, the following constraints, among others, were accepted. (1) The contaminant mixture (diesel oil and copper) would not replicate the contaminants in the Langesund and Frierfjords, but should effect a graded response within those organisms for whom the main route of entry was from the dissolved or emulsified state (i.e. via the gills or surface epithelium). (2) The community samples, captured within box-cores, would not reflect changes dependent on recruitment from the water column (e.g. many of the macrofauna) and would likely be affected by emigration of mobile epibenthic species; nevertheless, exposure to contaminants in the water was expected to impact at least the superficial layers of the sediment. We accepted that the time-scale of exposure in the mesocosm was short relative to some of the processes known to effect changes in benthic community structure.

During the mesocosm experiment, a further complication developed when mussels *Mytilus edulis* from the high-dose basin died prior to the start of the workshop and were subsequently replaced. This meant that the exposure period, and therefore the dose of contaminants (for the mussels), was not as originally planned, a point discussed by Widdows & Johnson (1988). Also, because of changes in the reproductive condition of mussels between the first and second transplants from field to mesocosm (25 April and 18 July 1986) the interpretation of measurements of cellular condition, which are particularly sensitive to changes in reproductive state, were rendered more complicated.

The summaries at the end of each of the main sections in this MEPS SPECIAL discuss the main conclusions, as agreed in the final days of the workshop. We present here an even briefer statement of the main highlights.

Biochemical processes

Biochemical measurements concentrated on processes likely to respond specifically to certain types of pollutant; those relating to 'Phase 1' enzymes of the microsomal cytochrome P-450-mediated mono-oxygenase system in the liver of the flounder *Platichthys flesus* were particularly successful in detecting a gradient of effects in the field. This conclusion applied to the activity of the enzyme ethoxyresorufin O-de-ethylase (EROD), and also to total microsomal cytochrome P-450, both of which increased in correlation with environmental levels of chlorobiphenyl and aromatic hydrocarbons (as deduced from levels of accumulation in mussel tissues), and as measured (PCB congeners) in the fish livers. In addition, 2 different techniques for measuring EROD activity were in good agreement. Further, a specific form of cytochrome P-450 – tentatively identified by Stegeman et al. (1988) as P-450E – which is the inducible form of the cytochrome under these circumstances, was confirmed by immuno-detection also to be correlated with the contamination gradient. Given the current understanding of the biochemistry of this system in fish, and its responsiveness, by induction, to certain types of organic xenobiotic compounds (Stegeman et al. 1986), these findings lend support to the use of the mono-oxygenases and their catalyst, cytochrome P-450, as effective measures of the effects of organic pollutants on fish.

Components of the analogous metabolic system in *Mytilus edulis* also responded in a predictable manner in the mesocosm experiment, when measured on microsomal preparations from the digestive gland (Livingstone 1988). However, measurements on mussel tissue from the field samples were more equivocal, reflecting our ignorance of biochemical details of the mono-oxygenase system in invertebrates. This contrasts with measurements of EROD activity and P-450 levels in fish from the mesocosm, which failed to show any consistent differences between exposure levels, an observation that can be interpreted in the light of the chemical determinations in the mesocosm, which confirmed that the contaminants present were not those normally thought capable of inducing the P-450 system in fish.

Another successful biochemical measurement made on *Mytilus edulis* from the mesocosm experiment concerned the distribution of copper amongst the metal-binding thioneins and higher molecular weight proteins of the digestive gland (Viarengo et al. 1988). Mussels exposed to certain trace metals in the laboratory are known to increase the amounts of metal bound to thioneins, and toxic damage is expected to occur when normal cellular controls, based in part on compartmentation of the protein-bound metal within lysosomes, break down, and/or when the metal begins to accumulate to high levels in association with the cytosolic proteins (George & Viarengo 1985). There was evidence of both these processes occurring within mussels in the mesocosm basins (Moore 1988), correlating both with measured levels of copper and with evidence of cellular damage. These results also confirm the difficulty in extrapolating laboratory results to more complex field situations, for the possible interactive effects of hydrocarbons on the normal processes controlling copper toxicity are not known. Nevertheless, results obtained at the workshop point to the utility of measures of protein-bound metals as monitors of the biological effects of trace metal contamination, even in the presence of hydrocarbons.

