Intergovernmental Oceanographic Commission

Workshop Report No. 53

IOC Workshop on the Biological Effects of Pollutants

Oslo, Norway, 11-29 August 1986

Unesco

IOC Workshop Reports

The Scientific Workshops of the Intergovernmental Oceanographic Commission are usually jointly sponsored with other intergovernmental or non-governmental bodies. In each case, by mutual agreement, one of the sponsoring bodies assumes responsibility for publication of the final report. Copies may be requested from the publishing bodies as listed below.

No.	Title	Publishing Body	Languages
1	CCOP-IOC, 1974, Metallogenesis, Hydrocarbons and Tectonic Patterns in Eastern Asia (Report of the IDOE Workshop on); Bangkok, Thailand 24-29 September 1973 UNDP (CCOP), 138 pp.	Office of the Project Manager UNDP/CCOP c/o ESCAP Sala Santitham Bangkok 2, Thailand	English
2	CICAR Ichthyoplankton Workshop, Mexico City, 16-27 July 1974 (Unesco Technical Paper in Marine Sciences, No. 20).	Division of Marine Sciences, Unesco Place de Fontenoy 75700 Paris, France	English (out of stock) Spanish (out of stock)
3	Report of the IOC/GFCM/ICSEM International Workshop on Marine Pollution in the Mediterranean, Monte Carlo, 9-14 September 1974.	IOC, Unesco Place de Fontenoy 75700 Paris, France	English French Spanish (out of stock)
4	Report of the Workshop on the Phenomenon known as "El Niño". Guayaquil, Ecuador, 4-12 December 1974.	FAO Via delle Terme di Caracalla 00100 Rome, Italy	English (out of stock) Spanish (out of stock)
5	IDOE International Workshop on Marine Geology and Geophysics of the Caribbean Region and its Resources, Kingston, Jamaica, 17-22 February 1975.	IOC, Unesco Place de Fontenoy 75700 Paris, France	English (out of stock) Spanish
6	Report of the CCOP/SOPAC-IOC IDOE International Workshop on Geology, Mineral Resources and Geophysics of the South Pacific, Suva, Fiji, 1-6 September 1975.	IOC, Unesco Place de Fontenoy 75700 Paris, France	English
7	Report of the Scientific Workshop to Initiate Planning for a Co-operative Investigation in the North and Central Western Indian Ocean, organized within the IDOE under the sponsorship of IOC/FAO (IOFC)/Unesco/EAC, Nairobi, Kenya, 25 March-2 April 1976.	IOC, Unesco Place de Fontenoy 75700 Paris, France	English French Spanish Russian
8	Joint IOC/FAO (IPFC)/UNEP International Workshop on Marine Pollution in East Asian Waters, Penang, 7-13 April 1976.	IOC, Unesco Place de Fontenoy 75700 Paris, France	English (out of stock)
9	IOC/CMG/SCOR Second International Workshop on Marine Geoscience, Mauritius, 9-13 August 1976	IOC, Unesco Place de Fontenoy 75700 Paris, France	English French Spanish Russian
10	IOC/WMO Second Workshop on Marine Pollution (Petroleum) Monitoring, Monaco, 14-18 June 1976.	IOC, Unesco Place de Fontenoy 75700 Paris, France	English French Spanish (out of stock) Russian
11	Report of the IOC/FAO/UNEP International Workshop on Marine Pollution in the Caribbean and Adjacent Regions, Port of Spain Trinidad, 13-17 December 1976.	IOC, Unesco Place de Fontenoy 75700 Paris, France	English Spanish (out of stock)
11 Suppl.	Collected contributions of invited lecturers and authors to the IOC/FAO/UNEP International Workshop on Marine Pollution in the Caribbean and Adjacent Regions, Port of Spain, Trinidad, 13-17 December 1976.	IOC, Unesco Place de Fontenoy 75700 Paris, France	English Spanish
12	Report of the IOCARIBE Interdisciplinary Workshop on Scientific Programmes in Support of Fisheries Projects, Fort-de-France, Martinique 28 November-2 December 1977.	IOC, Unesco Place de Fontenoy 75700 Paris, France	English French Spanish
13	Report of the IOCARIBE Workshop on Environmental Geology of the Caribbean Coastal Area, Port of Spain, Trinidad, 16-18 January 1978.	IOC, Unesco Place de Fontenoy 75700 Paris, France	English Spanish
14	IOC/FAO/WHO/UNEP International Workshop on Marine Pollution in the Gulf of Guinea and Adjacent Areas, Abidjan, Ivory Coast, 2-9 May 1978.	IOC, Unesco Place de Fontenoy 75700 Paris, France	English French
15	CPPS/FAO/IOC/UNEP International Workshop on Marine Pollution in the South-East Pacific, Santiago de Chile, 6-10 November 1978.	IOC, Unesco Place de Fontenoy 75700 Paris, France	English (out of stock)
16	Workshop on the Western Pacific, Tokyo, 19-20 February 1979.	IOC, Unesco Place de Fontenoy 75700 Paris, France	English French Russian
17	Joint IOC/WMO Workshop on Oceanographic Products and the IGOSS Data Processing and Services System (IDPSS), Moscow, 9-11 April 1979	IOC, Unesco Place de Fontenoy 75700 Paris, France	English
17 Suppl.	Papers submitted to the Joint IOC/WMO Seminar on Oceanographic Products and the IGOSS Data Processing and Services System, Moscow, 2-6 April 1979.	IOC, Unesco Place de Fontenoy 75700 Paris, France	English
18	IOC/Unesco Workshop on Syllabus for Training Marine Technicians, Miami, 22-26 May 1978 (Unesco reports in marine sciences, No. 4)	Division of Marine Sciences, Unesco Place de Fontenoy 75700 Paris, France	English (out of stock) French Spanish (out of stock) Russian
19	IOC Workshop on Marine Science Syllabus for Secondary Schools, Llantwit Major, Wales, U.K., 5-9 June 1978 (Unesco reports in marine sciences, No. 5).	Division of Marine Sciences, Unesco Place de Fontenoy 75700 Paris, France	English French Spanish Russian Arabic
20	Second CCOP-IOC Workshop on IDOE Studies of East Asia Tectonics and Resources, Bandung, Indonesia, 17-21 October 1978.	IOC, Unesco Place de Fontenoy 75700 Paris, France	English
21	Second IDOE Symposium on Turbulence in the Ocean, Liège, Belgium, 7-18 May 1979.	IOC, Unesco Place de Fontenoy 75700 Paris, France	English French Spanish Russian
22	Third IOC/WMO Workshop on Marine Pollution Monitoring, New Delhi, 11-15 February 1980.	IOC, Unesco Place de Fontenoy 75700 Paris, France	English French Spanish Russian
23	WESTPAC Workshop on the Marine Geology and Geophysics of the North-West Pacific, Tokyo, 27-31 March 1980.	IOC, Unesco Place de Fontenoy 75700 Paris, France	English Russian
24	WESTPAC Workshop on Coastal Transport of Pollutants, Tokyo, 27-31 March 1980.	IOC, Unesco Place de Fontenoy 75700 Paris, France	English (out of stock)
25	Workshop on the Intercalibration of Sampling Procedures of the IOC/WMO UNEP Pilot Project on Monitoring Background Levels of Selected Pollutants in Open-Ocean Waters, Bermuda, 11-26 January 1980.	IOC, Unesco Place de Fontenoy 75700 Paris, France	English (superseded by IOC Technical Series No. 22)
26	IOC Workshop on Coastal Area Management in the Caribbean Region, Mexico City, 24 September-5 October 1979.	IOC, Unesco Place de Fontenoy 75700 Paris, France	English Spanish
27	CCOP/SOPAC-IOC Second International Workshop on Geology, Mineral Resources and Geophysics of the South Pacific, Nouméa, New Caledonia, 9-15 October 1980.	IOC, Unesco Place de Fontenoy 75700 Paris, France	English
28	FAO/IOC Workshop on the effects of environmental variation on the survival of larval pelagic fishes Lima, 20 April-5 May 1980.	IOC, Unesco Place de Fontenoy 75700 Paris, France	English
29	WESTPAC Workshop on Marine biological methodology Tokyo, 9-14 February 1981.	IOC, Unesco Place de Fontenoy 75700 Paris, France	English
30	International Workshop on Marine Pollution in the South-West Atlantic Montevideo, 10-14 November 1980.	IOC, Unesco Place de Fontenoy, 75700 Paris, France	English (out of stock) Spanish
31	Third International Workshop on Marine Geoscience Heidelberg, 19-24 July 1982	IOC, Unesco Place de Fontenoy 75700 Paris, France	English French Spanish
32	UNU/IOC/Unesco Workshop on International Co-operation in the Development of Marine Science and the Transfer of Technology in the context of the New Ocean Regime Paris, 27 September - 1 October 1982	IOC, Unesco Place de Fontenoy 75700 Paris, France	English French Spanish

CONT'D ON INSIDE OF BACK COVER

Intergovernmental Oceanographic Commission

Workshop Report No. 53

IOC Workshop on the Biological Effects of Pollutants

Oslo, Norway, 11-29 August 1986

SC.88/WS/41

Unesco

TABLE OF CONTENTS

ANNEXES

SUMMARY

The Biological Effects Workshop which is the subject of this report was held over 8-30 August 1986 at the University of Oslo, Norway, under the auspices of the IOC and organized through the Group of Experts on the Effects of Pollutants (GEEP); 32 scientists from 11 countries participated.

Participants were engaged in measuring biological responses in material gathered along a gradient of contamination in a nearby fjord, and in material experimentally exposed to a mixture of contaminants in a mesocosm facility, and then in comparing their findings with chemical determinations carried out simultaneously. In this way, the performance, sensitivity and relative strengths and weaknesses of the biological procedures could be assessed in relation to common environmental gradients. The techniques tested included biochemical, cytochemical, histological and physiological responses, and procedures to measure features of benthic community structure. Both field and experimental studies were subject to rigorous statistical design and analysis, including coding of samples so that biological analyses were all performed "blind".

The Proceedings of the Workshop are to be published in full as a special volume of the journal Marine Ecology Progress Series and as a book. The following report summarises the aims, content and main highlights of the Workshop.

Chemical gradients. Concentrations of selected aromatic hydrocarbons in mussel tissues increased in line with the anticipated field contaminant gradient, with a 7-fold concentration difference between the end-point sites. Similar, though less marked, gradients were found for PCBs and some trace metals, and in crab, flounder and periwinkle tissues. The gradient was less clear-cut for contamination of sediments, though some sites exhibited significant differences. In the mesocosm experiment, four concentrations (including control) of a diesel oil and copper mixture were dosed into the water of basins containing the same range of biological material as sampled in the field. After a 3-4 month exposure period, clear chemical gradients were seen in mussel and crab tissues but not in flounder livers or transplanted sediments.

Biochemical responses. Biochemical measurements concentrated on processes likely to respond specifically to certain types of contaminant; those relating to the "Phase 1" enzymes of the microsomal cytochrome P-450-mediated mono-oxygenase system in the liver of the flounder 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), which increased over 10-fold between the end-point sites, and also to total microsomal cytochrome P-

450. Both measurements correlated well with environmental levels of chlorobiphenyls and aromatic hydrocarbons. In addition, two different techniques for measuring EROD activity were in good agreement.

Components of the analogous metabolic system in mussel digestive gland also responded in a predictable manner in the mesocosm experiment. However, the same analyses from the field samples were more equivocal, reflecting relative ignorance of the role 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, in line with previous suggestions that the contaminants dosed are not those normally thought capable of inducing the P-450 system in fish.

Another successful biochemical measurement made on mussels from the mesocosm experiment was of the distribution of copper amongst the metal-binding thioneins and higher molecular weight proteins of the digestive gland. Mussels exposed to certain trace metals are known to increase the amounts of metal bound to thioneins, and toxic damage is expected to occur when normal cellular controls break down and/or when the metal begins to accumulate to high levels in association with the cytosolic proteins. There was evidence of both these processes occurring within the mussels in the mesocosm basins, correlating both with measured levels of copper and with evidence of cellular damage.

Cellular responses. A range of cytochemical and histological procedures was carried out on mussels and winkles, to evaluate correlations with contaminant levels and to explore linkage between sub-cellular, cellular and tissue effects. Measurements of lysosomal enlargement and membrane stability are known to be sensitive to contamination by hydrocarbons, and these proved successful at the Workshop in identifying mussels from the field reference site as less impacted than at other sites. Gross changes in lysosomal structure and function were reflected in evidence of pathological damage to appropriate cells and tissues.

However, many of these measurements depend on reproductive condition. During the spawning period there may be considerable disturbance to sub-cellular function within the digestive gland. These considerations were paramount for the mesocosm material, probably confounded by a degree of food limitation in the basins, and contributed to the failure of the cellular techniques to demonstrate clear relationships in this material.

Physiological responses. Physiological studies concentrated mainly on those processes appropriate to the energy budget of an organism, i.e. measurements of feeding, absorption, respiration and excretion, integrated as components of the energy balance equation. The resulting "scope for growth", determined for mussels from field and experimental studies, proved to be very effective in delineating both contaminant gradients. Furthermore, on the basis of growing understanding of relationships between aromatic hydrocarbons and energy balance in mussels, it was possible to anticipate some effects (including lethal effects) of copper in combination with diesel oil in the mesocosms.

Whilst scope for growth can be a powerful tool when used for sessile suspension-feeding bivalve molluscs, a similar approach applied to winkles was unsuccessful in detecting consistent trends, probably due to the difficulties of measuring scope for growth in a mobile, discontinuous feeder.

Community responses. The Workshop evaluated a wide range of commonly used and recently proposed methods for interpreting benthic community structure (together with statistical tests for site differences), not only for macrofauna but also for meiofauna and microbes. It was found that commonly used diversity indices were poor at distinguishing field sites when compared with multivariate statistical techniques. Different ordination methods applied to species abundance and biomass arrays generally demonstrated good agreement, differences between various data transformations (which determine the relative weighting given to rare and common species) often being greater than between ordination methods. Also found to be useful were species-independent curve-plotting methods applied to the macrofauna (e.g. species abundance distributions and Warwick's ABC method).

The community techniques clearly identified the "cleaner" nature of the field reference site; elsewhere, stressed conditions were inferred for macrofaunal communities at some sites though this appeared to reflect seasonal anoxia in the deeper water rather than the (marginal) contaminant gradient. The meiofauna have an advantage over the macrofauna of faster response times to pollution incidents and, in the mesocosm experiment, the copepod meiofaunal component was the most sensitive of all groups to the different dosing levels (the macrofaunal response was marginal).

A major finding of the Workshop was the robustness of many of the data analyses to the aggregation of species into higher taxonomic units. This conclusion could lead not only to less labour-intensive approaches to monitoring pollutant effects on macrofauna, but should also increase the potential for analysis of meiofauna, which have hitherto been under-investigated because of taxonomic difficulties. It also suggests that taxonomic uncertainties over some sub-tropical and tropical benthic communities may not present the hurdle that is sometimes supposed to community studies of pollution effects in these areas.

Several pointers for follow-up activities can be drawn from the evident successes (and failures) of the Workshop.

(1) A wider practical evaluation of 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) Some procedures seen to have potential for monitoring will need to be simplified, to make them practicable when less sophisticated equipment is available.

(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 biological effects will encourage the continuing development and application of appropriate techniques and facilitate the training necessary to widen the experience and expertise of others.

The IOC Group of Experts on the Effects of Pollutants therefore plans to propose several regional activities, with the necessary mix of training and further development, in order gradually to enhance the quality of biological measurements undertaken in programmes of environmental pollution assessment; these plans are sketched in the following list.

1988 Participation in an FAO/UNEP/IOC Training Workshop on the Statistical Treatment of Benthic Community Data, planned for Piran, Yugoslavia, June 1988.

Convening an IOC/UNEP Practical Workshop on Biological Effects Techniques at the Bermuda Biological Station, Bermuda, September 1988.

Participation in a Training Workshop on Laboratory Bioassay Techniques, convened by CPPS/UNEP/IOC/FAO at Cartegena, Columbia.

1989 Co-convening, with ICES, a sea-going Workshop based on the R.V. *Meteor* (Federal Republic of Germany), in the North Sea, September 1989.

Participation in a CPPS/UNEP/IOC/FAO Training Workshop on Benthic Community Analysis, in Ecuador.

1990 Convening, with UNEP, a Training/Research Practical Workshop on Biological Effects Techniques, at the Third Oceanographic Institute, Xiamen, China

1991 Possible participation in further practical Workshops in the Philippines and the Caribbean

1. INTRODUCTION

The IOC Workshop on the Biological Effects of Pollutants was organised by the IOC Group of Experts on the Effects of Pollutants and held over three weeks in August, 1986 at the University of Oslo, Norway. The Proceedings of the Workshop are to be published in full as a book ("Biological Effects of Pollutants: The Results of a Practical Workshop", edited by B L Bayne, K R Clarke and J S Gray) which will include detailed accounts and discussions of all the procedures employed, and a comprehensive listing of all the data, both chemical and biological, compiled at the Workshop. The purpose of this Report is not to duplicate the Proceedings, but rather to summarise the aims, the content and the main highlights of the Workshop, and to draw the reader's attention to the lessons learnt and to the conclusions drawn. This is therefore a Summary Report; the Proceedings should be consulted for a detailed analysis of the Workshop content.

The Workshop was a practical one; that is to say, the participants were engaged in measuring biological responses in material gathered along a gradient of contamination in a nearby fjord, and in material experimentally exposed to a mixture of contaminants in a mesocosm facility, and then in comparing their findings with chemical determinations carried out simultaneously. As a result, we were able to evaluate the sensitivity of the biological procedures to environmental contamination, and to compare the performance, information gain and relative merits and de-merits of the techniques used. The Workshop was, to this extent, unique, representing the first attempt at such an inter-calibration of "biological effects techniques". The successes and failures of the Workshop are recorded in this Report in the form of Introductory and Summary statements for each of the four main topic groupings, *viz.* Biochemistry, Cell Biology/Pathology, Physiology and Community Ecology, together with Abstracts of all contributions to the Proceedings. In addition, two contributions to the Proceedings are reproduced here in their entirety, namely an introductory statement on the Background and Rationale to the Workshop and a concluding Overview of the Results of the Workshop.

The importance of deriving a suite of measures capable of identifying and of quantifying the biological effects of contamination is no longer in doubt. Relative to the techniques of chemical analysis that are employed in measuring contaminant levels in the sea, biological effects techniques are in an early stage of development. This is not an indication of any failure of will on the part of the biologists, but rather of the great complexity and variability associated with biological processes, and the consequent difficulties of providing a coherent and non-trivial means of assessing the biological response to any environmental variable. These difficulties are made the greater when the intention is to establish, not just the response, but also the extent (if any) of the damage caused to the biological resource. The Workshop represents an early step in an effort to identify the biological responses most capable of providing information on the damage caused by pollution. Future activities of GEEP, operating within the IOC/GIPME programme, will endeavour to build on this start.

