Intergovernmental Oceanographic Commission
Workshop Report No. 61

Second IOC Workshop on the Biological Effects of Pollutants

Bermuda, 10 September-2 October 1988

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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
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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
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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	IOC, Unesco Place de Fontenoy 75700 Paris, France	English (superseded by IOC Technical Series No. 22)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

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1. INTRODUCTION

The IOC-GIPME/GEEP Bermuda Workshop was held at the Bermuda Biological Station for Research, Inc. Ferry Reach, Bermuda (BBS) from September 10-October 2 1988. The Workshop had two main objectives:

(i) to apply, in a sub-tropical ecosystem, indices which had proved successful at a previous IOC-GIPME/GEEP Workshop in Oslo, Norway in August 1986, for assessing the biological impact of marine contamination, and to extend the range of techniques evaluated;

(ii) to offer advanced training in these approaches to a number of scientists from the IOC Regional Programmes, who might act as contacts for the organisation of appropriate training workshops in their own countries.

A list of participants who attended for some or all of the Workshop is appended as Annex I.

Bermuda was felt to be an appropriate location to further GIPME-GEEP's aims, in two key respects. Firstly, the anticipated low levels of contaminant inputs to the marine environment of Bermuda would provide a severe test of the sensitivity of the chosen biological effects techniques, in giving early warning of environmental deterioration. Secondly, Bermuda is sub-tropical and the impact of pollution on sub-tropical ecosystems is generally less well understood than in temperate or sub-Arctic ecosystems. Thus, the format of the Bermuda Workshop differed from that in the previous workshop in Oslo. While the Oslo Workshop had involved participants in applying their techniques to sample types which were familiar to them, these techniques could not be applied indiscriminately to samples from sub-tropical ecosystems. The Bermuda Workshop therefore required considerably more research on the "calibration" of responses in local marine organisms so that data from the field could be interpreted sensibly. In essence, this meant that more experimental and laboratory studies were required in Bermuda than in Oslo.

In addition, the provision of advanced training was an important consideration in Bermuda. The laboratory "calibration" studies were a convenient vehicle for this, and these studies were followed by sampling and analysis of field samples. The training component was enhanced by a series of seminars on the techniques under examination, which were regularly scheduled throughout the Workshop period; Annex II gives the seminar titles.

The Workshop was therefore structured as follows:

Week 1: Setting up of laboratories, acquisition of experimental test organisms and calibration of responses under lab. conditions. Transplantation of test organisms from clean to contaminated sites.

Week 2: Acquisition and analysis of field samples.

Week 3: Recovery and analysis of transplanted animals from field sites, completion of lab studies and concluding discussions.

The biological studies fell logically into three main areas:

(i) responses at the biochemical or sub-cellular level (for example, studies of the mono-oxygenase system and metal-binding proteins in reef fish);

(ii) responses at the whole organism level (for example, determination of energy balance in a molluscan species and a ciliate water-quality bioassay);

(iii) responses at the community level (both macrofaunal and selected meiofaunal communities were studied).

In addition, experience at Oslo had shown the importance of analytical chemical data describing pollutant distribution, and a fourth major area of activity was therefore to provide such data for inorganic and organic contaminants. Much of these data were provided through the co-operation of GEMSI, under a separate IOC contract with the BBS (see Burns, 1989).

The timetable summarised above varied, obviously, from group to group: those carrying out community response studies, for example, started almost immediately with field sampling while those engaged in biochemical studies tended to emphasize lab. studies at the earlier stages of the Workshop. The details of the field sampling programme are given in Annex III.

The following sections describe the rationale for selecting certain sites for field sampling, and summarize the (preliminary) results and conclusions from the Workshop. The final output will consist of a number of primary scientific papers to be published as a special issue of the Journal of Experimental Marine Biology and Ecology. Abstracts of some of these are attached as Annex IV; final publication is anticipated in early 1990.

2. CHOICE OF FIELD SAMPLING SITES

A major design objective of the field studies was to carry out *integrated* testing of techniques, that is, to select certain 'core' sites at which the full suite of biological effects measures would be deployed. This would facilitate linkage and inter-comparison of, for example, surface film, water column and sediment bioassay results with community structure and bivalve energetic studies, tied in to analytical chemistry of water, sediment and organism samples taken simultaneously at these precise locations.

The ideal sites would therefore be situated in a gradient of both metal and hydrocarbon (or other organic) pollution; they would have "native" populations of fish, macroinvertebrates and benthic infauna and epifauna; the water columns would be of similar depths, they would have similar sediment granulometry and would be amenable to the transplantation of organisms from other locations. The most important of these criteria was the existence of some kind of pollution gradient, and two areas were considered in this context. These were Hamilton Harbour and Castle Harbour (Fig. 2-1).