Cellular processes

A range of cytochemical and histological procedures was tried, in order not only to evaluate correlations with contaminant levels, but also to explore points of linkage between sub-cellular, cellular and tissue effects. Features of lysosomal structure and function (membrane stability, lysosomal enlargement) are known from laboratory and mesocosm experiments to respond to contamination by hydrocarbons (Moore et al. 1987), and these proved successful at the workshop in identifying *Mytilus edulis* from Field site 1 as less polluted than mussels from the other sites (Moore 1988). As expected, gross changes in lysosomal structure and function were reflected in evidence of pathological damage to the appropriate cells (e.g. the digestive cells of the digestive gland of mussels) and tissues (e.g. degeneration in the digestive tubules), as reported by Lowe (1988).

However, many of these measurements, when applied to *Mytilus edulis* and *Littorina littorea*, depend greatly on the reproductive condition of the organism. Mussels containing many ripe gametes, or those in the act of, or immediately after, spawning may exhibit considerable disturbance to sub-cellular (lysosomal) function within the digestive gland. (The reasons for this are unclear but are probably related to processes of nutrient mobilisation and general tissue reorganisation and repair that accompany the final stages of gametogenesis and spawning). These considerations were paramount in examining the material from the mesocosm experiment, probably confounded by a degree of food limitation in all basins (Widdows & Johnson 1988), and contributed to the failure of cellular techniques to demonstrate clear relationships in this material. The confoundings effects of reproductive state on many measures of cellular and physiological condition in mussels are well known (Bayne et al. 1978, Livingstone 1984) and need to be considered in any study that utilises evidence of cellular and sub-cellular pathology as indicators of pollution.

Physiological responses

The physiological processes adopted for the GEEP Workshop concentrated on those appropriate to the energy budget of the organism, i.e. measurements of feeding, absorption, respiration and excretion, integrated as components of the energy balance equation (Bakke 1988, Widdows & Johnson 1988). This approach offers insights into the physiological condition of the test animal, its potential (or scope) for growth and reproduction, and how a balance is maintained by the organism between differential environmental effects on individual physiological traits (reviewed by Bayne & Newell 1983). It is an approach that has proved useful in documenting effects of pollution on mussels (Widdows 1985).

Widdows & Johnson (1988) report the success of scope for growth determinations on *Mytilus edulis* in identifying a gradient of pollutant effect in the field samples as well as in the mesocosm experiment. Further, on the basis of a growing understanding of relationships between aromatic hydrocarbons and energy balance in mussels, Widdows & Johnson were able to anticipate some aspects of the effects (particularly lethal effects) of copper in combination with diesel oil in the mesocosms. When applied to mussels and other sessile suspension-feeding bivalve molluscs, scope for growth determinations can be very effective in documenting the effects of pollution.

However, as Bakke (1988) reports, a similar approach applied to *Littorina littorea* was unsuccessful in detecting a consistent trend, either in the field or in the mesocosm. This was due, at least in part, to problems associated with measuring the scope for growth in a mobile, discontinuous feeder such as *L. littorea*. In order for the individual physiological measurements, which make up the energy balance equation, to sum to an accurate estimate of the true scope for growth, they must represent a good integration of the component physiological traits over an appropriate time scale (e.g. one entire feeding cycle). Scope for growth is a powerful index of biological effects of pollution, but further research is necessary before it can be extended, with conviction, beyond its present application using suspension-feeding molluscs such as mussels.

Community responses

Techniques for describing the structural attributes of benthic communities are highly developed, and some are in common use to describe the effects of disturbance. There is a good background knowledge, for north temperate soft-sediment communities, of which species are potentially useful as indicators of disturbance, and estimates of community structure are often the end-products of biological monitoring because communities are perceived as integrating the effects of pollutants over long periods of time. Changes in community structure are also seen as representing features of ecological 'relevance', to a degree that is much less obvious, for example, with measures of biochemical effects. The GEEP Workshop aimed to evaluate a wide range of measures of community structure, together with appropriate statistical tests of the significance of observed differences, and to do so not only on the macrofauna but also the meiofauna and microbes.