2. BACKGROUND AND RATIONALE TO A PRACTICAL WORKSHOP ON BIOLOGICAL EFFECTS

2.1 BACKGROUND

The Group of Experts on the Effects of Pollutants (GEEP) was set up by the Intergovernmental Oceanographic Commission (IOC) within its programme entitled Global Investigation of Pollution in the Marine Environment (GIPME). At its first Session, in December 1984, this Group decided that there was an urgent need to convene a practical workshop which would evaluate some of the diverse means presently proposed as measurements of 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. 14, 1976). 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.

This topic, the biological effects of pollutants on marine organisms, has been the subject of considerable debate, the essence of which is captured in four publications in particular 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.

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

(ii) Participants at the Workshop should be research scientists who were currently working on these topics and 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.

(iii) 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 being evaluated would be encouraged.

(iv) 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 presumed ranking relative to the contamination gradient.

(v) Finally, the biological components of the Workshop should be complemented by thorough chemical analysis of the material in order to address the relationship between levels of contamination and the 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".

2.2 THE 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 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:

(i) Biochemical responses. 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.

(ii) Cytochemical responses. Measurements of the function of cellular organelles involved in sequestration and metabolism of toxicants.

(iii) Histopathology. Quantitative and descriptive techniques to assess the extent of pathological change to cells, tissues and organ systems.

(iv) Physiological responses. 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.

(v) Community attributes. 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 three-week Workshop. Some measures of the genetic consequences of pollution show promise as indices of biological response, but the timing of the 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 Annex IV.

2.3 THE SITE FOR THE WORKSHOP

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

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

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

(iii) The species commonly employed in research into the 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.

(iv) 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, Fig. 1, 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 (Klungsoyr 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 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 four available mesocosm basins are described in detail by Bakke et al. (1988). Briefly, the seawater inputs to these flow-through basins were dosed with three 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 flounder (*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 four months.

2.4 STATISTICAL PROCEDURES

Several points regarding the criteria for statistical design are worth emphasising here:

(i) 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.

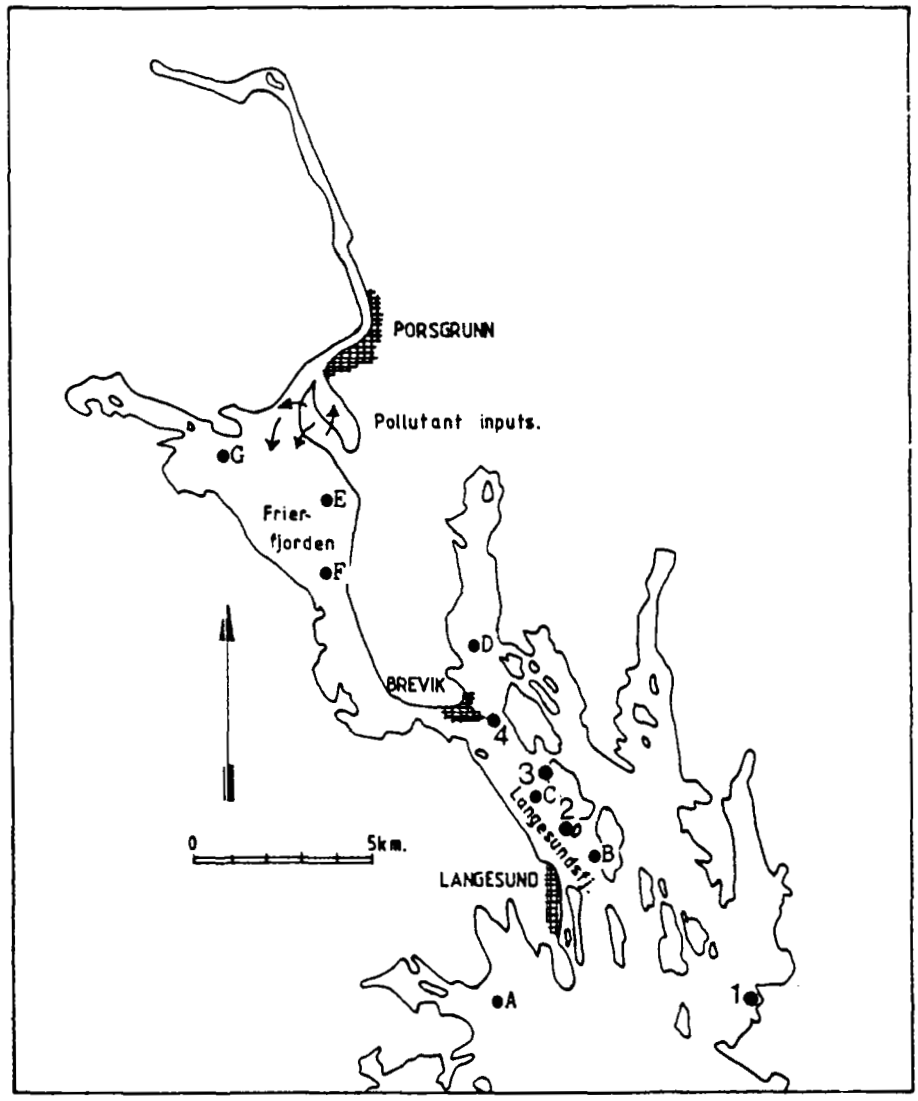

Fig. 1. Frierfjord and Langesundfjord, Southern Norway, the location of the field samples for the Workshop. Numbers 1-4 denote the sites for collection of Mytilus edulis, Platichthys flesus, Carcinus maenas *and* Littorina littorea, *and letters A-G the sites for collection of soft-sediment benthic communities (macrofauna, meiofauna and microbial samples).*

(ii) 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.

(iii) 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.

(iv) 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.

(v) Statistical analysis was undertaken centrally, for the full range of response data obtained during the 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.

2.5 THE WORKSHOP TIMETABLE

A sketch of the Workshop timetable is given here; further details of the timing 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 earnest 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 the macrofauna were identified and their abundance and biomass recorded (see 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. The meiofauna were identified and counted by Dr. C. Heip and colleagues (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-week dosing period, benthic community samples were taken from the experimental basins, and analysed in preparation for the Workshop by Drs. R. Warwick and M. Gee (meiofauana, 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.

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

(i) 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 (Klungsoyr 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).

(ii) 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).

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 over the three weeks 11-29 August, 1986. During the first week, participants studying sub-lethal responses of organisms, at the biochemical, cellular and physiological levels, analysed material from the field sites. Samples from the mesocosm experiment were analysed during the second week. During the first two 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.

2.6 ACKNOWLEDGEMENTS

The 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. 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. Support from the Institute of Marine Research, Bergen, Norway made possible a comprehensive chemical programme. The International Council for the Exploration of the Sea supported the participation of one research scientist, and the co-operation with ICES in this respect is gratefully acknowledged.

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 the *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 who were so helpful. In particular, 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 David Livingstone for organising the purchase of consumables and the use of heavy-duty equipment and to Roger Carter for computing support.

The Convenors of the Workshop groups, Richard Addison, Michael Moore, Judith Capuzzo and Richard Warwick, played a key role and we are grateful for their assistance. Finally, we wish to acknowledge the efforts of Professor Karsten Palmork and his colleagues at the Institute of Marine Research, Bergen and Professor Mo Abdullah, University of Oslo, for undertaking chemical analyses. Their efforts were an essential element in the Workshop; they served as convincing evidence that any study of the biological effects of pollutants must advance in the closest association with chemical analysis, both of environmental and biological material. It is only when chemical and biological assessments enrich each other in this way that a full appreciation of environmental pollution becomes possible.

3. BIOCHEMICAL METHODS

3.1 INTRODUCTION

Many biochemical techniques have been developed during the last few years to allow the detection of the effects of various pollutants on aquatic biota. Some of these are based on clinical chemical approaches originally developed to assess human health; others are derived from basic studies of the mechanism of action of specific toxicants (usually in mammalian systems). However, of all the approaches available, only a very few have shown any promise as techniques with which to assess the effects of pollution in the field. These are: (1) induction of enzymatic detoxification systems by certain organic contaminants and (2) induction of metallothionein by certain heavy metals. Undoubtedly, some of the success of these two approaches arises from the fact that they are, first of all, specific to a relatively small suite of pollutants; they are therefore likely to be insensitive to stress caused by factors other than pollution, such as temperature or salinity changes, disease, etc. (though the indices measured may vary with natural biological factors). Secondly, both approaches depend on detecting an *increase* rather than a depression of the variable being measured, and this simplifies the analytical chemistry involved. Finally, both approaches are based on a large body of information from mammalian studies, which provides a basic understanding of the mechanisms underlying the cause - effect relationship.

Studies of drug metabolism in mammals carried out during the 1950's and 60's showed that an enzyme system existed in liver which was

associated with the endoplasmic reticulum, required oxygen, involved a specific cytochrome (P-450, so-called from the absorption spectrum of its CO complex) and was inducible on exposure of the organism to various organic compounds. This enzyme system, "mixed function oxidase" (MFO) or, more recently, "mono-oxygenase", generally catalysed the conversion of lipophilic substrates to more polar products ("Phase I" reactions); these intermediates could be conjugated (by "Phase II" enzymes) to excretable products. In the 1970's and 80's, fish were shown to contain such enzyme systems, which were inducible by certain organic pollutants, including some chlorobiphenyls and polynuclear aromatic hydrocarbons. Activity of the "Phase I" enzymes in fish is now usually measured with substrates such as ethoxyresorufin or benzo(a)pyrene, which are converted to products which can easily be analysed with a high degree of specificity and sensitivity.

Hepatic mono-oxygenase induction in teleost fish has been used successfully on many occasions as an "effects monitoring" technique, and this proved true at this Workshop also. Mono-oxygenase induction in invertebrates is less well established as a monitoring tool though its potential was also examined in some detail at the Workshop. In addition, the possible application of some Phase II enzymatic measurements as indices of pollutant exposure was evaluated.

At the Workshop, measurements of the MFO system were made in fish (Platichthys flesus), molluscs (Mytilus edulis and *Littorina littorea*) and in crabs (*Carcinus maenas*).

Early studies on the toxicity of metals to fish showed that fish could acclimatise to increasing concentrations of some heavy metals. This led to the recognition that there existed in both vertebrates and invertebrates a group of proteins whose function was mainly to bind metals, usually those of Group IIB. The general characteristics of metallothionein are that it is of low molecular weight (around 6,000 - 12,000 depending on species and tissue), it contains relatively high concentrations of cysteine residues (up to 30 residue percent), and it is inducible on exposure of the organism to certain metals. Metallothionein is usually determined via its bound metal content (measured, for example, by atomic absorption) after a preliminary fractionation of tissue homogenate on a molecular weight basis (e.g. by gel filtration). Metallothionein measurements in fish (*Platichthys flesus*) and invertebrates (*Mytilus edulis*) were studied at the Workshop as potential "effects monitoring" tools.

3.2 MICROSOMAL XENOBIOTIC METABOLISING SYSTEMS

Several components of the microsomal mono-oxygenase enzyme system were measured in fish and molluscs. These included the "Phase 1" enzymes which carry out the first oxidations of lipophilic substrates, illustrated by ethoxyresorufin O-de-ethylase (EROD) and benzo(a)pyrene hydroxylase (B(a)PH) and the "Phase 2" enzymes, which act upon the products of oxidative metabolism. The latter are exemplified by epoxide hydrolase (EH) and glutathione S-transferase (GST). In addition, concentrations of cytochrome P-450, the complex of proteins which catalyses mono-oxygenase reactions, were measured. Finally, glutathione

concentrations were measured in some samples, since this compound is involved in the conjugation of some pollutant metabolites, and since its concentration may indicate in a more general way the response of the organism to pollutant stress.

Fish. EROD measurements in flounder liver gave the clearest and most sensitive response to expected pollutant gradients in the field samples. EROD activities measured by two independent methods increased approximately 7-fold between the reference site (1) and intermediately-polluted sites (2 and 3), and approximately 15-fold between sites 1 and 4 (the latter being expected to be the most polluted). This trend is consistent with the observed gradient of chlorobiphenyl and, less clearly, hexachlorobenzene and octachlorostyrene concentrations in flounder liver samples, and with chlorobiphenyl and aromatic hydrocarbon burdens in mussels (used to index the contamination gradient at these sites).

Two other measurements of mono-oxygenase activity showed similar trends, but differences between sites were less significant. B(a)PH increased about two- to three-fold between the reference site and all three contaminated sites, though there was no difference between B(a)PH activities in fish from any of the contaminated sites. Cytochrome P-450 specific content showed a trend similar to that of EROD, with statistically significant differences between the sites. Other measurements which in experimental mammalian studies have indicated mono-oxygenase induction, such as hepatic microsomal protein content or liver somatic index, showed no consistent pattern across the field sites.

The concentrations of cytochrome P-450E, an isozyme associated with EROD and B(a)PH activity, and determined immunochemically, followed the same trend in field samples as did EROD activities. This provides clear evidence that the environmental chemicals to which the flounder were exposed induced a specific cytochrome P-450 isozyme whose presence was reflected in induced EROD and B(a)PH activities.

Mono-oxygenase measurements in flounder from the mesocosm exposures showed no response to treatment. This could be an indication of low levels of inducing compounds, either higher molecular weight PAH or the PCBs, or it may indicate a possible effect of metal poisoning by Cu which was also a component of the contaminant dosing in the mesocosms. However, the EROD activities in all the mesocosm-exposed fish were similar to, or slightly below, those from fish from the field reference site. Cytochrome P-450 concentrations were also lower in mesocosm samples than in the reference field sample, and there was no evidence of induction of cytochrome P-450E. It therefore seems probable that the absence of induction reflects a low accumulation of, or exposure to, inducing compounds. This conclusion was supported by chemical analyses of tissue samples, which showed no difference in B(a)P levels between treatments.

Invertebrates. Consistent responses occurred in measurements of components of the mono-oxygenase system and epoxide hydrolase (EH) in molluscs from the mesocosm experiment, although the magnitude of the changes was considerably less dramatic than those generally observed in

fish. Cytochrome P-450 content of digestive gland microsomes in both mussel and periwinkle increased with increasing dose in the mesocosm exposures; a two- to three-fold increase was seen between control and high dose treatments. Similarly, up to a doubling of activity was seen in the cytochrome c (P-450) reductase of periwinkles and the EH of whole mussels with oil exposure in the mesocosms. In contrast, B(a)PH activity in whole mussels was elevated only in the medium dose treatment. All these observations are consistent with the results of previous studies on experimental exposures of mollusc species to hydrocarbons. In addition, the previously unstudied GST activity of the digestive gland of periwinkles was significantly (though not strongly) elevated in the high dose treatment, but no statistically significant response was seen in this enzyme in mussels; interpretation of this comparison is made difficult by the fact that a different assay was used.

In contrast to the mesocosm, the molluscan responses in the field were fewer and less consistent. Cytochrome P-450 content of the digestive gland of mussels was elevated at site 2, as was GST activity of whole mussels. However, the cytochrome P-450 content of periwinkles, and the cytochrome c (P-450) reductase and EH activities of mussels, were reduced at some of the polluted sites (2, 3 and 4). This difference between field and mesocosm results for the molluscs cannot be interpreted, but it indicates the very limited understanding that we have at present of the basic biochemistry of these enzyme systems in such organisms. It is interesting to note the different responses of molluscs and fish to mesocosm and field exposures, which may indicate that differences in inducing agents exist for these two groups of organisms.

The increased crab hepatopancreas GST activity at field sites 3 and 4 was consistent with the higher body burdens of various organic pollutants at these sites. However, the literature offers no guidance as to which of these organic contaminants (or groups of contaminants) may have caused induction. No such differences were observed in crabs from the mesocosm exposures, suggesting that the diesel oil added to these basins was not a GST inducer.

3.3 METALLOTHIONEIN

Metallothionein (MT) measurements were made on flounder liver and kidney samples from both field and mesocosm exposures. Although the technique used would not have detected copper metallothionein (Cu MT), it did measure endogenous zinc metallothionein (Zn MT). While it is not known whether water-borne Cu would accumulate as Cu MT in the liver or kidney of the flounder, any accumulated Cu should have disturbed Zn metabolism and hence altered the levels of Zn MT.

Fish from the reference site had consistently lower MT levels than those from other sites, but in general, MT levels in fish from the field sites were not correlated with measured metal concentrations in the same individuals. Furthermore, Cu was not accumulated from the mesocosm basins by experimentally-exposed fish, nor were liver or kidney MT levels elevated by the Cu dosing. (The lack of Cu accumulation in

the tissues perhaps indicates that the main route of uptake is through diet rather than by direct uptake from water).

Thus, for the present data, MT measurements on flounder liver or kidney did not reflect expected pollution gradients in the field, though it is worth noting that MT levels have reflected pollution gradients in other field situations, especially if considered in relation to measurements such as free metal concentrations in a cytosolic pool, and metals bound to high molecular weight (non-metallothionein) proteins.

Mytilus edulis samples were taken from both field sites and mesocosm exposures for MT determinations, together with total metal and high molecular weight protein-bound metal determinations. In the experimental mesocosm exposures, MT-bound Cu increased consistently with Cu exposure, but an even clearer effect was seen in total cytosolic protein-bound Cu. In field samples also, total cytosolic protein-bound Cu appeared to be a relatively sensitive monitor of metal pollution. Finally, evidence was obtained implying that Ca homeostasis was affected in animals from the contaminated field sites and, to a lesser extent, from the dosed mesocosm basins.

3.4 CONCLUSIONS

(i) Of the biochemical indices measured, flounder hepatic microsomal EROD activity and cytochrome P-450 isozyme concentrations showed the most sensitive response to the expected pollution gradient in the field. Since a mechanistic framework exists within which to interpret EROD and cytochrome P-450 induction in the field, in terms of the effects of body burdens of either chlorobiphenyls or PAH, these measurements can be recommended as indicators of environmental contamination by such compounds, with the reservations noted in (4) below.

(ii) Measurements of cytochrome P-450 and other enzymes of xenobiotic metabolism in marine invertebrates can show responses which can be related to pollutant distribution. However, the magnitude of these responses is smaller than those seen in fish and they are more difficult to interpret because of the inadequate current knowledge of marine invertebrate xenobiotic biochemistry. Thus, these measurements are not generally applicable at present, but they should be regarded as having some potential for use in the field, when the underlying biochemistry is better understood.

(iii) Several other measurements evaluated at the Workshop, including cytosolic GSH measurements, tissue GST activities and flounder intestinal MFO measurements, were either insensitive or too variable to have any immediate use as biochemical indicators of the effects of pollution.

(iv) It must be stressed that measurements of the sort described above cannot be applied indiscriminately. Their use requires careful evaluation, preferably in the light of complementary analyses, some of which should be chemical. The general application of such methods will

require intercalibration and validation when used in different environments and for different species.

