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Background and rationale to a practical workshop on biological effects of pollutants

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ABSTRACT: The Practical Workshop on Biological Effects of Pollutants (GEEP Workshop) was held in August 1986 at the University of Oslo, Norway, under the auspices of the Intergovernmental Oceanographic Commission's Group of Experts on the Effects of Pollutants (GEEP). The workshop was attended by 35 participants from 11 countries. This MEPS SPECIAL contains the major results and conclusions, and this first paper introduces and explains the background and rationale to the workshop.

BACKGROUND

The Group of Experts on the Effects of Pollutants (GEEP) was set up by the Intergovernmental Oceanographic Commission (IOC) as a component of its programme Global Investigation of Pollution in the Marine Environment (GIPME). At its first meeting, in December 1984, this group established that there was an urgent need to convene a practical workshop which would evaluate some of the diverse means presently proposed for measuring the effects of chemical contaminants on marine organisms. The need for progress on the development of such 'biological effects' measurements has been recognised for many years, and was inherent in the Comprehensive Plan for GIPME (IOC Technical Series No. 25, 1984). This document – and others before and since – recognised that in order to proceed from an appreciation of *'contamination'*, which is a physico-chemical phenomenon, to an assessment of *'pollution'*, with its biological emphasis, it was essential to be able to measure the impact of chemical contaminants on the biota in terms of meaningful biological responses.

The biological effects of pollutants on marine organisms have been the subject of considerable debate, the essence of which is captured in 4 publications – ICES (1978), GESAMP (1980), papers in McIntyre & Pearce (1980) and in Sheehan et al. (1984). Discussions at the first meeting of GEEP recognised that such debates had served an essential purpose in summarising the available literature, but that further progress depended on appropriate scientists evaluating various procedures together in a practical manner, based on the analysis of common material sampled from the same contamination gradient. The following considerations were judged to be particularly important.

(1) Methods to be evaluated at a practical workshop should cover the full spectrum of effort, from molecular approaches ('biochemical level'), through cellular and physiological procedures ('cell' and 'whole organism levels') to measures appropriate to the structure of communities of benthic organisms ('community level').

(2) Participants should be research scientists currently working on these topics who had expressed interest in relating their results to problems associated with the measurement of pollution impact. The necessity eventually to transfer expertise to others, who were not directly researching these problems, but were concerned with monitoring biological impact, was recognised but considered more appropriate to later workshops.

(3) Material should be taken from a known pollution gradient (complemented by experimental exposures) according to a strict sampling and analytical protocol and analysed, as far as possible, during the workshop itself. Wide-ranging discussion amongst the participants of the strengths and weaknesses of the various techniques evaluated would be encouraged.

(4) The workshop would be the subject of a rigorous statistical design and, importantly, all material analysed during the workshop would be handled 'blind', i.e. without the participants knowing its origin or its

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presumed ranking relative to the contamination gradient.

(5) The biological components of the workshop should be complemented by thorough chemical analysis of the material in order to address the relation between levels of contamination and biological response.

In summary, GEEP observed that '. . . there is now an urgent requirement for a convincing practical evaluation of the relevance of a variety of biological procedures to pollution assessment, in order to progress towards an agreed set of standard techniques for incorporation into national and international programmes of pollution monitoring'; and the group recommended that a workshop be organised '. . . to compare and evaluate in quantitative, practical terms, techniques currently available or proposed for measuring the biological effects of pollutants at levels from the cell to the community'.

TECHNIQUES TO BE EVALUATED

As a result of discussions over the past decade, a wide variety of procedures for measuring the biological effects of pollution have been proposed (McIntyre & Pearce 1980). In a report published in 1980 (GESAMP 1980) criteria were suggested to guide the selection of appropriate 'effects measurements' for use in assessing the environmental impact of pollutants. It was recognised that some of the relevant biological variables might offer a relatively high specificity to particular contaminants, whereas others may lack specificity but be more directly relevant to the fate of populations or communities of organisms; measures will differ also in their sensitivity to contaminants. In any programme designed to measure biological impact, it would be advantageous to include a suite of effects measurements to cover these, and other, differences in attributes.

In deciding what measurements to include in the GEEP Workshop, the criteria suggested by GESAMP (1980) were considered first, followed by more practical concerns, e.g. requirements for sample size, seasonal constraints and requirements for laboratory equipment. It was considered important that the great majority of the work needed to measure each biological response could be completed during the workshop itself, with minimal work left to be carried out after the workshop was completed. It was apparent at an early stage that samples for the assessment of benthic community structure would have to be taken, and subjected to faunal analysis, before the workshop; effort during the workshop would then be directed to the application of statistical and other techniques to the resulting data sets.

With these considerations in mind, the following topics were accepted for inclusion.

Biochemistry. Measurements at the sites of toxic action, of processes showing specificity for particular contaminants, viz. the cytochrome P450-mediated system of mixed function oxygenation of organic compounds, and the metal-binding proteins.