Our results suggested that commonly used diversity indices were rather poor at distinguishing between the field sites in Langesundfjord/Frierfjord when compared with multivariate statistical techniques. Multivariate techniques can be very powerful in summarising between-site differences, and there now exist appropriate objective significance tests. Different ordination methods tested (e.g. multidimensional scaling, MDS; principal components analysis, PCA) demonstrated good agreement, and the choice between these is probably one of personal preference, provided that the data ordinate well in a low number of dimensions; PCA sometimes performed poorly in this respect. An important finding was that differences due to the application of different transformations to the raw data were often greater than differences between ordination methods. Different transformations essentially apply different weightings to the common and rare species, and the choice between them cannot be made solely on statistical grounds. However, further statistical research is needed to define an appropriate range of transformations from which the investigator can select.

Some of the species-independent curve-plotting methods applied to the macrofauna were found to be useful, but generally lack a suitable statistical framework for testing site differences; this is another area requiring research. The meiofauna have an advantage over the macrofauna of faster response times to pollution incidents. In the mesocosm experiment the copepod component of the meiofauna was the most sensitive of all groups to different contaminant dosing levels. However, few techniques are currently available for evaluating the effects of disturbance on meiofauna or micro-organisms, and here again more research is needed to bring our knowledge of meiofaunal community responses to a level comparable with that of the macrofauna.

A major conclusion which emerges from the workshop is the robustness of many of the multivariate and univariate techniques of data analysis to the procedural aggregation of species into higher taxonomic units. This finding could lead not only to less labour-intensive (and therefore more cost-effective) approaches to monitoring the effects of pollutants on the macrofaunal components of communities, but it also opens up wider possibilities for similar analyses on meiofaunal and microbial community components which have hitherto been under-investigated because of taxonomic difficulties. This important finding also suggests that taxonomic uncertainties over some tropical and sub-tropical benthic communities may not present the hurdle that is sometimes supposed to the application of community techniques to measure pollution impact in these areas.

LINKAGES AND COHERENCE BETWEEN EFFECTS

The results of the GEEP Workshop have shown that various techniques are now available which measure different aspects of the biological effects of pollution and which can be collected together as a suite of procedures for use in programmes of impact assessment. However, these results also demonstrate the empirical basis for any such collection of effects measures; there are few causative links that can be identified between successful effects measures, particularly between those at different levels of biological organisation (biochemical, physiological etc.). The application of such measurements to the quantitative evaluation of the biological impact of pollution would gain in reliability, conviction and predictability, if a fundamental coherence could be demonstrated between the various components of effect.

There were suggestions from workshop discussions of areas where such linkages may be possible. For example, elevated free calcium within the digestive cells of mussels (Viarengo et al. 1988) is indicative of enhanced oxidative processes and may be causally related both to increased mono-oxygenase activity and to increases in cellular peroxidation, as indexed by high lipofuscin levels within lysosomes (Moore 1988). As suggested earlier, disruption of lysosomal function can be related to observed cell and tissue pathology within the mussel digestive gland. Good correlational evidence already exists to link lysosomal hydrolase latency with physiological scope for growth (Bayne et al. 1982) and more recent investigations suggest that this link may be caused by biochemical and cellular events which enhance lysosomal catabolism of intracellular proteins (Moore & Viarengo 1987) hence increasing the demands for protein synthesis and so, in turn, enhance the metabolic requirements for maintenance.

Coherence at another level already exists between particular aromatic hydrocarbons and PCB isomers, and the induction of EROD activity and of specific forms of cytochrome P-450, as confirmed at the workshop (Stegeman et al. 1988); further research is necessary to link these processes to cytological and other evidence of pathological damage to fish tissues (Malins et al. 1985). Current understanding of the processes which regulate metal-binding and sequestration within cells, and the interactions between individual trace metals (zinc, cadmium, copper) and the metallothioneins provide an equivalent coherence between pollutant and biochemical response (Viarengo et al. 1988) and also suggest causal links with the phenomena of fatty degeneration and lipidosis and possible interactions between metal and hydrocarbon impacts as affecting lysosomal function.