4. CELLULAR AND HISTO-PATHOLOGICAL METHODS

4.1 INTRODUCTION

Numerous cytological, histochemical and histological approaches have been used to detect pathological disturbances in aquatic organisms. Many of these have been based directly on techniques used in mammalian pathology, while others have been developed from studies on various cellular processes in invertebrates, and particularly in mussels. Some of these approaches have proved useful in the assessment of pollutant effects including (1) lysosomal membrane stability, (2) lysosomal enlargement, (3) liposfuscin accumulation, (4) stimulation of NADPH-ferrihemoprotein reductase, (6) digestive tubule dilation and degeneration, (7) degeneration of ovarian eggs, (8) degeneration of gills and (9) the incidence of granulocytomas. Most of these effects are probably generalized responses to toxic chemicals, although stimulation of NADPH-ferrihemoprotein reductase (cytochrome P-450 reductase) appears to be specifically induced by certain organic xenobiotics such as polycyclic aromatic hydrocarbons.

Some effects such as destabilization of lysosomal membranes can be mechanistically linked to lysosomal enlargement and lipofuscin accumulation, both of which are indicative of autophagy. Autophagy in turn can be linked directly to degeneration of digestive tubules. There is also a considerable body of evidence on lysosomal involvement in cell injury in mammals to support the occurrence of these types of relationships in marine invertebrates.

The digestive cells of the digestive tubules of mussels appear to be a particular target for the injurious action of many pollutants and the extensive lysosomal-vacuolar systems in these cells is a site of accumulation of metals. Lysosomes are also known to accumulate polycyclic aromatic hydrocarbons and nitrogenous heterocyclic compounds.

Standard histopathological approaches are useful in providing an overall picture of the degree of disturbance within the organ systems of the organism. The studies on cellular pathobiology and histopathology conducted at the Workshop used all of the approaches mentioned above and emphasised, where possible, the mechanistic linking of effects at the different levels of cell and tissue organisation. Many of the effects observed support the utility of some of the above techniques in the assessment of pollutant impact, and provide further evidence for the claim that digestive cells of mussels are a sensitive target for environmental xenobiotics. All of these studies were carried out on mussels, *Mytilus edulis*, and some on winkles, *Littorina littorea*.

4.2 REPRODUCTIVE CONDITION

Quantitative histological analysis of the reproductive tissues in mussels indicated gametogenic activity in both field and mesocosm

samples. However, in animals from the field sites, the proportion of storage tissue was relatively high in relation to the volume density of gametes. This indicated that reproductive activity was not dominant and was unlikely to interfere with the interpretation of environmental effects. In contrast, some of the experimental mesocosm conditions showed evidence of extensive reproductive activity, with the low exposure treatment having the least proportion of gametes. This factor may have contributed to these animals having a higher lysosomal membrane stability. The high exposure treatment and its control were quite different from the other three treatments indicating that, with regard to cellular condition, the high exposure treatment cannot realistically be compared with the low and medium exposures. The high exposure treatment was the only one that displayed evidence of xenobiotic-induced degeneration of gametes.

4.3 CELLULAR PATHOBIOLOGY

The lysosomal data indicated that mussels from field sites 2, 3 and 4 were impacted by pollutants. There was good agreement between the authors and also between the various lysosomal characteristics tested. Lysosomal enlargement, increased fragility, lipid accumulation and lipofuscin accumulation all emerged as good descriptors of pathological effects in the digestive cells. The mussel data were further supported by the evidence of increased lysosomal membrane fragility in the digestive cells of periwinkles from sites 2, 3 and 4. These types of pathological alteration were interpreted as being indicative of augmented autophagocytosis resulting in increased catabolism of macromolecules and ultimately in cellular atrophy. This latter consequence was supported, in part, by the evidence of tubule degeneration at site 3.

The picture that emerged from the experimental mesocosm investigation was less clearcut, with mussels from all treatments showing evidence of cellular perturbation, probably induced by relative shortage of food within the mesocosm basins. However, when the high-exposure treatment was compared with its control there was good agreement between the authors that this condition was impacted by pollutants, in terms of lysosomal disturbance and cellular degeneration.

NADPH-ferrihemoprotein reductase appeared to be a good indicator of xenobiotic effects, being directly correlated with the tissue concentrations of polycyclic aromatic hydrocarbons (PAHs). The specific cause of this induction of activity was not identified, but previous field and experimental investigations have indicated that a range of PAHs are capable of stimulating the activity of this enzyme.

4.4 HISTOPATHOLOGY

Examination of mussels from the field sites indicated that sites 2, 3 and 4 were impacted. This was based on evidence of increased incidences of granulocytomas, aggregations of brown cells, digestive tubule dilation and degeneration. In mussels from the experimental treatments there was good agreement between authors that the high

exposure condition was the most severely impacted. This was based on evidence of digestive tubule dilation and degeneration, as well as degenerative changes in the gills and kidney.

The incidence of parasitism in mussels from the field sites indicated that site 1 was more affected than the other sites. Authors' data coincided on this factor but did not give any indications regarding the cause of differences. It may be that mussels already impacted by xenobiotics are more prone to mortality if infected by parasites, or alternatively, other factors such as contact with intermediate or definitive hosts may be reduced at the impacted sites.

4.5 CONCLUSIONS

(i) The results obtained by all three authors showed good general agreement in the assessment of effects in both the field (Table 1) and mesocosm samples. Furthermore, in those instances where there was overlap in the variables being measured, the level of agreement was very close.

(ii) When the various hierarchical levels are considered, namely, molecular, subcellular, cellular, tissue and organ levels, some effects observed at the lower levels can be used to predict those observed at higher levels of organisation. For instance, on the basis of the increased fragility of the lysosomal membrane in the digestive cells of impacted animals, one would predict that lysosomal dysfunction would lead to increased autophagocytosis and increased vacuolar fusion as evidenced by lysosomal enlargement, accumulation of lipid and lipofuscin. This would in turn lead to the observed atrophy of the digestive cells resulting in the dilation and degeneration of digestive tubules. The consequence of degeneration of the digestive tubules would be the failure of digestive and storage functions in the digestive gland.

(iii) The findings indicate that the relationships between many of the observed pathological effects and the level of contaminants are not necessarily simple. In a number of instances the most heavily contaminated mussels were not the most impacted and this may be indicative of either interactive effects in complex mixtures of contaminants and/or the selection of more resistant forms at the most contaminated site.

(iv) The techniques used to investigate the cellular and tissue effects are in general fairly straightforward, but the level of both structural and functional information that is obtained is high. That effects observed at one level of cellular organization can be used to predict those observed at higher levels is good confirmation that the mechanistic assumptions are firmly based. As such, this strengthens the case for the use of many of these types of methods in monitoring for the biological effects of environmental pollutants.

Table 1. Ranking of field sites based on the main cellular variables measured in mussels. (Site 1 is the reference site)

Variable	Site ranking
NADPH-ferrihemoprotein reductase	1 = 2 < 3 = 4
Lysosomal membrane stability	1 > 4 > 2 = 3
Lysosomal enlargement	1 = 4 < 2 = 3
Lysosomal and cytoplasmic lipid	1 < 2 = 3 = 4
Lysosomal lipofuscin	1 < 2 = 3 = 4
Brown staining lysosomes	1 > 3 = 4
Pyramidal cell vacuolation	1 < 2 = 3 = 4
Tubule degeneration	1 < 3
Tubule dilation	1 = 3 < 2 = 4
Granulocytomas	1 < 3
Aggregations of brown cells	1 < 3 = 4
Parasitism	1 > 2 = 3 = 4

5. PHYSIOLOGICAL METHODS

5.1 INTRODUCTION

Physiological responses of marine organisms to pollutants are dependent on the bioavailability, uptake, accumulation and disposition of the contaminants within the body, and on the interactive effects of multiple contaminants. In this regard, physiological responses are integrators of sub-cellular and cellular processes, and may be indicative of the overall fitness of the individual organism; they contribute also to our understanding of possible consequences of pollution to the population. The most important physiological changes associated with contaminant exposure are those that may adversely affect the organism's growth and survival and, thus, its ability to contribute to the population gene pool. Physiological indices linked to the survival and growth potential of the individual (such as the bioenergetic variables, feeding, digestion and respiration), or to the reproductive and developmental potential of the population (such as reproductive effort and larval viability) are therefore potentially most effective in assessing the effects of contaminant gradients.

However, techniques appropriate to the measurement of the energy budget of an animal, i.e. rates of feeding, digestion, respiration and excretion, under physiological steady-state conditions, have been developed for relatively few organisms. For example, some suspension-feeding bivalve molluscs (such as the common mussel, *Mytilus edulis*) have proved well-suited to the laboratory determination of the so-called "balanced energy equation", or scope for growth; they are sessile, they do not suffer from prolonged handling stress, and it is technically straight-forward to estimate rates of feeding, which are virtually continuous, on suspended particulate material. Crabs (*Carcinus maenas*) on the other hand, are active predators, with very variable rates of respiration reflecting, in part, a discontinuous feeding cycle; to establish physiological steady-states for the reliable determination of energy balance in these circumstances is difficult, requiring many hours of continuous measurement to achieve integrated estimates of the appropriate rates. As a result, the bioenergetic approach to assessing the effects of pollutants has been limited in phylogenetic scope, although the principles involved should apply to a wide range of organisms.

At the Workshop the physiological energetics approach was applied to mussels from both the field and the mesocosm samples, and also to the periwinkle, *Littorina littorea*. Acknowledging the variability so apparent in "whole animal" physiology of crabs, respiration rates of isolated gill tissues were measured as a potential index of pollutant effect, and the same approach was adopted as a comparative exercise with the gill tissues of mussels. A standard bioassay technique was used to estimate the viability of embryos of mussels from the mesocosm experiment. A detailed analysis of the lipid composition of both mussel and crab tissue was carried out to provide a link between the cellular and physiological studies.

The objectives of this section can therefore be categorised as assessing the following physiological responses to field and experimental contaminant gradients: (1) bioenergetic parameters, such as scope for growth, as an integrative measure of energy allocation to somatic and reproductive processes; (2) specific components of a bioenergetic budget, such as respiration and excretion; (3) larval viability from controlled spawnings; (4) lipid composition of digestive gland in relation to nutrient storage and turnover.

5.2 SCOPE FOR GROWTH

A marked decline in scope for growth (SFG) was observed in *Mytilus edulis* with increasing body burdens of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in field samples, and of PAHs and copper in the mesocosm study. Clearance (= feeding) rate was the component of the energy budget that was primarily affected. Little difference in respiration rates, absorption efficiencies or excretion rates was evident with either field or mesocosm contaminants, with the exception of the high dose mesocosm basin where the first two were significantly reduced. In both mesocosm and field studies, results indicated simple multiplicative (i.e. proportionally additive) effects of the different contaminants.

SFG was responsive not only to contaminant gradients but also to differences in seston (food ration) concentration, reflected in the lower growth potential of *Mytilus* in the mesocosms than in the field. SFG has utility under both standardized conditions, where effects of contaminants are separated from other environmental factors such as seston concentration, and under *in situ* field conditions where the interaction of environmental factors and contaminants may be determined.

The concept of SFG can, in theory, be applied to all animals. In practice, however, many species show cyclic or erratic feeding and metabolic activities which make estimation of physiological parameters difficult without relying on integrated measures over several days. For SFG to be a useful tool in biological effects monitoring, the individual physiological components must be monitored within 24 hours, before significant recovery from contaminant exposure can occur. The mussel meets this criterion because it maintains clearance and respiration rates at relatively constant levels; in addition it does not suffer "handling stress". In both the field and mesocosm studies, SFG in *Mytilus* proved to be an extremely sensitive indicator of physiological response to contaminant gradients and agreed well with both measures of biochemical and cellular processes and predictions of impacts based on bioaccumulation data.

In the field study with *Littorina*, however, there was no clear relationship between SFG measurements and the contaminant gradient, with animals from one of the more impacted sites (4) exhibiting the highest readings and no differences being seen between the remaining sites. In the mesocosm experiment with *Littorina*, bioenergetic responses failed to discriminate among the exposure conditions, with the exception of reduced respiration rates in the dosed basins, relative to the control. The lack of consistent trends in SFG of *Littorina* in response to contaminants was the result of erratic feeding activity and unpredictable behavioural responses to fluctuations in food availability and quality. At present, its use as a monitoring tool in *Littorina* is premature and both method development and a better understanding of bioenergetic processes in such gastropod molluscs are required.

5.3 RESPIRATION AND EXCRETION

A comparison of rates of respiration and nitrogen excretion, presented in the form of either nitrogen quotients (NQ) or oxygen:nitrogen ratios, showed no significant differences for *Mytilus* or *Littorina*, with the exception of values calculated for *Mytilus* in the high dose mesocosm basin where ammonia excretion rates were significantly lower than for other treatments. In field populations nitrogen quotients calculated for *Mytilus*, and O:N ratios for *Littorina*, were significantly different at sites 2, 3 and 4 and indicated lower rates of protein catabolism. In the mesocosm experiment with the deposit-feeding bivalve *Nucula*, increased rates of protein catabolism were observed from the low and medium dose basins (there was no material available from the high dose). These differences in physiological measurements coincided with water column concentrations of diesel oil and copper in the basins but did not correlate with sediment

concentrations. Interspecific differences in the sensitivity of protein catabolism and protein turnover in response to contaminant gradients warrant further exploration to assess their potential as monitoring tools.

Tissue respiration rates are of limited applicability as physiological measures since they reflect the metabolic demands of specific tissues to contaminant gradients and are not an integrated (i.e. "whole animal") response. Their utility for monitoring will vary with the nature of the contamination and the sensitivity of the species. Gill respiration rates of *Carcinus* were elevated at the two most contaminated field sites (3 and 4); these changes coincided with increased body burdens of PAHs and PCBs, but did not coincide directly with changes in gill body burdens of copper (F. Thurberg, pers. comm.). Similar measurements on gill preparations from crabs in the mesocosm experiment were insensitive to the contaminant gradient. Gill respiration rates of *Mytilus* were insensitive to contaminant gradients in both field and mesocosm studies and did not correlate with scope for growth measurements. Gill respiration rates of *Mytilus* did correlate with measurements of whole animal respiration but they are not an adequate estimate of whole animal energetics.

5.4 LARVAL BIOASSAY

The larval bioassay, using *Mytilus edulis* incubated in each of the mesocosm basins, showed a pattern of larval development similar to responses in scope for growth of adults. The relative agreement of a short-term measure such as the bioassay with a measure that integrates responses over a longer time period is promising in selecting multiple techniques for pollution monitoring.

5.5 LIPID COMPOSITION

In the Langesundfjord, changes in lipid content and lipid:protein ratios of digestive glands of *Mytilus edulis* and *Carcinus maenas* reflected the pollution gradient. In the mesocosm experiment only *Mytilus* from the high dose basin showed elevations in lipid content and lipid:protein ratios.

Analysis of lipid class composition for field populations of *Mytilus* suggested decreased mobilization of triacylglycerols into phospholipid pools with potential consequences for membrane structure and function. *Mytilus* from the mesocosm experiment did not show consistent trends among the various conditions, in part reflecting differences in reproductive condition and nutritional status of the groups. Mussels from the high dose basin, however, were distinctly different from other conditions, having significantly lower phospholipid content, suggesting membrane destruction, and marked increases in both the neutral lipid:polar lipid and triacylglycerol:phospholipid ratios. The changes in lipid class distributions of *Mytilus* from both field and mesocosm studies are consistent with histological and histopathological changes and correlate well with body burden data for tissue concentrations of PAHs and PCBs.

Observations on *Carcinus* from the contaminated field sites suggested that alterations in the mobilization of triacylglycerols, sterol turnover, and tissue degeneration may have taken place. Responses to the contaminant gradient were consistent with changes in respiration rate and elevations in glutathione transferase activity. The responses were not consistent with PAH or PCB tissue concentrations. Crabs from the mesocosm experiment showed no evidence of alterations in lipid class distribution in spite of the clear trend in aromatic hydrocarbon tissue concentrations across the basins. This is consistent with other mesocosm studies of *Carcinus*, as no differences in either gill respiration rate or glutathione transferase activity were observed. In comparison with field populations of *Carcinus*, there was no evidence of metabolic turnover of aromatic hydrocarbons or of molting among any of the mesocosm crabs. Differential responses of *Mytilus* and *Carcinus* are possibly the result of differences in metabolic capacity for detoxification and in trophic transfer.

6. COMMUNITY STUDIES

6.1 INTRODUCTION

For determining effects of pollution at the community level, assemblages of benthic organisms have obvious advantages over the pelagos. Because of the movement of water masses, in spatial surveys one can rarely be certain how long a particular pelagic community has been in the vicinity of the pollution source, and in temporal studies the same assemblage cannot be sampled repeatedly at a particular place. Changes in benthic community structure have therefore been the mainstay in the monitoring of the biological effects of pollution. Traditionally, these have largely involved studies of species abundance distributions for the macrofauna (defined in practical terms as the large species which are retained on a 0.5 or 1 mm meshed sieve when the sediment is passed through it). Species vary in their degree of tolerance to pollution so that some will decrease in abundance, some remain unaffected, and some which may benefit from the changed conditions will increase (the so-called "pollution indicator" species). A large literature has developed describing how these patterns of species abundance respond to pollution.

Recently, McIntyre (1984) has commented on "the failure of the popular benthic community monitoring approach to deliver the goods in some cases". There are several probable reasons for this. Firstly, many natural environmental variables also modify community structure and it has not always been easy to separate these from anthropogenic effects. Secondly, studies of this kind are highly labour intensive and cannot readily be translated to regions of the world where the fauna is poorly known and a high level of taxonomic expertise is lacking. Thirdly, not enough effort has been made to identify predictable responses to pollution in other components of the benthic system such as the meiofauna (metazoans retained on a 63 μm sieve) or the microbial community (passing through the 63 μm sieve), or even to examine attributes of macrobenthic community structure other than species abundance.

The aim of the Workshop was to examine, compare and evaluate as wide a range as possible of both traditional and newly developed techniques for determining pollution effects on all components of the benthos, in an attempt to develop suitable protocols. The robustness of these techniques to levels of taxonomic discrimination lower than that of species has also been investigated, in order to increase their cost-effectiveness and their utility for worldwide application.

The study, as with those of individual organisms, had two major elements. Field samples were taken from a series of sublittoral soft-sediment stations in Frierfjord and Langesundfjord along a putative pollution gradient. The biological components were mainly analysed before the Workshop; the macrofauna in Oslo, the meiofauna in Gent (Belgium) and the microbes in Dartmouth (Canada). Activity during the Workshop and subsequent to it was mainly concerned with data analysis. A mesocosm study involved the transplantation of large boxes of undisturbed sediment from a sublittoral site at Bjørnhodet Bay, and these were subjected to three levels of pollutant contamination. Again, the biological components were analysed prior to the workshop, in the same laboratories for the macrofauna and microbes, but in Plymouth (England) for the meiofauna.

6.2 MATERIAL AND METHODS

Seven 0.1 m^2 replicate Day grab samples for macrofauna were taken at six sites (A,B,C,D,E,G) in Frierfjord/Langesundfjord, sieved at 1.0 mm and preserved in formalin. Five 5.6 cm internal diameter replicate Craib core samples for meiofauna were also taken from six sites (A,B,C,D,E,F), sieved at 63 μm and preserved in formalin. Sediment samples for microbial analysis were subsamples of one meiofaunal core from each site.