Cellular- and histopathology. Measurements of the function of cellular organelles involved in sequestration and metabolism of toxicants. Quantitative and descriptive techniques to assess the extent of pathological change to cells, tissues and organ systems.

Physiology. Measurements of the responses of isolated tissue and of whole organisms, viz. respiration, feeding and excretion rates, and the processes of energy balance and growth.

Community studies. Measurements pertinent to properties of community structure.

There are significant omissions from this list. Any structured suite of effects measurements should give due consideration to population variables, but appropriate measures (such as recruitment and mortality) require time scales that were not possible to include in a 3 wk workshop. Some measures of the genetic consequences of pollution show promise as indices of biological response, but the timing of the GEEP Workshop vis-a-vis seasonal reproductive cycles in the local fauna was thought to preclude the availability of suitable material for cytogenetic analysis. Measures of community function (metabolism, nutrient flux) were also technically difficult in the context of this workshop. Finally, when the preferred site of the workshop had been decided (see below) it became clear that adequate sample sizes of fish for the purposes of fish pathology would be impossible to achieve; measures of pathology were therefore confined to invertebrate material.

Having decided upon the response measures to be included in the workshop, scientists who were actively engaged in relevant research, and who had expressed an interest in applying the results of this research to problems of pollution impact, were identified and invited to participate. A list of participants is given in Appendix 4.

SITE FOR THE WORKSHOP

Four criteria were identified in selecting the site for the workshop.

(1) There should be a well-defined contamination gradient, made up of multiple contaminant inputs (metals and organic contaminants).

(2) There should be a good base of historical information on the physico-chemical properties of the site, including data on contaminant loads.

(3) The species commonly employed in research into biological responses to pollutants should be available, since practical evaluation of these responses would be effected most efficiently in the first instance on species familiar to the participants; the benthic faunal communities should also be familiar, avoiding major taxonomic difficulty.

(4) Adequate laboratory and support facilities (including a research ship) should be available at minimal cost. It was considered important that a large-scale experimental facility ('mesocosm') also be available, since exposures to known levels of contaminants under controlled conditions were to be an important complement to the field study.

Various sites were considered. The University of Oslo in Norway offered to host the workshop, and this offer was gratefully accepted. The most pertinent features of the sampling sites in Frierfjord and Langesundfjord are described by Follum & Moe (1988), who also quote from the data available on the contaminants in water, sediments and biota within these fjords; a contamination gradient from the top of Frierfjord to Langesund Bay was apparent. These data were supplemented by chemical analyses carried out as part of the workshop (Klungsøyr et al. 1988, Abdullah & Steffenak 1988), which confirmed the existence of a contamination gradient and provided an essential framework for an assessment of the biological results.

Early in the planning of the GEEP Workshop it was decided to carry out experimental exposures to contaminants, under controlled conditions, as a supplement to the field samples. The experimental mesocosm facility at Solbergstrand on Oslofjord was used for this purpose. Procedures employed at Solbergstrand in the 4 available mesocosm basins are described in detail by Bakke et al. (1988). Briefly, the seawater inputs to these flow-through basins were dosed with 3 concentrations of a mixture of diesel oil and copper, with the fourth basin acting as a control. The basins were stocked with a similar range of biological material to that sampled for the field sites, viz. mussels *Mytilus edulis*, crabs *Carcinus maenas*, periwinkles *Littorina littorea* and flounders *Platichthys flesus*, together with undisturbed box cores of soft sediment for the benthic infaunal studies. Organisms were exposed to the contaminant dosing for up to 4 mo.

STATISTICAL PROCEDURES

A later section discusses the criteria for statistical design and analysis underlying the GEEP Workshop (Clarke & Green 1988); several points require emphasis here.

(1) Great stress was laid on obtaining comparable material for all techniques, with standardised protocols for sampling from each site and for exposure of organisms in the experimental basins. Sampling from each field site took place on only one occasion. Mussels, crabs, periwinkles and flounder from a particular site were all collected within a few hours of each other, and subsets of the common pool of organisms for each species were randomly allocated to the various biological and chemical requirements. Randomisation was also considered to be an important principle in allocating the fauna and soft-sediment cores to the mesocosm basins, and in later sampling of them.

(2) For some of the effects measures, desired levels of replication were determined (a priori) from calculations on the 'power' to detect anticipated changes in response, along the field gradient. Response precision was increased by standardising (where possible) on the same narrow size-ranges of organisms over all field and experimental samples. By contrast, field sites were suitably widely defined, with proper spatial representation in collection.

(3) A major rationale for the controlled experimental exposures was to demonstrate causal links between pollutant and effect, complementing the correlative information from the field study. Another motivation was to compare the sub-lethal responses to those of the benthic community, under a common dosing regime; the hydrography of the fjordic environment precluded any such juxtaposition for the field survey. In addition, the highest exposure level in the mesocosm experiment was chosen to extend the contaminant gradient beyond that anticipated for the field study, with the intention of defining dose-response relations more clearly.

(4) The coding of site and basin designations, ensuring that participants undertook 'blind' analyses, was seen as important in facilitating objective testing of hypotheses (specified a priori) concerning direction and magnitude of change in specific biological responses.