A major problem in any attempt to equate biochemical responses to effects on individuals and thence on communities lies in the difficulty of relating changes in the reproductive behaviour and effectiveness of the individual organism to population-level responses of recruitment, growth and mortality, a problem discussed by Underwood & Petersen (1988). Of course, some parameters of population dynamics are the direct result of individual growth rates, summed across all individuals in the population, and are therefore amenable to estimation from physiological determinations of the scope for growth. Also, much effort within community ecology is being focussed on abundance/biomass changes of sets of species, in order to reduce the redundancy present in comprehensive species-abundance data sets. There is therefore a convergence of interest towards population processes, from both the whole organism and the community level approaches. Nevertheless, to measure these population-level processes as affected by pollution requires more time than was available at the workshop; there is a clear need here for further research.

In the design of the Workshop it was hoped that sites could be chosen within the fjords that would allow direct comparison of effects measurements on individuals with those on communities – not to be able to link these causally, but to explore possible differences in sensitivity to pollutants. Even within a hydrographic system of small tides and weak tidal currents, however, stratification of the water column and differences in the time scales upon which pollutants act within the different levels of biological organisation render such comparisons difficult. A further difficulty which confounds this problem concerns the choice of species on which biochemical and physiological measurements are made relative to those species which are perceived to be important to the community (see Underwood & Petersen 1988). The rationale for choosing certain species for toxicological assessment does not normally include their potential role in helping to structure their communities (though mussels are selected, in part, for their importance in littoral and sub-littoral hard sediment communities) and more research is needed to focus on such 'key' species if a correlational link between individual and community level measurements is deemed important.

SOME LESSONS LEARNT

The GEEP Workshop was a success in many ways, not least because it brought together a group of scientists who were skilled in various research areas but who seldom had the opportunity of working closely together with scientists experienced in other sub-disciplines of ecotoxicology. The result was not only to widen appreciation of each other's problems, but also to focus attention on the inter-relatedness of the various procedures and approaches being evaluated and so to help towards the coherence that is so important. For these reasons, as well as the more immediate returns of intercalibration and comparison of specific techniques, all participants agreed on the need for further workshops of this type. Such workshops will need to be held in different climatic and environmental regimes, in order to explore the prospects for general application of biological effects measurements. The basic design of the GEEP Workshop, viz. a spatial gradient of contamination, together with experimental exposures in a mesocosm facility, could usefully be adopted in future workshops, but some specific lessons were learnt in Oslo that should also be taken into consideration. Most of these are discussed by Clarke & Green (1988); some key considerations are mentioned below.

It is essential to erect prior hypotheses about the behaviour of particular effects measurements along the anticipated contaminant gradient, which are then testable by 'blind' analysis. The more subjective the technique (e.g. visual interpretation of some cytochemical and pathological tests; presence of indicator species in community samples) the more important is this blind analysis. In an ideal case, a prior hypothesis would be erected and calculations performed on the power of the appropriate statistical test, in order to identify the required sample size; pilot samples are very desirable here.

The GEEP Workshop made plain the importance of site selection in the field survey. In order to detect a spatial gradient of effect, control ('reference') sites are needed that match the physical features (substrate type, water depth, salinity range, degree of exposure) with those of the putative impacted sites. The so-called 'nuisance' biological variables (e.g. animal size/age, reproductive condition) should also be closely matched in order to reduce the variance in the results. At the workshop we failed to control water depth adequately at the benthic community sites and so partially confounded contaminant with depth gradients. Again, a pilot survey can be an important step in final site selection.

Adequate replication is, of course, essential, but 2 aspects are worth emphasising. Firstly, the problem of 'pseudo-replication' (Hurlbert 1984) must be avoided by ensuring that replicate samples are taken on a spatial scale appropriate to the aims of the study. Secondly, the balance of replication effort should be at the highest level relevant to the particular technique. For example, it is better to replicate over more animals (or pools of animals) rather than to indulge in too many replicate determinations on a common pool. For benthic community studies the preferred strategy is to do less detailed analyses on more cores, with subsampling from these cores for smaller organisms if necessary. An important result of the workshop, discussed briefly above, was to determine how much redundancy there might be in full species counts (Gray et al. 1988, Heip et al. 1988, Warwick 1988).