For the mesocosm experiment at Solbergstrand, twenty 0.25 m^2 USNEL box core samples of sediment were taken from Bjørnehodet Bay and transferred undisturbed in plastic liners to the mesocosm basins, five being allocated at random to each of the four treatments: C - control, L - low, M - medium and H - high dosing of a copper and diesel oil mixture. After an exposure period of approximately three months, two 9.3 cm internal diameter cores for macrofauna, four 5.6 cm cores for meiofauna and one 0.5 ml surface sediment sample for microbes were taken from four of the five boxes in each basin, and processed as above.

All macrofauna were identified to species level, counted and weighed (wet wt.). Only four of the seven field replicates were analysed. The field meiofauna were enumerated to major taxon level in four of the five replicates at each site, the copepods were identified to species in three of these replicates and the nematodes in two replicates. Only the nematodes and copepods were enumerated (to species level) in the mesocosm experiment, based on subsamples of 16% of each sediment core, the four subsamples from each box being combined. Microbes were only identified to major taxa both in the field and mesocosm samples, and abundance and biomass (from volume measurements) were determined. A benthic biomass size-spectrum was constructed from

the microbial, meiofauna and macrofauna data.

6.3 ANALYSIS OF DATA

Four distinct stages in the identification of pollution effects on benthic communities can be recognised, each with its own set of analytical techniques:

(i) Multivariate methods used to discriminate between sites based on their faunal attributes. Three broad categories of techniques have been applied.

Classification. At the Workshop, hierarchical agglomerative clustering based on group-averaging of Bray-Curtis similarity measures was used throughout. TWINSPAN was used for Indicator Species Analysis (field meiofauna only).

Ordination. Several techniques were compared, including Multidimensional Scaling (MDS), Detrended Correspondence Analysis (DECORANA), Principal Components Analysis (PCA) and Reciprocal Averaging (RA).

Discrimination tests. The significance of differences between field sites or mesocosm treatments was tested using Analysis of Similarity (ANOSIM), Roy's Greatest Root Criterion and Malhanobis' Distance tests; the latter were followed up by Canonical Discriminant Analysis (CDA).

The effects of various strengths of transformation of the abundance and biomass data on the results of the multivariate analyses were tested.

(ii) Univariate methods used to determine levels of disturbance or "stress" at given sites. These can be divided into two categories.

Methods which can only be used in a comparative manner between sites along a spatial or temporal gradient. Those which have been applied are : number of taxa (S), total abundance (A), total biomass (B), A/S (abundance ratio), B/A (size ratio), abundance and biomass group distributions, dominance distributions, diversity and evenness indices (H', D, J, Hill's diversity numbers), comparison of functional (e.g. trophic) groups, biomass spectra.

Methods which can be applied to single sites without the need for reference samples. Those which have been applied are: identification of indicator organisms (species or higher taxa), abundance/biomass comparison curves (ABC).

(iii) Methods of correlating 1. and 2. above with pollution levels. Care must be taken to identify such natural confounding variables as depth, sediment type, physical disturbance etc. when relating the observed differences in faunal attributes between sites to chemical measurements of pollution levels, or proximity to pollution sources. The method used for multivariate analyses (ordinations) was to

superimpose levels of pollutants and some of the more obvious natural variables onto the site configurations to provide visual correlations. The univariate indices have been related to pollutant levels using standard statistical techniques (principally for the microbial studies).

(iv) Methods to establish whether pollution *causes* the observed pattern of site differences. The level of community reponse to measured levels of pollutants in controlled experiments was studied in an attempt to provide this test, which at the Workshop constituted the mesocosm experiment. Methods of analysing this data were essentially the same as those given in 1. and 2. above.

6.4 RESULTS

Field macrofauna

(i) The different multivariate analyses using a variety of transformations were in general agreement with each other, although there were some differences in detail. Three major groups of sites were seen: B+C+D, E+G and A. Some analyses separated the replicates at D from those of B+C (B and C were never divided) and most analyses separated E from G, though the latter were always closer to each other than any other site. To test the idea that species in the intermediate abundance classes were mainly responsible for the observed structure, MDS analyses were performed on the 19 species in these classes. Although these reproduced the structure of the full data set rather closely, they did so no better than any randomly selected subset of 19 species. This indicates strong site differences and a high degree of redundancy in the data. Such redundancy could be exploited by laboratories with limited taxonomic expertise, or in regions of the world where the fauna is poorly documented, if identification to species level were not necessary and sufficient information remained using higher taxonomic groupings. The Workshop data for both species abundance and biomass were aggregated at the family and phylum level. At the family level, MDS on the abundance data produced results which were virtually identical to the species level configurations, the same being true for the untransformed biomass data; with the 4th root transformed biomass data there was an appreciable *improvement* in the separation of replicates from certain sites. At the phylum level, groupings based on abundance data that were apparent at the species and family level largely broke down, but the biomass data faithfully reproduced these groupings with surprisingly little loss of information.

(ii) Comparison of a wide variety of univariate measures between sites indicated that the communities at sites B and C were the most stressed, followed in decreasing order by D, E, G and A. Of the methods which did not require inter-site comparison, indicator taxa suggested that sites B,C and D were stressed and that sites A and G were unpolluted, whereas the pollution status at site E was equivocal. ABC plots indicated that sites C and D were intermediate between the "moderately polluted" and "grossly polluted" conditions, sites B and E were "moderately polluted" and sites A and G "unpolluted"; aggregation of the data to family level gave exactly the same results.

(iii) Two pollution variables (metal and PAH concentrations) and two natural variables (water depth and median sediment grain size) were superimposed on the site configurations in the species abundance MDS (under 4th root transformation). Neither sediment type, metals or PAH correlated closely with the site configuration, whereas water depth did. Furthermore, the sites where the univariate measures indicated the highest degree of stress (B, C and D) were not those with the highest measured levels of pollutants but were at the greatest water depths. The most parsimonious explanation for differences between communities in the multivariate analyses is therefore that they result from the well-known effect of water depth on benthic community type, and the clear indications of stress derived from the univariate techniques at the deeper sites do not result from inputs of pollutants at the head of the fjord but to some other depth-related character. Seasonal anoxia in the deeper basins of the fjord is well-documented, and is the most likely cause of stress at these deep sites. For pollution surveys of heterogeneous geographic regions where the differences in non-pollution related ("nuisance") variables between sites are great, it is postulated that multivariate analyses at the higher group (e.g. phylum) level may more closely reflect the pollution gradient. It is well documented that pollution modifies community structure at this higher level, whereas differing environmental conditions (e.g. water depth and sediment granulometry) may affect community structure more by replacement at the species level.

Field meiofauna

A major drawback to the use of meiobenthos in programmes for monitoring the effects of pollution has been that the necessary expertise in identifying these organisms to species level is lacking in most laboratories, and in any case a taxonomic literature approaching adequacy is only available in N. Europe and perhaps N. America. An important element of the meiofauna study was therefore to analyse the data assuming that less taxonomic rigour was possible.

(i) As with the macrofauna, the different multivariate analyses gave generally similar results, with some differences in detail. The analyses were very robust to the aggregation of the nematode and copepod species data into higher taxa. The use of nematode feeding groups and the abundances of major taxa also produced similar results. With different numbers of replicates for nematodes, copepods and major taxa it was difficult to compare the resolving power of the different hierarchical groupings of taxa, but generally copepod species were best in separating all sites in at least some analyses, although the nematodes were more robust to aggregation into higher taxa.

(ii) Many of the univariate measures listed earlier are not applicable or have not yet been well developed for use with meiofauna; heavy reliance has therefore been placed on diversity measures. For a range of diversity indices, site F had a significantly lower diversity of nematodes and copepods both for species and higher taxa. For meiofauna groups, this trend was not apparent for all diversity indices. Plotting *k*-dominance curves for nematode species indicated that site F clearly had the lowest diversity, followed by E and then B, and A, C and D had the highest diversity and were indistinguishable from each other.

Similar plots for copepods showed that D had the highest diversity, but otherwise there was no clear separation of curves.

(iii) The marked separation of site F in the multivariate analyses, and its low meiofaunal diversity relative to the other sites, coincided with the much higher PAH levels at that site and the much higher levels of certain metals, particularly cadmium. This site was of intermediate water depth, so that its distinctive characteristics did not relate to naturally occurring depth-related environmental variables. Thus there was correlative evidence that the meiofauna were affected by pollution. In many of the multivariate analyses the remaining sites A-E were grouped in the same way as for the comparable macrofaunal analyses. Therefore, naturally occurring depth-related factors rather than pollution were implicated, although, unlike the macrofauna, the meiofaunal assemblages at the deeper sites (B, C and D) showed no signs of stress.

Field microbes

(i) No multivariate analyses were performed on the microbial data.

(ii) There were significant differences in abundance of bacterial rods+cocci and microflagellates among sites A-F, with the combined inner sites (D,E,F) having significantly lower abundances of bacterial filaments, rods+cocci and microflagellates than the combined Langesundfjord sites (A,B,C). The same trend was apparent in the biomasses of all three microbial groups.

(iii) Microbial abundance and biomass did not correlate closely with water depth or sediment granulometry. Concentrations of lead and manganese in the sediments were negatively correlated with microflagellate biomass, and zinc concentrations were negatively correlated with bacterial biomass. However, pairwise comparisons showed that the most highly polluted site F was not significantly different from sites D and E in the abundances of any of its microbial components, and no causal inferences could be drawn from these correlations.

Field biomass spectra

(i) For the field sites, MDS was used for the macrofaunal size range only, treating the x 2 geometric size classes as "species" in a normal analysis. Sites B, C and D were grouped together, as were sites A and E, reflecting a similar split to that found for macrofauna and meiofauna taxa.

(ii) For the complete size spectrum, there was an overall decrease in biomass of most size classes within the microbial, meiofaunal and macrofaunal categories, from sites A-E. Spectra had a higher degree of "bumpiness" at sites D, E and F than at A, B and C, with more pronounced biomass troughs between meiofauna and macrofauna peaks.

(iii) The differences between the size spectra were not obviously correlated with pollution levels, and unfortunately no macrofauna samples were available from the most heavily polluted site F, where any pollution-induced modifications to the spectrum would have been most apparent. For the macrofaunal component, MDS of the size classes corroborated the equivalent taxonomic analyses, suggesting the same causation. There was a trend to reduced average size of organisms in the macrofaunal assemblages which showed the most stress.

Mesocosm macrofauna

(i) Most multivariate analyses failed to demonstrate clear differences between treatments. An exception was a MANOVA analysis on the first five principal components of (log) species abundances, the treatment means being ordered C-L-M-H on the first canonical variate axis (a 1 in 12 chance of occurring if there were no treatment effects). On balance, evidence for community change with treatment must be regarded as marginal.

(ii) Most of the univariate analyses used failed to demonstrate treatment effects, perhaps due to the fact that the small core size failed to sample the large biomass dominants adequately. However, the high dose treatment had an uneven distribution of species abundance groups, which is indicative of moderate organic enrichment. Seven species in the middle abundance groups showed trends of increasing or decreasing abundance with treatment level commensurate with their predicted behaviour based on previous studies of organic enrichment in the field.

(iii) Differences in species composition and community structure between mesocosm treatments were minimal, certainly not approaching the differences between the field sites. The main reasons for this may be the short time scale of the experiment relative to the generation time of the macrofaunal organisms, problems of macrofaunal recruitment to the mesocosm boxes, the fact that pollutants did not penetrate the sediments over the timescale of the experiment and the small sample size. For these reasons it was not possible to test in this experiment whether the measured pollutant levels in the field were capable of causing community differences.

Mesocosm meiofauna

(i) For both nematode and copepod species abundances, multivariate analyses revealed some significant differences between the high dose and other treatments. PCA produced better separation of the H replicates than other ordinations, the treatment level means on PC2 again ranking as C-L-M-H. However, further analysis revealed the two-dimensional PCA configuration to be a poor representation of the higher-dimensional structure, by contrast with MDS, as seen when groupings from cluster analyses were superimposed on the two-dimensional ordinations.

(ii) For the nematodes, there were no significant differences in species diversity betwen treatments, but for the copepods there were significant global differences, which principally resulted from the contrast between the H replicates and the others. Diversity profiles

determined from *k*-dominance curves showed no clear cut treatment effect for nematodes, but a strong effect for copepods, the curves forming a decreasing sequence from high to low diversity in the C-L-M-H basins (in this case the chances of this are 1 in 24, under a hypothesis of no treatment affect, because there is a predictive direction). For the nematodes, total abundance in the H replicates was significantly higher than in M and C, and copepod abundance was higher in H than in all other treatment levels.

(iii) The decrease in diversity of the copepod component of the meiofauna with increasing treatment level was due to a disproportionate increase in abundance of certain species (notably *Tisbe* spp., but also other species) rather than to selective mortalities. A possible mechanistic explanation for the pollution effects lies in a general response to organic enrichment brought about by the addition of hydrocarbons and/or by mortalities of macrobenthic species; certain opportunistic species, notably *Tisbe* spp., having a higher colonising potential than others. The toxicity of the pollutants was not an important element in the response, but may have had an effect if the experiment had run for longer or if the pollutants had penetrated into the sediments. The situation in the high dose treatment may therefore represent an early successional stage in the pollution response and did not parallel the condition at the polluted site F in Frierfjord, where overall densities were much *lower* than at other sites, and none of the species known to have opportunistic characteristics were found. Therefore, as with the macrofauna, this experiment was not able to determine whether measured levels of pollutants in the field were capable of causing the observed community responses.

Mesocosm microbes

(i) No multivariate analyses were performed on the microbial data.

(ii) Microbes were sampled half-way through the experiment as well as at its termination. Analysis of variance of the various microbial groups indicated that only benthic diatoms showed any significant differences in abundance between treatments. There was a significant increase in diatom abundance in all treatments during the course of the experiment, but pairwise differences between treatments were not consistent on the two sampling occasions, nor did they reflect the gradient of dosing levels. There were no differences in biomass of any microbial groups between treatments at the termination of the experiment.

(iii) Lack of penetration of pollutants into the mesocosm sediments, or grazing by the increased meiofaunal levels in the high dose boxes, are possible explanations for the lack of response in microbial abundance or biomass with treatment level.

Mesocosm biomass spectra

(i) MDS based on geometric size classes within the macrofaunal size range separated the H replicates from the remainder, but ANOSIM showed that there were only significant differences between H and M, and

H and C, and then only at the 10% level. As with the equivalent taxonomic analysis, these results were therefore equivocal.

(ii) There were no significant differences in the structure of the overall size spectrum which could be attributed to dosing level.

6.5 CONCLUSIONS

(i) It is appropriate here to consider the relative merits of the different size-categories of organisms which were examined for the Workshop community studies, both in terms of the practicalities of sampling and processing, and in terms of their resolving power when the same analytical techniques are applied to them.

(ii) Macrofauna and meiofauna can be compared on the same terms since sampling regimes and species concepts are similar. Macrofaunal community reponses to pollution have been much more widely studied and a variety of univariate stress indices has been developed, many of which are probably not applicable to the meiofauna (especially those related to size distributions such as B/A and ABC curves, and the use of "indicator" taxa). Their relative longevity means that the community structure reflects environmental conditions integrated over a long period (years rather than months). Taxonomic literature enabling identification to the species level is also available for many regions of the world, the necessary expertise to do this is present in many laboratories, and methodologies for sampling and processing are well developed. None of these conditions pertain to the meiofauna, but they too have certain advantages. Sampling is less labour intensive in that sample size is smaller, so that sieving need not be done at sea. The taxonomic problems are undoubtedly greater if identification to species level is required, but this Workshop has demonstrated that lower levels of taxonomic discrimination produce results which for many purposes are as good as species analyses, for both the meiofauna and macrofauna. Also, the shorter generation times of meiofauna have certain advantages in terms of their faster potential response time to pollution incidents. Meiofaunal studies are hampered by the lack of suitable univariate measures of stress which are applicable to them, and more work needs to be done to develop such techniques. Meiofauna have advantages over macrofauna in experiments to determine cause and effect relationships: because of their size and turnover time, community responses are measurable on the spatial and temporal scales which can be reproduced in such experiments. Also, because of direct benthic development, there are no recruitment problems which are apparent in mesocosm experiments on macrofauna with planktonic larvae. At the species level, copepods were better than the macrofauna or any other meiofaunal taxon at discriminating between sites in the field, and were by far the most sensitive component of the fauna in the mesocosm experiment.

(iii) Techniques using microbial communities and size spectra are much less well developed, but this Workshop has shown their potential to give qualitatively similar results to the taxonomic analyses of the metazoa. The need to identify microbes only into broad and easily recognisable categories, and the completely ataxonomic size-spectrum analysis, have obvious labour-saving advantages, and here again much

more work needs to be directed towards the development of appropriate techniques.

In the determination of pollution effects, a criticism frequently levelled at benthic community studies, in comparison with those of individual organisms, is that the former are highly labour-intensive. In macrofauna studies of the traditional kind, usually many hours have been spent in trying to separate certain difficult groups, such as small spionid, cirratilid or capitellid polychaetes, into species. Analysis of the Workshop data suggests that very little, if any, information is lost by working at the level of families, which are readily recognisable by ecologists with moderate experience. Useful information is still present when working at the phylum level, and this may also have some conceptual advantages in certain situations. More case studies need to be undertaken to establish the validity of these more cost-effective approaches.

7. AN OVERVIEW OF THE WORKSHOP

7.1 INTRODUCTION

The Workshop that is the subject of this report 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 that were tested during the three weeks 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 (which may be described as 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 contaminant impact on a marine fauna.

Our reasons for stating these caveats here are three-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 gained by so doing 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 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, perhaps, 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, we are conscious of the fact that this Workshop was held, by design, in an area already well studied with regard to its polluting inputs, and 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 Workshop was a considerable success. In this Overview we will 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.

7.2 A SUMMARY OF THE MAIN HIGHLIGHTS

An essential element in the design of the 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 four stations at which flounders, crabs, mussels and winkles were sampled in order of increasing contamination from sites 1 to 4; see 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 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) in their contribution. 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) the interpretation of measurements of cellular condition, which are particularly sensitive to changes in reproductive state, were rendered more complicated.

The summaries which are reproduced earlier discuss the main conclusions, as agreed in the final days of the Workshop. We offer here an even briefer statement of the main highlights.

Biochemical processes

Biochemical measurements concentrated on processes likely to respond specifically to certain types of contaminant; those relating to the "Phase 1" enzymes of the microsomal cytochrome P-450-mediated mono-oxygenase system in the liver of the flounder 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, two 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 contaminants on fish.

Components of the analogous metabolic system in the mussel 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 relative ignorance of

the 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 mussels 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 the mussels in the mesocosm basins (Moore 1988), correlating both with measured levels of copper and with evidence of cellular damage. These results also illustrate a long-standing 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 mussels 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 mussels and winkles, 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 the basins (Widdows & Johnson 1988), and contributed to the

failure of the 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 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 (Widdows & Johnson 1988, Bakke 1988). This approach offers insights into the physiological condition of the 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 the effects of pollution on mussels (Widdows 1985).