(5) Statistical analysis was undertaken centrally, for the full range of response data obtained during the GEEP Workshop, ensuring consistency of approach to hypothesis testing and description. The data on community structure were analysed in common by all participants working on benthos; the opportunity was taken to compare the performance of a wide range of univariate and multivariate statistical techniques on the same data sets.

WORKSHOP TIMETABLE

We give here a sketch of the GEEP Workshop timetable; further timing details of the field programme can

be found in Follum & Moe (1988) and details of the experimental programme in Bakke et al. (1988).

Practical effort began in January 1986, with a cruise on the research ship F/F 'Trygve Braarud' (University of Oslo) to the Frierfjord/Langesundfjord in order to sample the benthic community macrofauna. Between January and August 1986 the macrofauna were identified and their abundance and biomass recorded (Gray et al. 1988), in order for the data to be available for statistical analysis during the workshop. Meiofauna and bacteria were sampled from the same sites in April. Meiofauna were identified and counted by Heip et al. (1988); bacterial analyses were carried out according to Schwinghamer (1988). Also in April 1986, the mesocosm facility at Solbergstrand was set up as described by Bakke et al. (1988). In mid-July, after an 11 wk dosing period, benthic community samples were taken from the experimental basins, and analysed in preparation for the GEEP Workshop by R. Warwick and M. Gee (meiofauna, see Warwick et al. 1988) and M. Aschan (macrofauna, see Gray et al. 1988). Other species were not sampled from the mesocosm basins until the second week of the workshop. Tables of all benthic data analysed for the workshop are given in Appendix 3.

Material for chemical analysis was collected on a number of occasions during the course of the mesocosm experiment and, for field studies, collected concurrently with the biological material. Analysis for organic and trace metal contaminants took place as follows.

(1) GC/MS analysis of selected aromatic hydrocarbons and polychlorinated biphenyls from samples of water, sediments, mussels and crabs (whole tissues) from the mesocosm experiment, and mussels and crabs from Langesundfjord (Klungsøyr et al. 1988). Also, analysis of aromatic hydrocarbons in sediment samples from the benthic sampling sites in Frierfjord/Langesundfjord, and of selected chlorinated hydrocarbons from samples of fish (flounder) livers at the Langesundfjord sites (Addison & Edwards 1988). Fluorescence hydrocarbon measurements were made on water samples from the mesocosm basins (Bakke et al. 1988) and on digestive gland tissues of periwinkles from field and mesocosm studies (Livingstone 1988).

(2) Analysis of selected metals in sediments, whole tissues of mussels and crabs, and flounder livers (Abdullah & Steffenak 1988), for both mesocosm and field samples (mesocosm only, for crabs); also for mussel digestive glands in field and mesocosm studies (Viarengo et al. 1988).

Tables of these chemical data, and plots of the contaminant gradients for both field and mesocosm samples, are presented in Appendix 1

Between April and the start of the workshop in August 1986, and as a result of detailed correspondence between participants, all major consumable items were ordered and made available in Oslo. Requirements for large apparatus were identified and availability within the University of Oslo confirmed.

The workshop was held from 11 to 30 August, 1986. During the first week, participants studying sub-lethal responses of organisms, at the biochemical, cellular and physiological levels, analysed material from field sites. Samples from the mesocosm experiment were analysed during the second week (plots of mean responses, over all field sites and mesocosm exposures, are gathered together in Appendix 2). During the first 2 weeks the results of the macro- and meio-faunal species identifications were compiled on the computer and analysed by participants studying benthic community changes. The third and final week was spent completing the practical work, carrying out statistical analyses of the results and agreeing preliminary interpretations. Only when the results of the biological analyses were completed, in the final week, were the sample codings and the results of the chemical analyses made known to participants, so allowing interpretation of the data in the context of contaminant levels in the environment and in the biota.

CONCLUDING REMARKS

The GEEP Workshop would not have been possible without the support, financial and otherwise, of a large number of people and institutions. The Intergovernmental Oceanographic Commission approved the concept of the workshop and provided financial support; we are particularly grateful to Dr Gunnar Kullenberg, Head of IOC Marine Pollution Research and Monitoring Unit. Direct financial support was also provided by the United States National Oceanic and Atmospheric Administration, the Norwegian State Pollution Control Board, the Norwegian Department of Applied Research, and the United Kingdom Water Research Centre.

The University of Oslo hosted the workshop and made freely available, not only the required laboratory space and facilities, but also the use of their research ship 'Trygve Braarud' and access to their computers. We were welcomed into the laboratories of Marine Biology, Marine Chemistry and Biochemistry, and we are grateful to all the staff. We would like to acknowledge the efforts of Odd-Arne Follum, Kjell Moe and Mikaela Aschan. The Norwegian Institute for Water Research kindly made available laboratory space at the mesocosm facility in Solbergstrand. The Institute for Marine Environmental Research (now the Plymouth Marine Laboratory) also offered considerable support behind the scenes, and we are particularly grateful to

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