An essential feature of any study of the biological effects of pollutants is a close integration between chemical and biological analyses; the GEEP Workshop benefitted enormously from the careful and thorough chemical analysis of sediments and faunal samples, as it did also from the continuous availability of expert statistical advice and analysis. In designing the sampling strategy there should be the closest possible linking between biological and chemical measurements, since this expands the range of possible correlative techniques available to discriminate pollutant-induced effects from uncontrolled physical and biological differences, unrelated to contaminant load. Therefore, chemical analyses should be conducted on the same animals as used in the biological tests or, failing that, on a random subset of the pool of animals used. By the same token, each replicate core taken for sediment chemistry should be adjacent to (or preferably a subsample of) each replicate taken for faunal analysis.

Mesocosm experiments have an important role to play in the current stage of development of biological effects techniques but their limitations, already well recognised, need to be borne in mind. For example, the number of basins available at the Solbergstrand (and most other) mesocosm(s) rendered it impossible to replicate basin treatments, making it incumbent on us to hold constant across basins the potentially confounding 'nuisance' variables such as flow rates, particle concentrations, etc.

The mesocosm experiment was clearly well-suited to studies with mussels, and also to certain meiofaunal groups, such as copepods, which have short generation times and direct benthic recruitment. However, we were less successful in arranging adequate exposure of flounders and crabs to the pollutants since these animals could not be offered food contaminated at levels appropriate to the particular exposure conditions. In future experiments of this type, careful attention will be needed to the presumed routes of uptake of contaminants both into the fauna and into any sediments that may be employed for community impact assessments.

FINAL COMMENTS

Interdependence of biological and chemical measurements

Pollution effects are defined as interactions between chemicals and the biota. Analytical chemistry and the biological determination of toxic effects represent 2 approaches to the same problem; it is not possible to interpret biological changes without knowing the level of associated chemical contamination, just as it is impossible to assess the significance of observed concentrations of chemicals in the absence of information on their biological impact. It follows that any serious study of the biological effects of pollution must have a matching programme of chemical analysis.

This inter-relatedness operates at a number of levels, only some of which were considered at the GEEP Workshop. For example, a convincing interpretation of the lack of any induction of the flounder P-450 monooxygenase system in the mesocosm experiment relies, firstly, on the knowledge that there were no differences in the levels of organic contaminants in the fish livers from different treatments (Addison & Edwards 1988) and, secondly, on the suggestion that the specific hydrocarbons to which these fish were exposed did not include those most likely to induce components of the xenobiotic detoxication system (Stegeman et al. 1988). Similar arguments apply to interpretations of the data on metallothionein concentrations, in the light of observed concentrations of metals within the fish tissues. Current research on the molecular conformations that couple specific isomers of polychlorinated biphenyls to the components of the mixed function oxygenase system represents a further stage in our understanding of these chemical/biological interactions.

Another example (Widdows & Johnson 1988) concerns the interaction between observed toxicity and the quantitative structure/activity relations (QSARs) of the contaminants involved. By careful analysis of this interaction it may be possible to detect the influence of additional, possibly unsuspected, pollutants in certain field situations. Indeed, knowledge of how the fundamental chemical properties of potentially polluting compounds affect the processes of bioaccumulation and toxic damage represents a rich field for investigation – one of the few with any possibility of generalising from the large array of organic xenobiotics to predictions of biological effect.