Widdows & Johnson (1988), in their contribution to this volume, report the success of the scope for growth determinations on mussels in identifying a gradient of pollutant effect in the Workshop 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, they were able to anticipate some aspects of the effects (particularly the 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 winkles 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 *Littorina*. 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). The scope for growth is a powerful index of the 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 contaminants 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 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, or MDS; principal components analysis, or 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 indices. In the mesocosm experiment the copepod component of the meiofauna was the most sensitive of all groups to the different contaminant dosing levels. However, few techniques are currently available for evaluating the effects of disturbance on the 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 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 associated 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.

7.3 LINKAGES AND COHERENCE BETWEEN EFFECTS

The results of the 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 the Workshop 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 intra-cellular 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 contaminant 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 on to 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 made on individuals with those made on communities, not to be able to link these causally, but to explore possible differences in sensitivity to contaminants. 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. However, 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.

7.4 SOME LESSONS LEARNT

We consider that the 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 others 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 were agreed on the need for further workshops of this type. Clearly, 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 Oslo 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 followed. Most of these are discussed by Clarke & Green (1988); some of the key considerations will be mentioned here.

It is essential to be able 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; the 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 Workshop made plain the importance of site selection in the field survey. In order to detect a spatial gradient of effect, control (or "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 this Workshop we failed adequately to control water depth 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 two 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 sub-sampling 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, Warwick 1988, Heip et al. 1988).

An essential feature of any study of the biological effects of pollution is a close integration between chemical and biological analyses; the Oslo 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 contaminant-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 to the Workshop 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 the 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 fed 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.

7.5 FINAL COMMENTS

Interdependence of biological and chemical measurements

Pollution effects, which were the primary focus of this Workshop, involve the interaction between chemicals and the biota. Analytical chemistry and the biological determination of toxic effects represent two 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 chemical concentrations in the absence of information on their biological impact. It follows that any serious study of the biological effects of pollution must have in association a matching programme of chemical analysis.

This inter-relatedness operates at a number of levels, only some of which were considered at the Workshop. For example, a convincing interpretation of the lack of any induction of the flounder P-450 mono-oxygenase 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 the 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 relationships (QSARs) of the contaminants involved. By careful analysis of this interaction it may be possible to detect the influence of additional, possibly unsuspected, contaminants 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 and 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 (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 the implications this has for effecting a biological response. For example, the uptake of hydrocarbons into so-called "storage" lipids, e.g. the 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. A careful reading of the papers in this volume will identify the areas of uncertainty as well as those areas where we can be hopeful of successful application of the results of research. Perhaps the greatest danger is that a distancing will develop between the appropriate research and its application, for two reasons.

Firstly, the scientists doing the 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 research workers 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 Oslo 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 the 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 Workshop took place 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 to this report). 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 that are 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 that occur with contamination. The latter lack specificity and tend to be more empirically based; the former can, uniquely, 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 tools 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 ten years 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 Oslo Workshop. In looking forwards, the IOC Group of Experts on the Effects of Pollutants recognise the following points of emphasis:

(i) 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.

(ii) 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.

(iii) 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.

(iv) 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 Workshop and gradually enhance the quality of biological measurements undertaken in programmes of environmental pollution assessment.

ANNEX I

AGENDA

1. INTRODUCTION
2. BACKGROUND AND RATIONALE FOR THE WORKSHOP
3. BIOCHEMICAL METHODS
4. CELLULAR AND HISTO-PATHOLOGICAL METHODS
5. PHYSIOLOGICAL METHODS
6. COMMUNITY STUDIES

ANNEX II

ABSTRACTS OF SPECIFIC STUDIES

TABLE OF CONTENTS

1. SAMPLING AND CHEMICAL ANALYSIS

The Oslo Workshop: field sampling

O A Follum and K A Moe

The field sampling for the Oslo Workshop was carried out in Frierfjord and Langesundfjord in southern Norway. Both of these are stratified fjords with shallow sills. The Skiens river enters the Frierfjord and influences both the stratification and the pollution of the fjords. Chemical contamination of the water and sediments are well documented; a decreasing contaminant gradient can be observed from the top of the Frierfjord towards Langesund Bay. Procedures for sampling material for the Workshop are described.

The Oslo Workshop: a mesocosm experiment

T Bakke, O A Follum, K A Moe and K Sørensen

Four indoor mesocosms at the Marine Research Station, Solbergstrand were stocked with equal numbers of *Platichthys flesus, Carcinus maenas, Mytilus edulis, Littorina littorea* and *Nucula tenuis* as well as subtidal soft bottom sections taken with an USNEL box corer. For four months prior to the Workshop three of the basins were continuously exposed to a mixture of diesel oil (125, 32 and 6 $\mu g\ l^{-1}$ total hydrocarbons in the water) and copper (nominal 20, 5 and 0.8 $\mu g\ l^{-1}\ Cu^{2+}$), with the fourth mesocosm basin as a control (measured total hydrocarbons of 3 $\mu g\ l^{-1}$). The sediment sections were sampled by corers for community faunal analysis prior to the Workshop; sampling of *N. tenuis* and the epifauna was carried out during the Workshop.

The Oslo Workshop: organic chemical analyses

J Klungsøyr, S Wilhelmsen, K Westrheim, E Saetvedt and K H Palmork

Gas chromatographic/mass spectrometric (GC/MS) analyses of selected two- to five-ring aromatic hydrocarbons (AHs) were performed for water, sediments, mussels and crabs in the mesocosm experiment at Solbergstrand. The experiment involved dosing of three different concentrations of diesel oil and copper to the mesocosm basins, and the chemical analysis showed that elevated abundance of compounds from the diesel oil could be detected in water and organisms but not in sediments. Mussels and crabs sampled along a pollution gradient in Langesundfjord were analysed for selected aromatic hydrocarbons by GC/MS and for selected PCBs by GC. The concentrations formed a clear gradient from the "cleanest" site in Langesund Bay (site 1: total concentration in mussel whole tissue of selected AHs of 2.2 $\mu g\ g^{-1}$ dry wt., and total PCBs of 0.08 $\mu g\ g^{-1}$ dry wt.), through increasing levels of contamination at sites 2 and 3 (AHs 5.9 and 11.5, PCBs 0.18 and 0.23), to the most contaminated site at the head of Langesundfjord, closest to the industrial activity (site 4: AHs 15.5 and PCBs 0.28). The mussels had the highest concentrations of aromatic hydrocarbons, especially unsubstituted four- and five-ring PAHs, while the crabs had the highest concentrations of PCBs (ranging from 0.16 to 0.48 $\mu g\ g^{-1}$).

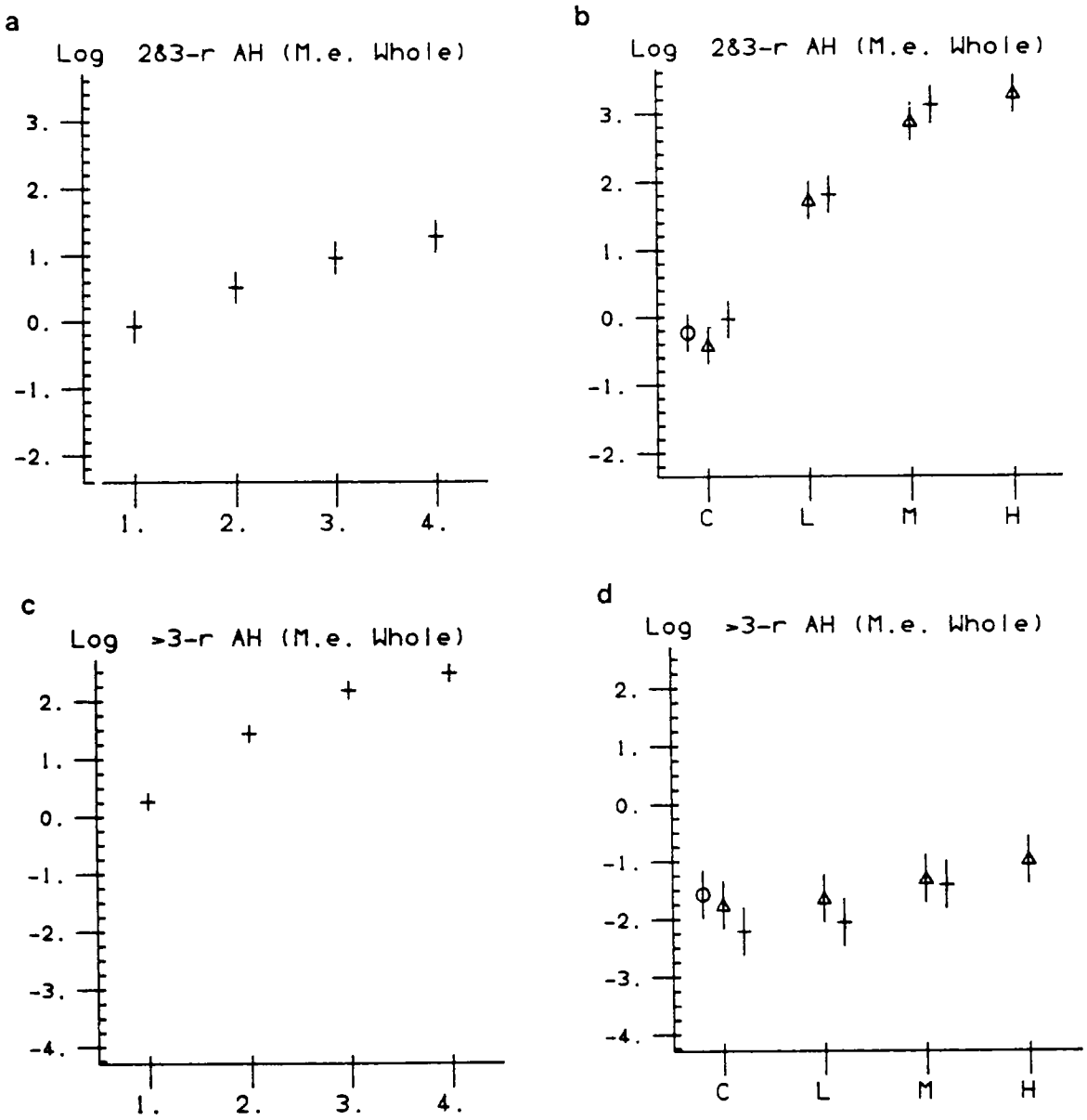

Fig. 2. **Klungsoyr et al.** *Total concentrations (ug g^{-1} dry wt.) of: a),b) selected 2- and 3-ringed aromatic hydrocarbons, and c),d) selected 4- and 5-ringed AHs, in* Mytilus edulis *whole tissue. The x-axis denotes sampling location: a),c) the four Langesundfjord sites 1-4, and b),d) the four Solbergstrand mesocosm basins, subject to control (C), low (L), medium (M) and high (H) dosing of a diesel oil and copper mixture. In all cases, symbols denote a mean from duplicate samples (each of 10 animals), and bars denote exact 95% confidence intervals based on a pooled error estimate from all four sites/conditions; all readings were $\log_e$-transformed prior to computations. a),c) In the mesocosm experiment, chemical data were taken at three times: o - 16 May, Δ - 13 June, + - 4 August (dosing commenced 24 April; sampling for biology was on 18-21 August). b),d) Field sampling for chemistry and biology was coincident, on 11-14 August.*

The Oslo Workshop: trace metal analyses

M I Abdullah and I Steffenak

As a counterpart to the biological analyses of the GEEP Oslo Workshop, chemical analyses of trace metals were carried out on sediments and on organs and tissues of fish (flounder), mussels and crabs, from the field sampling sites in Langesundfjord/ Frierfjord and the experimental exposures in the Solbergstrand mesocosm facility. The field sites were not characterised by strong metal gradients, but both tissue and sediment analyses revealed differences between sites, particularly between the "end-points" of the anticipated contaminant gradient (e.g. mean sediment concentrations ranged from 26 to 72 $\mu g\ g^{-1}$ dry wt. for Cu, 70 to 240 for Pb, 0.7 to 3.4 for Cd; mussel whole tissue concentrations from 14 to 17 for Cu, 3.5 to 7.9 for Pb, 1.5 to 2.7 for Cd). In the mesocosm experiment, the increasing copper dosing levels in the basin waters were closely matched by copper accumulation in mussel tissues (ranging from 7 to 59 $\mu g\ g^{-1}$) and, to a lesser extent, crab tissues. The copper did not appear to accumulate in the fish livers nor in the top layers of the sediment used for the benthic community studies.

2. BIOCHEMICAL METHODS

Glutathione S-transferase in marine invertebrates from Langesundfjord, Norway

R F Lee

Glutathione S-transferase (GST), an enzyme system which conjugates glutathione to a variety of xenobiotics with electrophilic centres, was found in the digestive glands of *Littorina littorea* (snail) and *Mytilus edulis* (mussel) and the hepatopancreas of *Carcinus maenas* (crab). GST activity was significantly higher in crabs from two polluted sites in Langesundfjord relative to reference site crabs but no such differences were found for mussels, in spite of large differences in PAH and PCB tissue concentrations between the sites. In a mesocosm experiment involving diesel oil and copper dosing, no significant effects were observed on crab and mussel GST activity though *L. littorea* showed significantly higher GST activity at the highest contaminant dose. Since few studies have been done on GST induction in marine invertebrates, it is not clear how differences in GST activity in crabs from the field sites can be related to the pollutants present.

Responses of microsomal NADPH-cytochrome c reductase activity and cytochrome P-450 of the digestive gland of the mussel, *Mytilus edulis*, and the periwinkle *Littorina littorea*, to environmental and experimental exposure to pollution

D R Livingstone

Digestive gland microsomal components were measured in mussels and periwinkles exposed to different levels of chemical contaminants in

an experimental facility (diesel oil/copper mixture) and along a field pollution gradient in Langesundfjord, Norway. Cytochrome P-450 content increased in mussels and periwinkles with experimental exposure and was elevated in mussels from some of the more contaminated field sites. Increases in P-450 content were accompanied by a blue shift in the P-450 λmax suggesting isoenzyme synthesis. NADPH-cytochrome c (P-450) reductase activity increased in mussels and periwinkles with experimental exposure but showed only decreases in the field. The "418" peak of the P-450 carbon monoxide difference spectrum increased in mussels and periwinkles with both experimental and field contaminant exposure. It is concluded that mussels were affected by organic chemical contamination, and to an extent increasingly so with increasing contamination, but that application of these methods in environmental monitoring is limited by a lack of understanding of the basic nature and function of the molluscan MFO system.

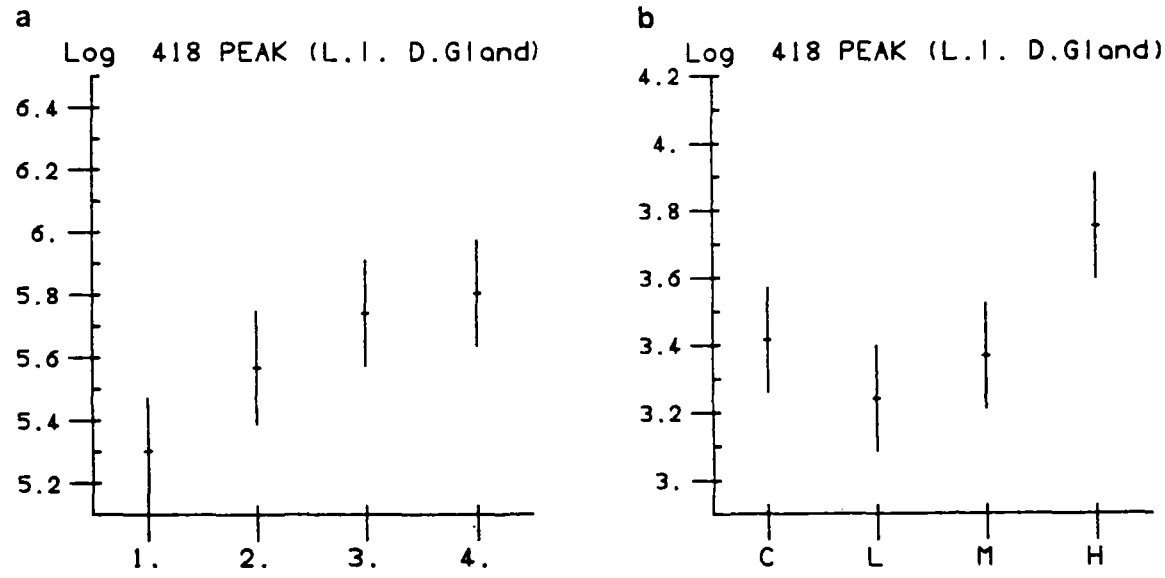

Fig. 3. **Livingstone.** *The '418' peak of the P-450 carbon monoxide difference spectrum (arbitrary units), for* Littorina littorea *digestive gland microsomal samples. Means and 95% confidence intervals of log-transformed data from: a) Langesundfjord sites 1-4 (n = 8 replicate tissue pools per site, of approx. 6 animals per pool), b) experimental basins C,L,M,H (n = 6).*

PAH-metabolizing enzymes in whole mussels as biochemical tests for chemical pollution monitoring

P Suteau, M Daubeze, M L Migaud and J F Narbonne

Enzyme activities related to PAH metabolism, benzo(a)pyrene hydroxylase (B(a)PH), epoxide hydrolase (EH) and glutathione S-transferase (GST), in subcellular preparations from whole mussels, were assayed as possible biochemical tests for pollution monitoring. No

response was observed along a field pollution gradient in Langesundfjord, owing to the variability within each biochemical measurement and to the limited number of samples/animals assayed per site. In mesocosm exposures to a diesel oil and copper mixture, inductions of B(a)PH and EH activities were related to some extent to the PAH gradient. These specific biochemical variables were more sensitive than a general biochemical variable related to the physiological state of the animal, the cytosolic glutathione content.

Hepatic microsomal mono-oxygenase activity in flounder (*Platichthys flesus*) from polluted sites in Langesundfjord and from mesocosms experimentally dosed with diesel oil and copper

R F Addison and A J Edwards

Hepatic ethoxyresorufin O-de-ethylase (EROD) and benzo(a)pyrene hydroxylase (B(a)PH) activity in flounder (*Platichthys flesus*) increased along a pollution gradient in Langesundfjord. EROD activity in fish from a reference site was around 100 pmoles min^{-1} mg microsomal $protein^{-1}$, increasing five-fold at two moderately polluted sites and a further two-fold at a more highly polluted site. B(a)PH activity was around 30 pmoles min^{-1} mg $protein^{-1}$ in fish from the reference site, and was more than doubled at the polluted sites. Liver residue concentrations of several industrially-derived chlorinated hydrocarbons also increased along the pollution gradient. The data support the conclusion that fish hepatic mono-oxygenase activity may indicate exposure to organic pollutants in the field.