An important aspect of chemical/biological interaction, though one not addressed at the workshop, concerns the partitioning (between dissolved and particulate forms) and speciation of contaminants, and how these factors affect biological accumulation and response. These questions have been considered elsewhere (e.g. Readman et al. 1984) but we draw attention here to one aspect with a more specific biological emphasis, namely the partitioning of contaminants within the organism, and its implications for effecting a biological response. For example, uptake of hydrocarbons into so-called 'storage' lipids, e.g. nutrient and gonadal reserves, may result in long residence times at low toxicity to the animal, release to the sites of toxic action being conditional on the organism actively mobilising its reserves in the normal course of its reproductive behaviour, or at times of nutrient limitation. On the other hand, hydrocarbons associated with more labile hydrophobic components, such as membrane lipids and cellular macromolecules, may be fluxed within the animal over shorter time scales and with more immediate toxic effects. Better insight into these processes will depend on knowledge of what factors control the chemical behaviour of such compounds within the complex cellular environment of the animal and will demand that chemists and biologists work very closely together.

On a wider scale, interactions between biological activity and chemical behaviour in the environment are important for any general understanding of the impact of pollutants. The role of microbial activity in altering the bioavailability of contaminants, the effects of bioturbation of sediments in the release and transport of pollutants, and the processes by which suspended particles, both as living phytoplankton and as detritus, scavenge material from the water column, all affect the distribution of contaminants and are likely to affect the processes of biological uptake and subsequent toxic damage. As adequate analytical techniques, both chemical and biological, are developed, so there is a growing need to use them to address these questions at the interface of environmental chemistry and associated biological response.

Some cautions

The pressures to adopt biological measurements within environmental monitoring programmes are great and, as a consequence, so are the temptations to expect (and to claim) more of the available biological techniques than is scientifically reasonable. Careful reading of the papers in this MEPS SPECIAL will identify the areas of uncertainty as well as those areas where we can be hopeful of successful application of research results. Perhaps the greatest danger is that a distancing may develop between appropriate research and its application, for two reasons.

Firstly, scientists doing research most relevant to biological effects measurements are primarily motivated by finding out how the various biological systems work, not by the possibility of discovering a new technique for biological monitoring. Nevertheless, when this research does result in a useful product there is a sense of achievement. It is important that the scientists are given the opportunity to explore the application of their findings in a research environment, where novel aspects of the different areas of study can emerge. The GEEP Workshop achieved this in its mix of participants and in its balance between investigation and inter-calibration. It is important to the health of the subject that this balance is maintained.

The second factor that might tend to distance research from its application is the demand that techniques must in some way be fixed, standardised to the point of inflexibility, before they may be used in earnest in biological effects assessment. Here again there is a balance to be struck. To seek finality in this way is to forego the benefits that can accrue from continued development and also to ignore the inherent variability in biological systems that will always demand a flexible approach. On the other hand some standardisation of procedure is necessary if results are to be comparable between different studies. The convenors of the workshop are aware of this need for balance and hope, through future practical workshops, to help develop the most relevant procedures in a way that preserves a flexibility of application which will foster their widest possible use.

The way forward

The GEEP Workshop was held in the context of discussions and review, taking place over many years, of the application of biological procedures to pollution assessment (see the introductory paper, Bayne et al. 1988). In looking forward, it is of interest to consider how the subject has developed, for example by comparing contemporary approaches with some of the recommendations made at the important workshop held in Beaufort, North Carolina, USA in 1978 (McIntyre & Pearce 1980). The result is to recognise the influence of these earlier recommendations, although the subject is now in many areas more focussed.

The biochemical procedures proving to have most potential for biological effects measurements are those concerned with specific molecular mechanisms of detoxication, rather than those involving general enzymatic or other changes occurring with pollution. The latter lack specificity and tend to be more empirically based; the former can provide specificity whilst also indicating likely higher-order effects on cells and tissues. Cellular approaches have benefitted from the introduction of quantitative techniques, made possible by the application of microdensitometry, image analysis and appropriate statistical software. The most powerful cytological procedures are those that draw on biochemical understanding of toxicity and detoxication processes and link these to subcellular effects which may, in turn, affect cellular and tissue condition.

Physiological approaches continue to focus on aspects of energy balance and growth, though these approaches are still applied over a rather narrow taxonomic range. The measurements of individual physiological traits, such as respiration or excretion, are confirmed in most cases as not yielding useful information. A further trend in physiological studies, though not well represented in this workshop, is towards the recording of individual variability, particularly of aspects relevant to the overall fitness of the individual organism, and the linking of this information to measures of genetic variability within populations; this is an approach foreseen at Beaufort but one that needs further research. The problems associated with linking individual physiological performance to population processes have already been discussed. In line with the recommendations of 10 yr ago, community studies continue to emphasise structural attributes and considerable advances have been made in appropriate statistical procedures.

Necessarily, some of the approaches recommended at Beaufort and elsewhere could not be assessed at the GEEP Workshop. In looking forwards, the IOC Group of Experts on the Effects of Pollutants recognise the following points of emphasis.

(1) A wider practical evaluation of possible biological effects techniques is needed, to embrace climatic and environmental regimes not yet studied in detail and to include a greater taxonomic coverage for biochemical, cytological and physiological approaches. This will require refinement of procedures so that they can be reliably replicated and transferred to other species.

(2) This widening of approach will require that procedures seen to have potential for monitoring will need to be simplified in some cases, to make them practicable in circumstances where the provision of equipment might be less than in the most sophisticated laboratories.

(3) The development of current techniques for wider regional application will be aided by involving scientists from tropical and sub-tropical areas, with experience of local faunas and facilities.

(4) The maintenance of a community of effort amongst the scientists involved in research into the biological effects of pollutants will encourage the continuing development and application of appropriate techniques and facilitate the training necessary to widen the experience and expertise of others.

In this way it is hoped that we can build on the experience of the GEEP Workshop and gradually enhance the quality of biological measurements undertaken in programmes of environmental pollution assessment.

LITERATURE CITED

Addison, R. F., Edwards, A. J. (1988). Hepatic microsomal mono-oxygenase activity in flounder *Platichthys flesus* from polluted sites in Langesundfjord and from mesocosms experimentally dosed with diesel oil and copper. Mar. Ecol. Prog. Ser 46: 51-54

Bakke, T (1988). Physiological energetics of *Littorina littorea* under combined pollutant stress in field and mesocosm studies. Mar Ecol. Prog. Ser 46: 123-128

Bayne, B. L., Clarke, K. R., Gray, J. S. (1988). Background and rationale to a practical workshop on biological effects of pollutants. Mar Ecol. Prog. Ser. 46: 1-5

Bayne, B. L., Holland, D. L., Moore, M. N., Lowe, D. M., Widdows, J. (1978). Further studies on the effects of stress in the adult on the eggs of *Mytilus edulis*. J. mar. biol. Ass. U.K. 58: 825-841

Bayne, B. L., Newell, R. C. (1983). Physiological energetics of marine molluscs. In: Wilbur, K. M., Salenddin, A. S. M. (eds.) The Mollusca, Vol. 4, Physiology, Part 1 Academic Press, London, p. 407-515

Bayne, B. L., Widdows, J., Moore, M. N., Salkeld, P., Worrall, C. M., Donkin, P. (1982). Some ecological consequences of the physiological and biochemical effects of petroleum compounds on marine molluscs. Phil. Trans. R. Soc. Lond. B 297: 219-239

Clarke, K. R., Green, R. H. (1988). Statistical design and analysis for a 'biological effects' study. Mar. Ecol. Prog. Ser. 46: 213-226

George, S. G., Viarengo, A. (1985). A model for heavy metal homeostasis and detoxication in mussels. In: Vernberg, F. J., Thurberg, F. P., Calabrese, A., Vernberg, W (eds.) Marine pollution and physiology: recent advances. Univ. S. Carolina Press, Columbia, p. 125-143

Gray, J. S., Aschan, M., Carr, M. R., Clarke, K. R., Green, R. H., Pearson, T H., Rosenberg, R., Warwick, R. M. (1988). Analysis of community attributes of the benthic macrofauna of Frierfjord/Langesundfjord and in a mesocosm experiment. Mar. Ecol. Prog. Ser 46: 151-165

Heip, C., Warwick, R. M., Carr, M. R., Herman, P. M. J., Huys, R., Smol, N., Van Holsbeke, K. (1988). Analysis of community attributes of the benthic meiofauna of Frierfjord/Langesundfjord. Mar. Ecol. Prog. Ser 46: 171-180