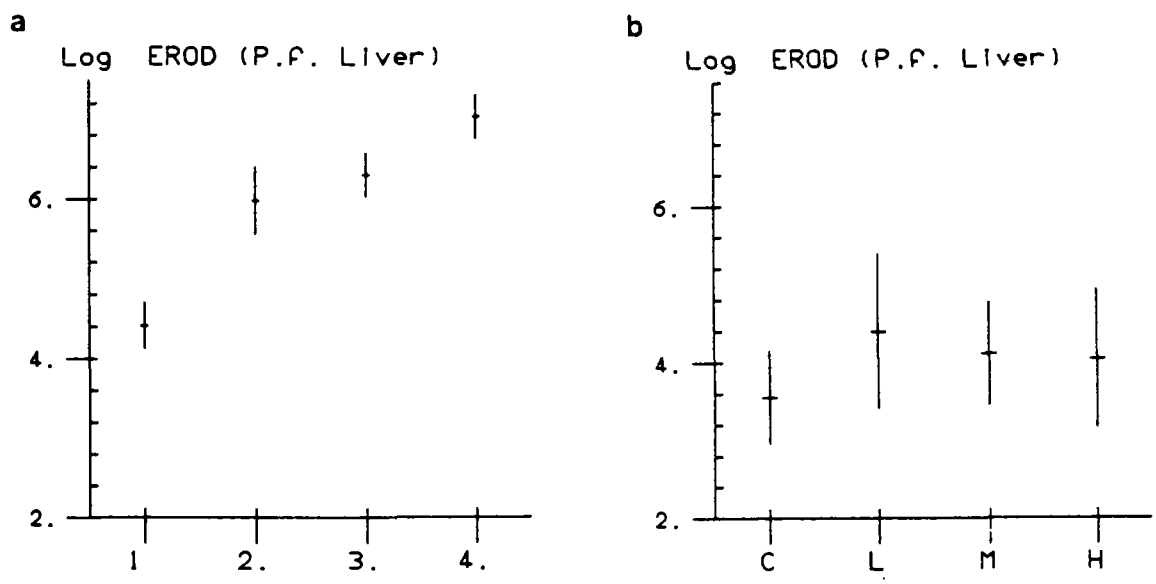

Fig. 4. **Addison and Edwards.** *Ethylresorufin O-deethylase (EROD) activity, in pmoles min^{-1} (mg microsomal protein)$^{-1}$, for* Platichthys flesus *liver microsomal samples. Means and 95% confidence intervals of log_e-transformed data from: a) Langesundfjord sites 1-4 (n = 11,5,12,12 fish respectively), b) experimental basins C,L,M,H (n = 11,4,9,5 fish).*

Exposure of flounder to diesel oil and copper in mesocosms over several months did not induce EROD or B(a)PH. Enzyme activities were all below those at the field reference site (EROD and B(a)PH around 70 and 20 pmoles min^{-1} mg $protein^{-1}$ respectively). Total PAH concentrations in liver (fluorescence measurements) did not vary with treatment, suggesting that the experimentally exposed fish did not accumulate inducing compounds.

Apparent cytochrome P-450 induction as an indication of exposure to environmental chemicals in the flatfish *Platichthys flesus*

J J Stegeman, B R Woodin and A Goksoyr

Flounder (*Platichthys flesus*) from a pollution gradient in Langesundfjord, Norway, and from experimental exposures to diesel oil in mesocosm basins, were analyzed for evidence of specific cytochrome P-450 induction. Ethoxyresorufin O-de-ethylase (EROD) activity and the content of microsomal cytochrome P-450 in liver were positively correlated with the field pollution gradient, as indicated by residues of PAH and PCB in mussels at the four sites. Monoclonal antibody 1-12-3 to the PAH- and PCB-inducible scup P-450 isozyme (P-450E) recognized a single protein band in *P. flesus* liver microsomes. The amount of this protein correlated positively with levels of EROD activity and microsomal P-450 content in the field-sampled fish. By contrast, fish from control and treated mesocosm basins all possessed relatively low levels of EROD activity and immunodetected protein. Levels of high molecular weight PAH known to induce teleost P-450 were likewise low in these basins, although there was a high content of other aromatics. We conclude that both EROD activity and levels of the *P. flesus* counterpart to P-450E indicate induction by environmental chemicals in *P. flesus* from Langesundfjord.

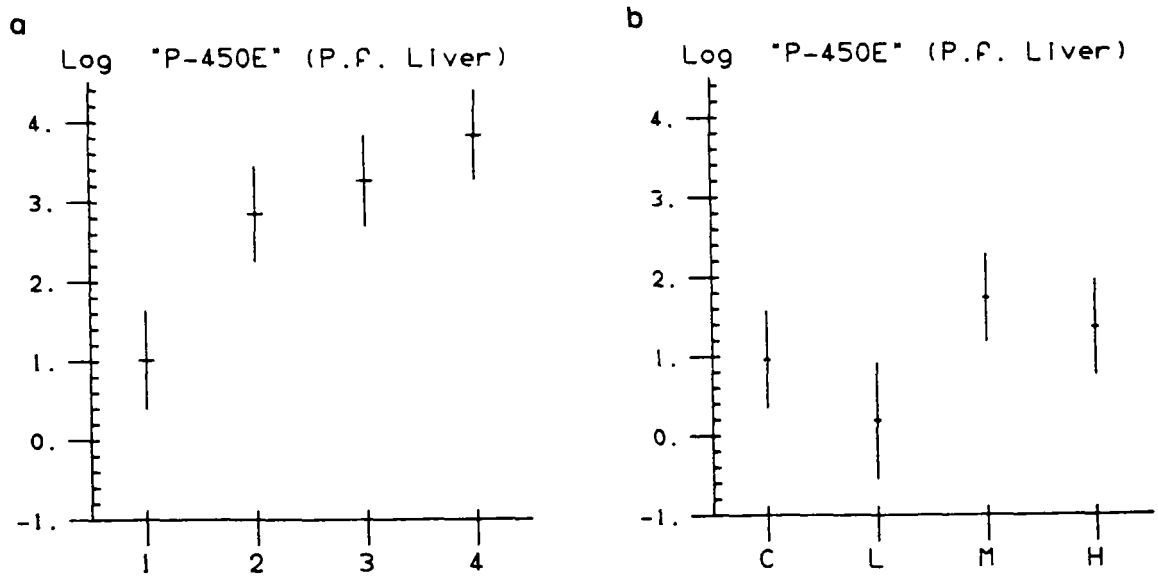

Fig. 5. Stegeman and Woodin. *Immunoblot analysis of* Platichthys flesus *liver microsomes with monoclonal antibody 1-12-3 to scup cytochrome P-450E. Means and 95% confidence intervals of $\log_e$-transformed P-450E equivalents (pmol mg^{-1}) from: a) Langesundfjord sites 1-4 (n = 10,12,11,12 fish respectively), b) experimental basins C,L,M,H (n = 10,7,12,11 fish).*

Intestinal glutathione S-transferase activity in flounder *Platichthys flesus* collected from polluted and reference sites in Langesundfjord, Norway

P A Van Veld and R F Lee

Intestinal glutathione S-transferase activities were measured in flounder *Platichthys flesus* collected from Langesundfjord, Norway. Though enzyme activities were generally higher in fish collected from three polluted sites by comparison with a reference site, the only significant differences in activities between the three sites ran counter to the anticipated pollution gradient. The results suggest that intestinal glutathione S-transferase activity may not be a sensitive indicator of pollution exposure under field conditions.

Integrated cellular stress indices in trace metal contamination: their critical evaluation in a field study

A Viarengo, G Mancinelli, G Martino, M Pertica,
L Canesi and A Mazzucotelli

The metal bound to metallothioneins and cytosolic proteins significantly increased in the digestive gland of mussels exposed in a mesocosm experiment to different levels of a copper and diesel oil mixture. Analysis performed on mussels sampled at four field sites in Langesundfjord, Norway showed that these animals were contaminated by metals, although to a limited degree. The concentration of metal bound to thioneins was essentially the same in the digestive gland extracts from mussels sampled at reference and polluted sites, but the values of copper bound to cytosolic proteins significantly increased at the field sites characterized by heavier metal pollution. These results indicate that the parameters studied (metals bound to cytosolic proteins and to metallothioneins) can be considered as satisfactory integrated stress indices in evaluating the biological impact of heavy metal contamination.

It was also shown that calcium concentration significantly increased in the digestive gland of mussels exposed to pollutants in the field as well as in the highest dosed mesocosm basins. The possibility of further application of this finding as a general stress index is discussed.

Metallothionein and metal levels in flounder (*Platichthys flesus*) from four sites in Frierfjord (Norway) and in flounder dosed with water-borne copper

J Overnell and M Abdullah

Concentrations of metallothionein (MT) and of zinc, copper, iron, manganese, cadmium and lead were measured in liver and kidney of flounder from four sites in Langesundfjord, subject to a pollution gradient, and from four mesocosm basins dosed with varying levels of copper and diesel oil. Little difference was found between the MT levels in samples from the three polluted field sites, though kidney MT levels were significantly higher than at the field reference site.

Tissue zinc levels were highest in the most heavily polluted fjord site but there was no correlation between hepatic MT and total hepatic zinc. Copper in the mesocosm basins was not taken up by the fish and deposited in the livers nor was liver or kidney MT elevated by the treatment.

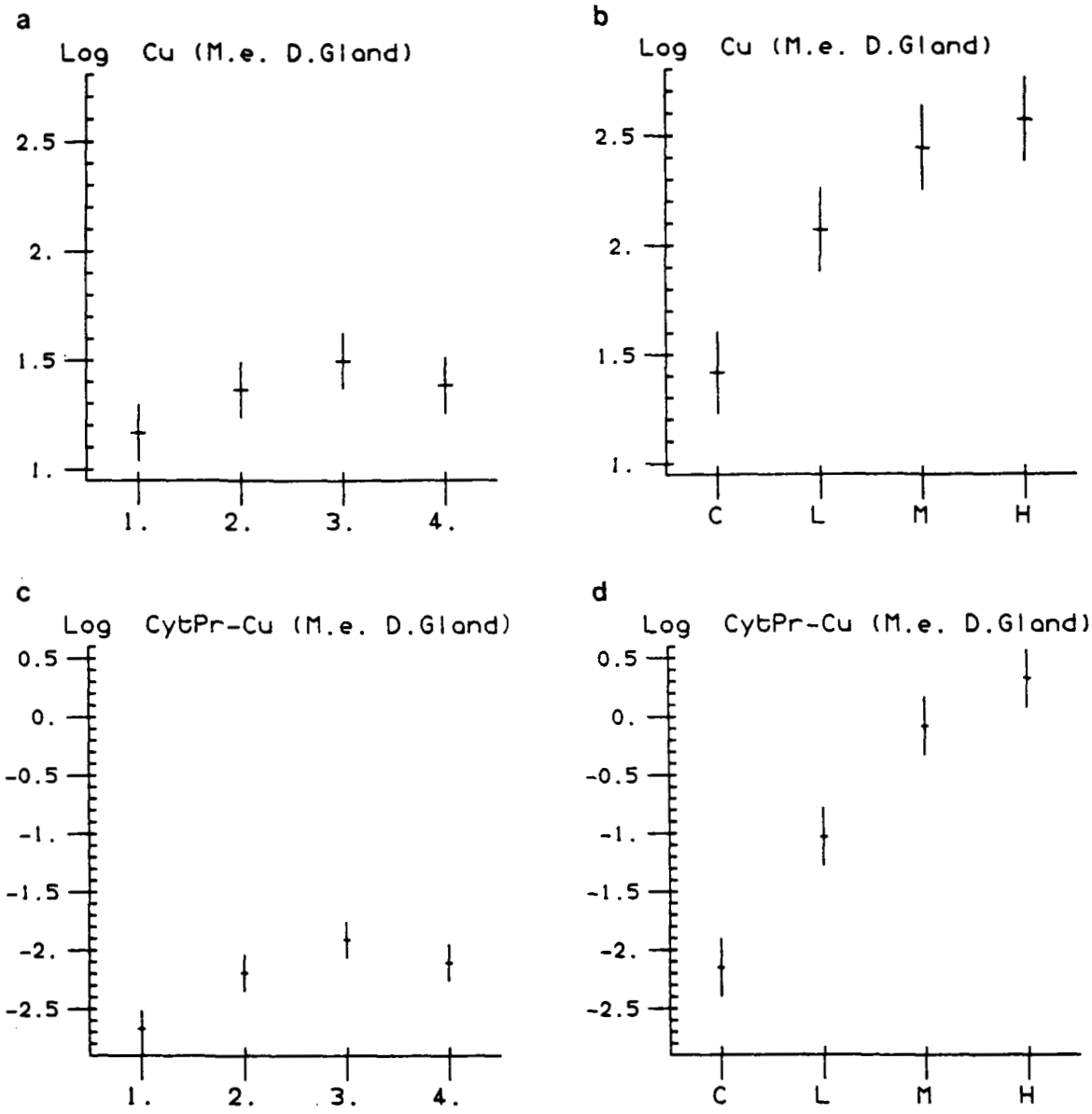

Fig. 6. **Viarengo et al.** *a),b) Cu concentration (µg g^{-1} wet wt.) and c),d) Cu bound to cytosolic proteins (µg g^{-1} wet wt.), in* **Mytilus edulis** *digestive gland tissue. Means and 95% confidence intervals of $\log_e$-transformed concentrations, based in all cases on n = 4 replicate tissue pools, each of 10-12 animals, from: a),c) Langesundfjord sites 1-4, b),d) experimental basins C,L,M,H.*

3. CELLULAR AND HISTO-PATHOLOGICAL METHODS

Cytochemical responses of the lysosomal system and NADPH-ferrihemoprotein reductase, in the digestive cells of the mussel *Mytilus edulis* and the periwinkle *Littorina littorea*, to environmental and experimental exposure to xenobiotics

M N Moore

Lysosomal characteristics and smooth endoplasmic reticulum (SER) associated NADPH-ferrihemoprotein (cytochrome P-450) reductase were measured cytochemically in the digestive cells of mussels and periwinkles exposed to environmental contaminants in Langesundfjord, Norway (PAHs, PCBs, metals) and to xenobiotics in an experimental facility (diesel oil and copper mixture). Lysosomal membrane stability was reduced in both mussels and periwinkles with increasing xenobiotic contamination in the field. Lysosomal and cytoplasmic unsaturated neutral lipid concentration was increased in contaminated mussels in the field and the lysosomal accumulation was associated with enlargement of the secondary lysosomes; the latter was inversely correlated with lysosomal membrane stability. The lipofuscin content and number of tertiary lysosomes was increased in the contaminated mussels in the field. These pathological changes are indicative of enhanced lysosomal autophagy and fatty degeneration. NADPH-ferrihemoprotein reductase activity was elevated in mussels from the two most heavily contaminated field sites. Activity of this enzyme was directly correlated with total PAHs.

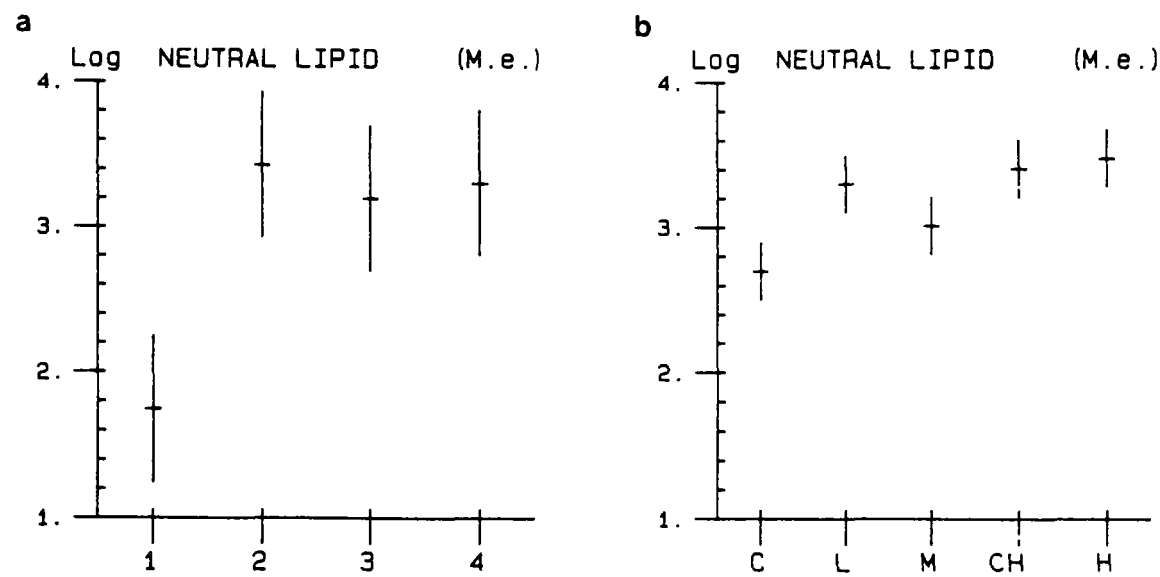

Fig. 7. **Moore.** *Cytoplasmic content of unsaturated neutral lipid (measured as integrated extinction), in* Mytilus edulis *digestive cell lysosomes. Means and 95% confidence intervals from log$_e$-transformed data from: a) Langesundfjord sites 1-4, b) experimental basins C,L,M,H, (n = 10 replicate animals in all cases).*

The lysosomal data from the experimental exposures was less clear and was difficult to interpret. Poor nutritional conditions possibly masked pollutant effects. However, the mussels from the highest exposure concentration showed elevated lipofuscin. The observed sensitivity of the tests for lysosomal enlargement and lysosomal lipid, combined with their relative simplicity, indicate their potential for the detection of environmentally induced pathology, although better understanding of the mechanisms of toxicity would enhance their utility.

Alterations in the cellular structure of mussels resulting from exposure to environmental contaminants under field and experimental conditions

D M Lowe

This study quantified cell and tissue changes in mussels, exposed to diverse contaminants under field and experimental conditions, and correlated the changes with tissue levels of PAHs, PCBs and metals. The results indicated effects on the reproductive tissues in mussels exposed to high levels of contaminants, both in the field and in the mesocosm experiment. High contaminant concentrations also induced the formation of pathologically enlarged secondary lysosomes in the digestive epithelium of mussels and caused a disturbance in lipid levels, resulting in alterations in digestive cell architecture.

Histopathological changes related to chemical contamination in mussels (*Mytilus edulis*) from field and experimental conditions

M Auffret

A comparative histopathological study assessed tissue changes in mussels (*Mytilus edulis*) from a contaminated Norwegian fjord and from mesocosm basins where a contaminant gradient had been simulated. Parasitism by larval trematodes was observed in both circumstances, but was not related to contaminant levels. Mussels from the field exhibited granulocytomas in the interstitial tissues, with a greater incidence at polluted sites than at a reference site, suggesting that granulocytomas could be a consequence of chronic exposure to contaminants. This pathological condition was not observed in experimentally treated mussels, which nevertheless had severe tissue alterations, especially in the digestive tubules and the gills, under exposure to high levels of a diesel oil and copper mixture. It is concluded that this type of histopathological analysis can provide useful information on the health of mussels, and that this can be used successfully in the comparison of field samples.