Hurlbert, S. H. (1984). Pseudoreplication and the design of ecological field experiments. Ecol. Monogr. 84: 187-211

Klungsøyr, J., Wilhelmsen, S., Westrheim, K., Saetvedt, E., Palmork, K. H. (1988). The GEEP Workshop: organic chemical analyses. Mar. Ecol. Prog. Ser. 46: 19-26

Livingstone, D. R. (1984). Biochemical differences in field populations of the common mussel *Mytilus edulis* L. exposed to hydrocarbons: some considerations of biochemical monitoring. In: Bolis, J. (ed.) Toxins, drugs and pollutants in marine animals. Springer-Verlag, Berlin, p. 161-175

Livingstone, D. R. (1988). Responses of microsomal NADPH-cytochrome c reductase activity and cytochrome P-450 in digestive glands of *Mytilus edulis* and *Littorina littorea* to environmental and experimental exposure to pollutants. Mar Ecol. Prog. Ser. 46: 37-43

Lowe, D. M. (1988). Alterations in cellular structure of *Mytilus edulis* resulting from exposure to environmental contaminants under field and experimental conditions. Mar. Ecol. Prog. Ser 46: 91-100

McIntyre, A. D., Pearce, J. B. (eds.) (1980). Biological effects of marine pollution and problems of monitoring. Rapp. P.-v. Reun. Cons. int. Explor Mer 179

Malins, D. C., Krahn, M. M., Myers, M. S., Rhodes, L. D., Brown, D. W., Krone, C. A., McCain, B. B., Chan, S.-C. (1985). Toxic chemicals in sediments and biota from creosote-polluted harbor: relationships with hepatic neoplasms and other hepatic lesions in English sole *(Parophrys vetulus)*. Carcinogenesis 6: 1463-1469

Moore, M. N., Viarengo, A. (1987). Lysosomal membrane fragility and catabolism of cytosolic proteins: evidence for a direct relationship. Experientia 43: 320-323

Moore, M. N., Livingstone, D. R., Widdows, J., Lowe, D. M., Pipe, R. K. (1987). Molecular, cellular and physiological effects of oil-derived hydrocarbons on molluscs and their use in impact assessment. Phil. Trans. R. Soc. Lond. B 316: 603-623

Moore, M. N. (1988). Cytochemical responses of the lysosomal system and NADPH-ferrihemoprotein reductase in molluscan digestive cells to environmental and experimental exposure to xenobiotics. Mar Ecol. Prog. Ser. 46: 81-89

Readman, J. W., Mantoura, R. F. C., Rhead, M. M. (1984). The physico-chemical speciation of polycyclic aromatic hydrocarbons (PAH) in aquatic systems. Fres. Z. Anal. Chem. 319: 126-131

Stegeman, J. J., Kloepper-Sams, P. J., Farrington, J. W. (1986). Mono-oxygenase induction and chlorobiphenyls in the deep-sea fish *Coryphaenoides armatus*. Science 231. 1287-1289

Stegeman, J. J., Woodin, B. R., Goksoyr, A. (1988). Apparent cytochrome P-450 induction as an indication of exposure to environmental chemicals in the flounder *Platichthys flesus*. Mar. Ecol. Prog. Ser 46: 55-60

Underwood, A. J., Peterson, C. H. (1988). Towards an ecological framework for investigating pollution. Mar. Ecol. Prog. Ser 46: 227-234

Viarengo, A., Mancinelli, G., Martino, G., Pertica, M., Canesi, L., Mazzucotelli, A. (1988). Integrated cellular stress indices in trace metal contamination: critical evaluation in a field study. Mar. Ecol. Prog. Ser. 46: 65-70

Warwick, R. M. (1988). Analysis of community attributes of the macrobenthos of Frierfjord/Langesundfjord at taxonomic levels higher than species. Mar. Ecol. Prog. Ser 46: 167-170

Widdows, J. (1985). Physiological responses to pollution. Mar. Pollut. Bull. 16: 129-134

Widdows, J., Johnson, D. (1988). Physiological energetics of *Mytilus edulis*: Scope for Growth. Mar. Ecol. Prog. Ser. 46: 113-121