4. PHYSIOLOGICAL METHODS

Physiological energetics of *Mytilus edulis*: Scope for Growth

J Widdows and D Johnson

Mussels (*Mytilus edulis*) were sampled from four field sites in Langesundfjord and from four experimental groups exposed to a range of water-accommodated diesel oil and copper concentrations in a mesocosm study. Measurements of physiological responses, such as feeding rate, food absorption efficiency, respiration rate and excretion rate, were integrated by means of the energy balance equation and performance was assessed in terms of the "scope for growth". The results showed a significant decline in the scope for growth both along the pollution gradient in Langesundfjord and with increasing exposure to copper and diesel oil in the mesocosm experiment. Feeding rate was the primary component of the energy budget that accounted for the decline in scope for growth with increasing pollution.

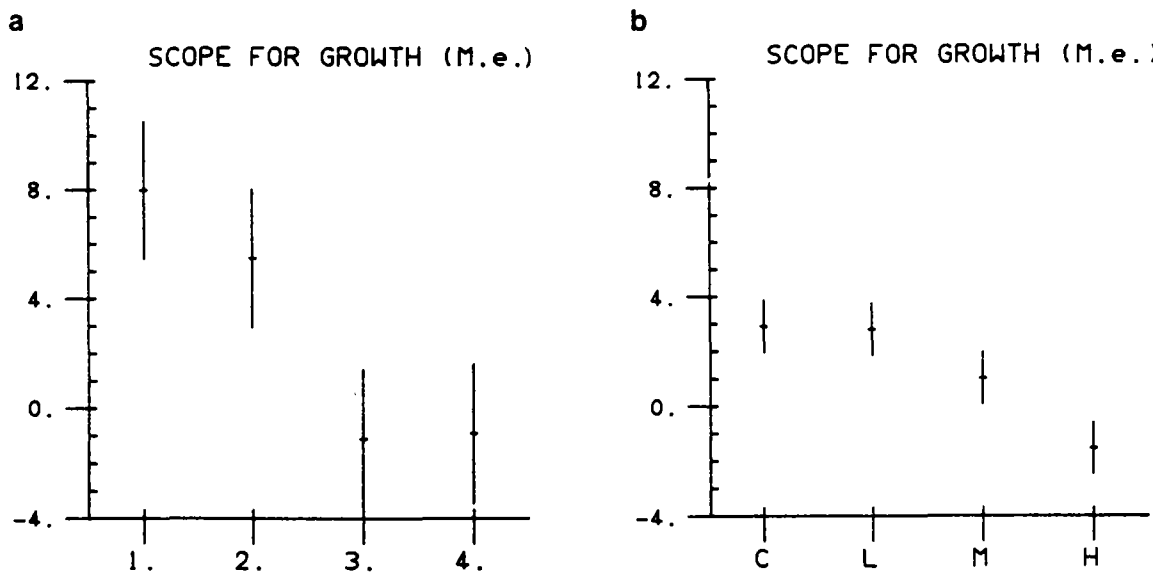

Fig. 8. **Widdows and Johnson.** *"Scope for growth" (J g^{-1} h^{-1}) a net energy balance, for* Mytilus edulis *from: a) Langesundfjord sites 1-4, b) experimental basins C,L,M,H. Values are means and 95% confidence intervals based on n = 16 replicate animals in all cases. (Note that, unlike many of the other biological measures, no transformation was needed to remove variance-mean dependencies.)*

Energy physiology in *Littorina littorea* under combined pollutant stress in field and mesocosms

T Bakke

Rates of energy turnover processes (ingestion, food absorption efficiency, ammonia excretion and respiration) and their integration into an energy budget (scope for growth) was estimated in samples of periwinkles (*Littorina littorea* L.) taken from four populations in

Langesundfjord, Norway, and from four populations which had been kept in mesocosms at different levels of exposure to oil and copper for four months. The physiological measurements separated the most contaminated field population from the others, as having an elevated scope for growth. The measurements failed to discriminate among the mesocosm treatments. The use of energy physiology of *L. littorea* in biological effects monitoring is at present premature, and demands both method development and better understanding of the factors regulating the energy conversion processes.

Physiological measurements on *Nucula tenuis* and on the isolated gills of *Mytilus edulis* and *Carcinus maenas*

B L Bayne and F P Thurberg

Measurements on the deposit-feeding bivalve mollusc *Nucula tenuis* indicated an increase in protein catabolism in the medium and low dose mesocosm basins, registered as increased nitrogen quotients; the implications for pollutant effect assessment are unclear, but further research on deposit-feeding species is warranted. For *Mytilus edulis*, gill oxygen uptake rates did not differ between samples, either from the field or from the mesocosm; oxygen consumption rates of isolated gills correlated with consumption rates for the whole organism, but showed no relationship with clearance rate. The rates of oxygen consumption by the isolated gills of *Carcinus maenas* showed the effects of contamination at one of the field sites, but were unresponsive to contaminant levels within the mesocosm. In these experiments, measures of physiological function in isolated tissues were relatively insensitive to stress from contaminants.

Development of *Mytilus edulis* embryos: a bioassay for polluted waters

D Johnson

Water from the four mesocosm basins was subjected to an embryo development bioassay using *Mytilus edulis*. The method used for the bioassay was based on the standard ASTM method but modified to suit local equipment availability since the bioassay was not originally planned in the Workshop programme. The end point of the bioassay was the development to D-stage which was achieved in the control mesocosm water after 72 hours. Significant ($P = 0.05$) effects were detected in the highest dose water. The Net Treatment Mortality in the medium dose mesocosm (5 $\mu g\ l^{-1}$ copper and 28.5 $\mu g\ l^{-1}$ petroleum hydrocarbons) was 50%; this corresponds well with the EC_{50} for mussel embryo in copper at 5.8 $\mu g\ Cu\ l^{-1}$.

Lipid composition of the digestive glands of *Mytilus edulis* and *Carcinus maenas* in response to contaminant gradients

J McDowell Capuzzo and D F Leavitt

In field studies in Langesundfjord, Norway, changes in lipid content and lipid:protein ratios of digestive glands of *Mytilus edulis* and *Carcinus maenas* were reflected along the pollution gradient, with populations of *Mytilus* showing elevations in both parameters at the

three most contaminated sites (2, 3 and 4) in comparison to the reference site (1), and populations of *Carcinus* showing elevations only at site 3. In mesocosm experiments only *Mytilus* from the high dose basin showed elevations in lipid content and lipid:protein ratios; *Carcinus* in the medium dose basin showed a decrease in both parameters.

Analysis of lipid class composition for field and mesocosm samples of *Mytilus* reveal differences in response to contaminant gradients that reflect alterations in mobilization of triacylglycerols to phospholipid pools, reductions in phospholipid content, and nutritional condition. Changes in lipid class distributions of *Mytilus* from both field and mesocosm experiments correlate well with body burden data for tissue concentrations of aromatic hydrocarbons and/or polychlorinated biphenyls. Lipid class distributions of field samples of *Carcinus* indicate alterations in the mobilization of triacylglycerols, sterol turnover, and reductions in phospholipid content. The responses, which suggest that crabs from site 3 are the most impacted, are not consistent with contaminant data from the field sites. Crabs from mesocosm experiments show no evidence of alterations in lipid class distribution in spite of a consistent trend in aromatic hydrocarbon tissue concentrations along the gradient. Differential responses of field and mesocosm populations of *Mytilus* and *Carcinus* are possibly the result of metabolic capacity for detoxification and differences in trophic transfer.

5. COMMUNITY STUDIES

Analysis of community attributes of the benthic macrofauna of Frierfjord/Langesundfjord, Norway, and in a mesocosm experiment

J S Gray, M Aschan, M R Carr, K R Clarke, R H Green, T H Pearson, R Rosenberg and R M Warwick

The sublittoral macrofauna was sampled along a putative pollution gradient at six sites in Frierfjord/Langesundfjord, Norway. Data were subjected to a variety of multivariate statistical analyses which discriminate between sites on their faunistic attributes, and univariate measures of community stress were determined. The multivariate analyses produced generally similar results. Univariate stress measures in combination ranked the sites in order of increasing disturbance. Measured levels of pollutants in the sediments correlated poorly with the multivariate 2-D configurations and with the univariate measures of disturbance. It was concluded that water depth was the overriding factor controlling community structure, and that this masked any possible effects of pollution. The three deepest sites displayed the most obvious signs of stress, and this was attributed to seasonal anoxia in the deeper parts of the fjord. In order to establish cause and effect relationships between measured levels of pollutants (copper and hydrocarbons) and community responses, box-cores of sublittoral sediment were subjected to four levels of contamination in a mesocosm experiment. Although some of the "classical" intuitive methods indicated possible community responses, the objective methods largely

failed to reveal clear-cut differences in community structure between treatment levels. The relative merits of the "classical" and objective approaches are discussed.

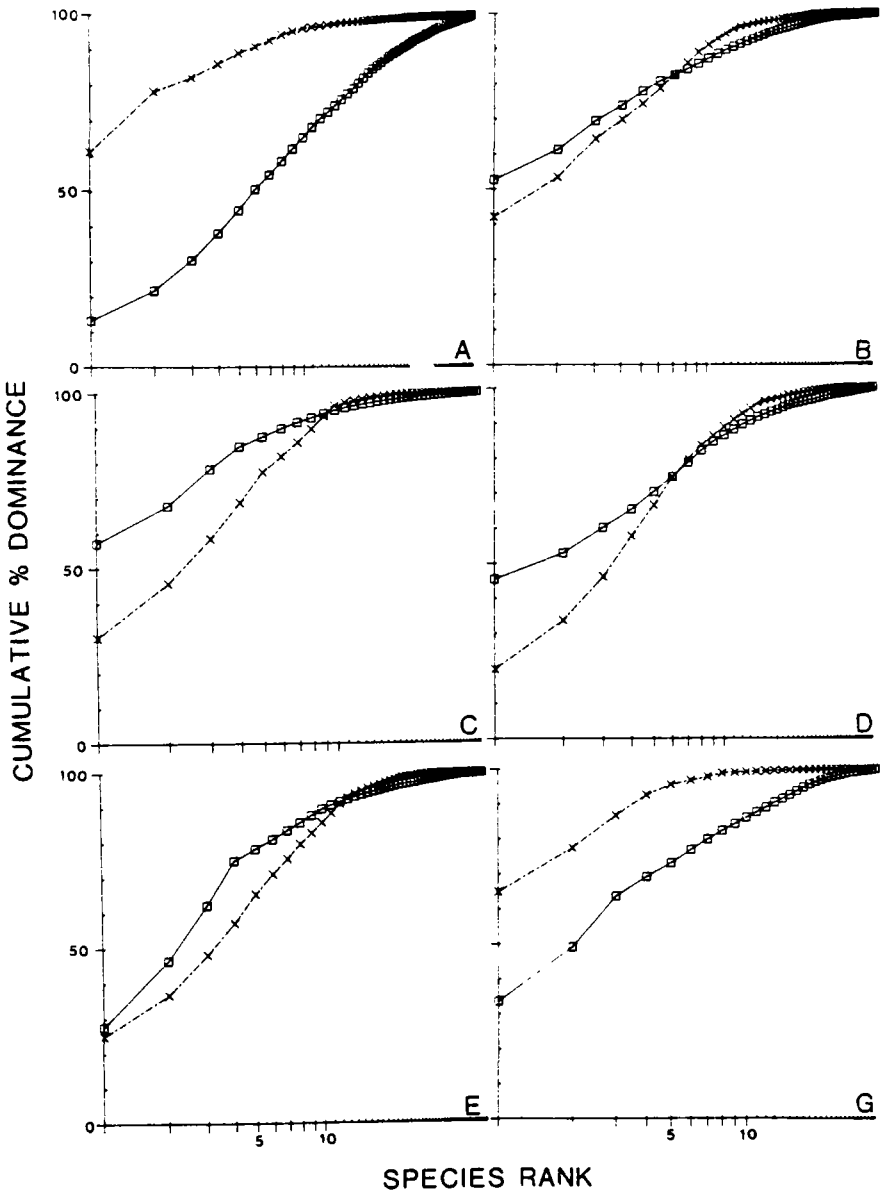

Fig. 9. **Gray et al.** *Abundance-Biomass Comparison (ABC) curves for the benthic macrofauna communities at the six Frierfjord/Langesundfjord sites A-E,G; pooled data from four 0.1 m² Day grabs at each site. The percentage of the pooled sample accounted for by the n most dominant species, in terms of abundance (squares) or biomass (crosses), is plotted against log(n), for n = 1,2,3,...,110. Sites A and G are interpreted as "undisturbed" (biomass curve lies above the abundance curve throughout), B and E as "moderately stress((curves closely coincident) and C and D intermediate between this an(e "grossly stressed" condition (latter inferred if the biomass curve is below the numbers curve throughout).*

Analyses of community attributes of the macrobenthos of Frierfjord/Langesundfjord, Norway, at taxonomic levels higher than species

R M Warwick

Multivariate and univariate analyses were made of macrobenthic species abundances and biomass from Frierfjord/Langesundfjord, Norway, with the data aggregated to family and phylum levels. For families, there was no loss of information compared with the species analyses, which augurs well for the worldwide application of the techniques and for the improvement of their cost-effectiveness. For phyla, multivariate analyses varied in the degree to which they reproduced species and family configurations depending on whether abundance or biomass was used and on the strength of data transformation, but generally the agreement was surprisingly good. It is suggested that the results of multivariate analyses based on higher taxa may more closely reflect gradients of contamination or stress than those based on species data, the latter being more affected by natural ("nuisance") environmental variables.

Analyses of community attributes of the benthic meiofauna of Frierfjord/Langesundfjord, Norway

C Heip, R M Warwick, M R Carr, P M J Herman, R Huys, N Smol and K Van Holsbeke

The sublittoral meiofauna was sampled along a putative pollution gradient at six sites in Frierfjord/Langesundfjord, Norway. Data were subjected to mutivariate statistical analyses which discriminate between sites on their faunistic attributes, and univariate measures of community stress were determined. Most multivariate techniques produced similar results. The copepod component of the meiofauna discriminated between sites better than the nematodes at the species level, but the nematodes were more robust to analyses based on data aggregated to higher taxonomic levels. It is concluded that pollution monitoring at the community level using higher taxonomic groupings of meiofauna is viable, and renders such studies much less time-consuming and more cost-effective than more standard procedures. Appropriate taxonomic levels for the two major meiofaunal taxa (nematodes and copepods) and for the total meiofauna are discussed. There is a paucity of validated univariate measures of community perturbation available for use with meiofauna. Traditional diversity measures were rather uninformative, but the community at one site was identified as being adversely affected by pollution.

A mesocosm experiment on the effects of hydrocarbon and copper pollution on a sublittoral soft-sediment meiobenthic community

R M Warwick, M Carr, K R Clarke, J M Gee and R H Green

Meiofaunal assemblages in undisturbed box-core samples of sublittoral sediment were subjected to four dose levels of pollutants (copper and hydrocarbons) in a mesocosm experiment. Multivariate analyses of species abundance data for nematodes and copepods showed

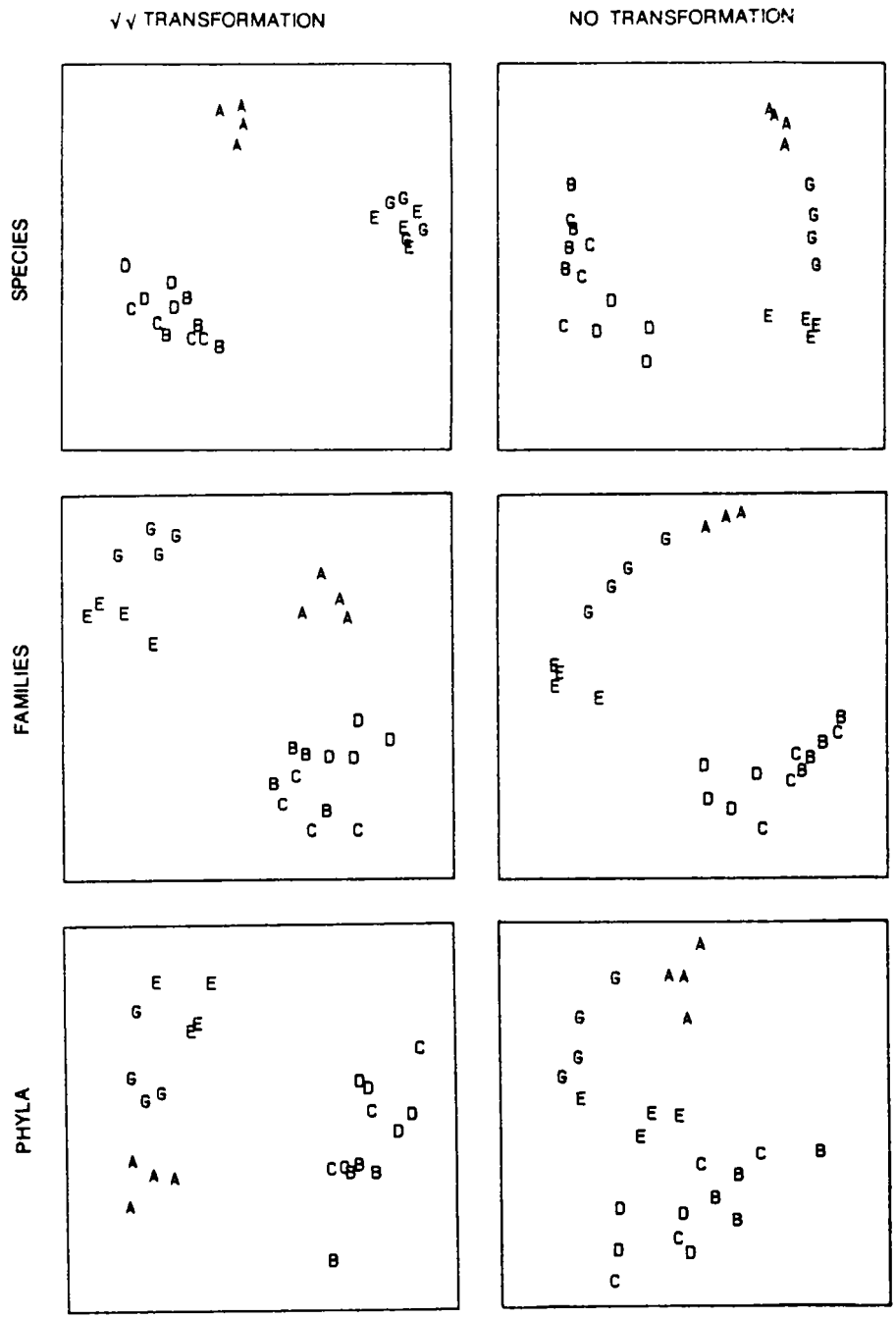

Fig. 10. **Warwick.** *Top figures: non-metric multi-dimensional scaling (MDS) ordinations from the 24 samples x 110 species array of benthic macrofaunal biomasses, from Frierfjord/Langesundfjord sites A-E,G (four replicate grab samples per site). Proximity of samples on a plot implies similarity of species composition; note that the configurations can be arbitrarily rotated or inverted. Data were double root-transformed (left-hand plot) or untransformed (right-hand plot) prior to computation of Bray-Curtis similarities between sites; the double root transform gives more emphasis to less-dominant species. Middle and bottom graphs contrast the same ordination procedures when taxonomic identification is only to the family level (52 families) and to phylum level (8 phyla); the ability to discriminate the clusters A,E,G and (B+C+D) is scarcely reduced at the higher taxonomic levels.*

only marginal differences in species composition between treatments at the end of the experiment, principally between the high-dose treatment and the others. Diversity profiles for the nematodes were virtually unaffected, but copepods showed a graded response of decreasing diversity with increasing dose level, both intrinsically and relative to the nematode diversity profiles. Changes in copepod diversity were brought about by disproportionate increases in abundance of certain species rather than selective losses, a situation which parallels the effects of particulate organic enrichment.

Influence of pollution along a natural gradient and in a mesocosm experiment on sediment microbial numbers and biomass

P Schwinghamer

Microbial communities in sediments from six locations in Frierfjord/Langesundfjord in southern Norway, and from an experimental mesocosm facility at Solbergstrand on the Oslofjord, were sampled and analysed to determine effects of hydrocarbon and metal contamination. The fjord sites showed clear trends of increasing microbial abundance, especially in microflagellates, with increasing distance from known pollution sources. Concentrations of Pb and Mn in the sediments were negatively correlated with microflagellate biomass, and Zn was negatively correlated with bacterial biomass. Results from the mesocosm experiment were less clear.

Influence of pollution along a natural gradient and in a mesocosm experiment on biomass-size spectra of benthic communities

P Schwinghamer

Biomass-size spectra of benthic communities at six sites in Frierfjord/Langesundfjord in southern Norway and in four experimental mesocosm basins at Solbergstrand, Norway, are presented. The fjord sites were selected to represent a contaminant gradient based upon known sources of pollution. Three of the mesocosm basins were exposed to different dosing levels of a mixture of diesel oil and copper for approximately three months and the fourth basin served as a control. The communities from the fjord sites showed differences in their size-structure that could be related, in part, to their proximity to pollution sources. The mesocosm communities showed no significant differences in their size-structure that could be attributed to dosing level.

6. STATISTICS

Statistical design and analysis for a 'biological effects' study

K R Clarke and R H Green

Statistical aspects of "biological effects" field surveys are discussed, with particular reference to the Oslo Workshop. Recommendations are made on design criteria, for example, selection of

sites and samples, and replication strategies (including formulae for sample size determination). The role of transformations is discussed, both for univariate sub-lethal response data and the multivariate data arising from benthic community studies. Statistical analysis is categorised into testing methods, for establishing biological differences between field sites, and descriptive techniques, for representation of those differences. The former includes a non-parametric randomisation test for use with site-species arrays and the latter a survey of various multivariate ordination and clustering methods. A final section outlines a procedure for comparison of different pollution indices, combining their power to detect specific contaminant inputs with their associated "costs".

Towards an ecological framework for investigating pollution

A J Underwood and C H Peterson

Three aspects of the study of effects of pollution in marine systems are discussed. First is the evaluation of relative sensitivities and reliabilities of different methods of detecting pollution, including a brief contrast of processes operating in the mesocosms and in the field. Second is the problem of interpretation of pollution, i.e. determining the importance of the observed effects of pollution to the biological system. Species selected for detecting pollution may not provide useful information about the economic effects on exploited parts of natural systems, nor about trophic structure of a community, nor about future sizes of populations of important species. The choice of appropriate species as indicators or detectors of pollution also requires determination of how representative they are of other species likely to be affected by pollution. Finally, there is the problem of prediction of future consequences of pollution. Some methods used to detect pollutants might be useful as early warnings of future deleterious effects, although the usefulness of these measures may be lessened by the decoupling of reproductive rates of many marine invertebrates from the eventual recruitment to adult populations. Other measures such as patterns in whole assemblages of species can usually only detect pollutants after sufficient time has elapsed for populations to have changed. Nevertheless, these offer more direct measurements of the importance of pollution to the continued functional well-being of the system. A mixture of different types of measures allows the best synthesis of predictive power while providing the most useful information for interpretation of the consequences of pollution to a marine system.

ANNEX III

REFERENCES

Abdullah, M.I., Steffenak, I. (1988). The Oslo Workshop: trace metal analyses. Mar. Ecol. Prog. Ser. (in press)

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. (in press)

Bakke, T. (1988). Energy physiology in *Littorina littorea* under combined pollutant stress in field and mesocosm studies. Mar. Ecol. Prog. Ser. (in press)

Bakke, T., Follum, O.A., Moe, K.A., Sørensen, K. (1988). The Oslo Workshop: a mesocosm experiment. Mar. Ecol. Prog. Ser. (in press)

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: Salenddin, A.S.M., Wilbur, K.M. (eds.) The Mollusca, Vol. 4, Physiology, Part 1. Academic Press, New York

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. Roy. 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. (in press)

Follum, O.A., Moe, K.A. (1988). The Oslo Workshop: field sampling. Mar. Ecol. Prog. Ser. (in press)

George, S.G., Viarengo, A. (1985). A model for heavy metal homeostasis and detoxication in mussels. In: Vernberg, F.J., Thurberg, F.P., Calabrise, A., Vernberg, W. (eds.) Marine Pollution and Physiology: Recent Advances. Univ. S. Carolina Press, Columbia, p. 125-143

GESAMP (1980). Monitoring biological variables related to marine pollution. UNESCO, Paris. Reports and Studies No. 12

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, Norway, and in a mesocosm experiment. Mar. Ecol. Prog. Ser. (in press)

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, Norway. Mar. Ecol. Prog. Ser. (in press)

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

ICES (1978). On the feasibility of effects monitoring. International Council for the Exploration of the Sea, Co-operative Research Report No. 75.

Klungsøyr, J., Wilhelmsen, S., Westrheim, K., Saetvedt, E., Palmork, K.H. (1988). The Oslo Workshop: organic chemical analyses. Mar. Ecol. Prog. Ser. (in press)

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 of the digestive gland of the mussel, *Mytilus edulis*, and the periwinkle *Littorina littorea*, to environmental and experimental exposure to pollution. Mar. Ecol. Prog. Ser. (in press)

Lowe, D.M. (1988). Alterations in the cellular structure of mussels resulting from exposure to environmental contaminants under field and experimental conditions. Mar. Ecol. Prog. Ser. (in press)

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

McIntyre, A.D. (1984). What happened to biological effects monitoring? Mar. Pollut. Bull. 15: 391-392

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 a 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. Roy. Soc. Lond. B. (in press)

Moore, M.N. (1988). Cytochemical responses of the lysosomal system and NADPH-ferrihemoprotein reductase, in the digestive cells of the mussel *Mytilus edulis* and the periwinkle *Littorina littorea*, to environmental and experimental exposure to xenobiotics. Mar. Ecol. Prog. Ser. (in press)

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

Schwinghamer, P. (1988). Influence of pollution along a natural gradient and in a mesocosm experiment on sediment microbial numbers and biomass. Mar. Ecol. Prog. Ser. (in press)

Sheehan, P.J., Miller, D.R., Butler, G.L., Bourdeau, P., Ridgeway, J.M. (ed.) (1984). Effects of pollutants at the ecosystem level. SCOPE 22, Wiley

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). Cytochrome P-450 isozymes as an indication of exposure to environmental chemicals in the flatfish *Platichthys flesus*. Mar. Ecol. Prog. Ser. (in press)

Underwood, A.J., Peterson, C.H. (1988). Towards an ecological framework for investigating pollution. Mar. Ecol. Prog. Ser. (in press)

Van Veld, P.A., Lee, R.F. (1988). Intestinal glutathione S-transferase activity in flounder *Platichthys flesus* collected from polluted and reference sites in Langesundfjord, Norway. Mar. Ecol. Prog. Ser. (in press)

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

Warwick, R.M. (1988). Analysis of community attributes of the macrobenthos of Frierfjord/Langesundfjord, Norway, at taxonomic levels higher than species. Mar. Ecol. Prog. Ser. (in press)

Warwick, R.M., Carr, M.R., Clarke, K.R., Gee J.M., Green, R.H. (1988). A mesocosm experiment on the effects of hydrocarbon and copper pollution on a sublittoral soft-sediment meiobenthic community. Mar. Ecol. Prog. Ser. (in press)

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

Widdows, J., Johnson, D. (1988). Physiological energetics of *Mytilus edulis*: Scope for Growth. Mar. Ecol. Prog. Ser. (in press)

ANNEX IV

LIST OF PARTICIPANTS

1. CHEMISTRY

M Abdullah, Department of Marine Zoology and Marine Chemistry, Biology Institute, University of Oslo, P.O. Box 1064, 0316 Blindern, Oslo 3, NORWAY

K H Palmork, Institute of Marine Research, Nordnesparken 2, 5011 Nordnes-Bergen, NORWAY

2. BIOCHEMISTRY

R F Addison, Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, Novia Scotia B2Y 4A2, CANADA

A J Edwards, Department of Pharmacology, Dalhousie University, Halifax B3H 4H7, CANADA

R F Lee, Skidaway Institute of Oceanography, P.O. Box 13687, Savannah, Georgia 31416, USA

D R Livingstone, Plymouth Marine Laboratory (West Hoe), Prospect Place, The Hoe, Plymouth PL1 3DH, UK

J F Narbonne, Laboratory of Food Toxicology, University of Bordeaux I, Avenue des Facultes, 33405 Talence Cedex, FRANCE

J Overnell, Institute of Marine Biochemistry, St. Fittick's Road, Aberdeen AB1 3RA, Scotland, UK

J J Stegeman, Biology Department, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, USA

P Suteau, Laboratory of Food Toxicology, University of Bordeaux I, Avenue des Facultes, 33405 Talence Cedex, FRANCE

P Van Veld, Grace Cancer Drug Center, Roswell Park Memorial Institute, Buffalo, NY 14263, USA

A Viarengo, Institute of General Physiology, Corso Europa 26, 16132 Genova, ITALY

3. CELLULAR AND HISTO-PATHOLOGY

M Auffret, Laboratory of Aquatic Animal Pathology, IFREMER, BP 337, 29273 Brest Cedex, FRANCE

M N Moore, Plymouth Marine Laboratory (West Hoe), Prospect Place, The Hoe, Plymouth PL1 3DH, UK

D M Lowe, Plymouth Marine Laboratory (West Hoe), Prospect Place, The Hoe, Plymouth PL1 3DH, UK

4. PHYSIOLOGY

T Bakke, Norwegian Institute for Water Research, P.O. Box 333, Blindern, Oslo 3, NORWAY

B L Bayne, Plymouth Marine Laboratory (West Hoe), Prospect Place, The Hoe, Plymouth PL1 3DH, UK

D Johnson, Water Research Centre, Environment Laboratory, Henley Road, Medmenham, Marlow, Buckinghamshire, SL7 2HD, UK

J McDowell Capuzzo, Department of Biology, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, USA

F P Thurberg, National Marine Fisheries Service NOAA, Northeast Fisheries Centre, Milford Laboratory, Milford, CT 06460, USA

J Widdows, Plymouth Marine Laboratory (West Hoe), Prospect Place, The Hoe, Plymouth PL1 3DH, UK

5. COMMUNITY STUDIES

J S Gray, Department of Marine Zoology and Marine Chemistry, Biology Institute, University of Oslo, P.O. Box 1064, 0316 Blindern, Oslo 3, NORWAY

C Heip, Delta Institute for Hydrobiological Research, Vierstraat 28, 4401EA Yerseke, THE NETHERLANDS

T H Pearson, Scottish Marine Biological Association, Dunstaffnage Marine Research Laboratory, Oban, Argyll, Scotland, UK

C H Peterson, Institute of Marine Sciences, University of North Carolina at Chapel Hill, Morehead City, North Carolina 28557, USA

R Rosenberg, Fishery Board of Sweden, Institute of Marine Research, S-45300 Lysekil, SWEDEN

P Schwinghamer, Department of Fisheries and Oceans, Science Branch, P.O. Box 5667, St. John's, Newfoundland A1C 5X1, CANADA

A J Underwood, Institute of Marine Ecology, Zoology Building, University of Sydney, NSW 2006, AUSTRALIA

R M Warwick, Plymouth Marine Laboratory (West Hoe), Prospect Place, The Hoe, Plymouth PL1 3DH, UK

6. STATISTICS

M R Carr, Plymouth Marine Laboratory (West Hoe), Prospect Place, The Hoe, Plymouth PL1 3DH, UK

K R Clarke, Plymouth Marine Laboratory (West Hoe), Prospect Place, The Hoe, Plymouth PL1 3DH, UK

R H Green, Department of Zoology, University of Western Ontario, London, Ontario N6A 5B7, CANADA

7. ORGANISATIONAL REPRESENTATIVES

M Auffret (ICES) - see above for address

Li Fengchun (UNEP), Third Institute of Oceanography, P.O. Box 70, Xiamen, Fujian, CHINA

E R Long (NOAA), NOAA National Ocean Service, Ocean Assessments Division, Pacific Office, 7600 Sand Point Way, NE BIN C 15700, Seattle, Washington 98115, USA

S Soria (UNEP), Marine Science Centre, University of the Philippines, Diliman, Quezon City, Metro Manila, THE PHILIPPINES

8. LOCAL SUPPORT

M Aschan, Biology Insitute, University of Tromsø, 9000 Tromsø, NORWAY

O A Follum, SFT, P.O. Box 8100 Dep, 0032 Oslo 1, NORWAY

J Klungsøyr, Institute of Marine Research, Nordnesparken 2, 5011 Nordnes-Bergen, NORWAY

K A Moe, CMS, Billingstadsletten 19, 1362 Billingstad, NORWAY

K Sørensen, Norwegian Institute for Water Research, P.O. Box 333, Blindern, Oslo 3, NORWAY

I Steffenak, Department of Marine Zoology and Marine Chemistry, Biology Institute, University of Oslo, P.O. Box 1064, 0316 Blindern, Oslo 3, NORWAY

9 SECRETARIAT

G Kullenberg, Intergovernmental Oceanographic Commission, 7, place de Fontenoy, 75700 Paris, FRANCE.

No.	Title	Publishing Body	Languages
32 Suppl.	Papers submitted to the UNU/IOC/Unesco Workshop on International Co-operation in the Development of Marine Science and the Transfer of Technology in the Context of the New Ocean Regime Paris, 27 September-1 October 1982	IOC, Unesco Place de Fontenoy 75700 Paris, France	English
33	Workshop on the IREP Component of the IOC Programme on Ocean Science in Relation to Living Resources (OSLR) Halifax, 26-30 September 1983	IOC, Unesco Place de Fontenoy 75700 Paris, France	English
34	IOC Workshop on Regional Co-operation in Marine Science in the Central Eastern Atlantic (Western Africa) Tenerife 12-17 December 1983	IOC, Unesco Place de Fontenoy 75700 Paris, France	English French Spanish
35	CCOP/SOPAC-IOC-UNU Workshop on Basic Geo-scientific Marine Research Required for Assessment of Minerals and Hydrocarbons in the South Pacific Suva, Fiji, 3-7 October 1983	IOC, Unesco Place de Fontenoy 75700 Paris, France	English
36	IOC/FAO Workshop on the Improved Uses of Research Vessels Lisbon, 28 May - 2 June 1984	IOC, Unesco Place de Fontenoy 75700 Paris, France	English
36 Suppl.	Papers submitted to the IOC-FAO Workshop on Inproved Uses of Research Vessels Lisbon, 28 May-2 June 1984	IOC, Unesco Place de Fontenoy 75700 Paris, France	English
37	IOC/Unesco Workshop on Regional Co-operation in Marine Science in the Central Indian Ocean and Adjacent Seas and Gulfs Colombo, 8-13 July 1985	IOC, Unesco Place de Fontenoy 75700 Paris, France	English
38	IOC/ROPME/UNEP Symposium on Fate and Fluxes of Oil Pollutants in the Kuwait Action Plan Region Basrah, Iraq, 8-12 January 1984	IOC, Unesco Place de Fontenoy 75700 Paris, France	English
39	CCOP (SOPAC)-IOC-IFREMER-ORSTOM Workshop on the Uses of Submersibles and Remotely Operated Vehicles in the South Pacific Suva, Fiji, 24-29 September 1985	IOC, Unesco Place de Fontenoy 75700 Paris, France	English
40	IOC Workshop on the Technical Aspects of Tsunami Analyses, Prediction and Communications Sidney, B.C., Canada, 29-31 July 1985 (in press)	IOC, Unesco Place de Fontenoy 75700 Paris, France	English
41	First Workshop of Participants in the Joint FAO/IOC/WHO/IAEA/UNEP Project on Monitoring of Pollution in the Marine Environment of the West and Central African Region (WACAF/2) Dakar, Senegal, 28 October - 1 November 1985	IOC, Unesco Place de Fontenoy 75700 Paris, France	English
42	IOC/UNEP Intercalibration Workshop on Dissolved/Dispersed Hydrocarbons in Seawater Bermuda, USA, 3-14 December 1984 (in press)	IOC, Unesco Place de Fontenoy 75700 Paris, France	English
43	IOC Workshop on the Results of MEDALPEX and Future Oceanographic Programmes in the Western Mediterranean Venice, Italy, 23-25 October 1985	IOC, Unesco Place de Fontenoy 75700 Paris, France	English
44	IOC/FAO Workshop on Recruitment in Tropical Coastal Demersal Communities Ciudad del Carmen, Campeche, Mexico, 21-25 April 1986	IOC, Unesco Place de Fontenoy 75700 Paris, France	English Spanish
45	IOCARIBE Workshop on Physical Oceanography and Climate Cartagena, Colombia, 19-22 August 1986	IOC, Unesco Place de Fontenoy 75700 Paris, France	English
46	Reunión de Trabajo para Desarrollo del Programa «Ciencia Oceanica en Relación a los Recursos No vivos en la Región del Atlantico Sudoccidental Porto Alegre, Brazil 7-11 de Abril de 1986 (in press)	IOC, Unesco Place de Fontenoy 75700 Paris, France	Spanish
47	IOC Symposium on Marine Science in the Western Pacific: The Indo-Pacific Convergence Townsville, 1-6 December 1986 (in press)	IOC, Unesco Place de Fontenoy 75700 Paris, France	English
48	IOCARIBE Mini-Symposium for the Regional Development of the IOC-UN (OETB) Programme on "Ocean Science in Relation to Non-Living Resources (OSNLR)"	IOC, Unesco Place de Fontenoy 75700 Paris, France	English Spanish
49	AGU-IOC-WMO-CPPS Chapman Conference: An International Symposium on "El Niño" Guyaquil, Ecuador, 27-31 October 1986	IOC, Unesco Place de Fontenoy 75700 Paris, France	English
50	CCAMLR-IOC Scientific Seminar on Antarctic Ocean Variability and its Influence on Marine Living Resources, particularly Krill (organized in collaboration with SCAR and SCOR) Paris, France, 2-6 June 1987	IOC, Unesco Place de Fontenoy 75700 Paris, France	English
51	CCOP/SOPAC-IOC Workshop on Coastal Processes in the South Pacific Island Nations, Lae, Papua-New Guinea, 1-8 October 1987	IOC, Unesco Place de Fontenoy 75700 Paris, France	English