Existing information suggested that Hamilton Harbour should show a gradient of pollution originating in the town of Hamilton; this might come from cruise shipping, urban run-off, storm sewage, etc. (Domestic sewage, both treated and untreated, is discharged to the North Atlantic: BBS, unpublished data and maps.) One published report (Jickells and Knap, 1984) provided direct evidence for a contamination gradient: a gradient of Cu and Zn and to a lesser extent, Cd, was observed in water and sediments sampled at points westward from inner Hamilton Harbour. Although these samples had been taken in 1982, there was no reason to expect that the situation had changed. No data were available describing sediment hydrocarbon concentrations in this area. The most recent analyses in Hamilton Harbour water (K. Burns, unpublished data) carried out in August 1988 showed no gradient. This may not have been surprising, however, as water column concentrations fluctuate rapidly over short periods, and may reflect recent events such as storms, spills, blow-outs, etc. It was considered that since urban runoff is probably a major source of some contaminant metals such as Cd (Jickells et al., 1986) hydrocarbons might arise from the same source; thus, sediment hydrocarbon concentrations might parallel those of some metals.

The disadvantage of using Hamilton Harbour as a study site was its high turbidity in some places (due to heavy shipping) and this could result in a dearth of biota, for example in the immediate vicinity of the Hamilton waterfront. However, zones of reasonable depth (10 m approx), which were not in the main shipping channel and likely to support stable benthic communities, could be found. A provisional sampling transect utilising these locations was therefore selected in advance of the Workshop; it comprised a point in inner Hamilton Harbour (expected to be significantly contaminated, H2); two points westward which were expected to be moderately affected (H3 and H4), and two "reference" points. A "harbour reference" location (H5) was chosen closer to the boundary between Hamilton Harbour and the Great Sound, which on the basis of strong tidal movements was expected to be relatively free of contamination. A "lagoon reference" point (H1), outside Hamilton Harbour and the Great Sound, was chosen as further control site (see Fig. 2-1).

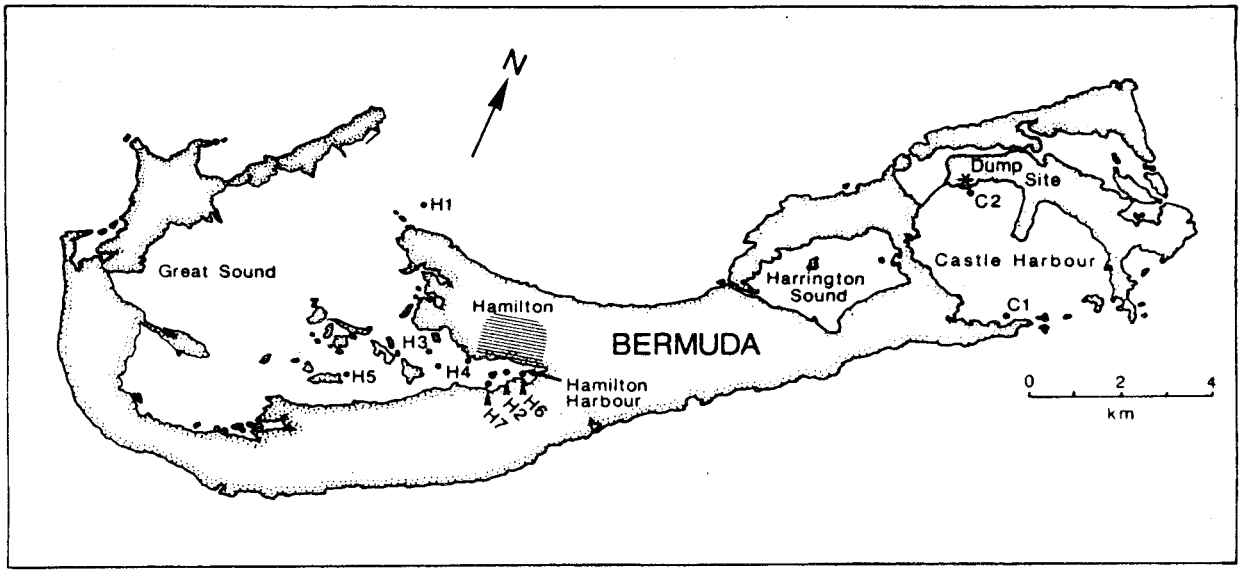

Fig. 2-1. Map of Bermuda, indicating the field sampling sites in Hamilton Harbour (H1-H7) and Castle Harbour (C1,C2).

Mill's Creek (NE Hamilton Hbr.) was considered briefly as a study site. An industrial dump inland and a yacht club and marina in the area would be expected to contribute miscellaneous contaminants including antifouling paints, gasoline, etc. Tidal movements in the area were strong (BBS unpublished data and maps) and the creek is shallow; these factors suggested that conditions in the area would be dynamic and unlikely to lead to any significant contaminant accumulation.

Castle Harbour was also considered. There is a heavy equipment dump inland from the north shore, and previous work (Jickells and Knap 1984) had shown the presence of a sharp (though unstable) Cd gradient in the water off the dump. Furthermore, Dr. Knap had new (unpublished) data describing metal concentrations in sediments in the area which would be made available to GEEP. Since the Cd levels here were similar to those found in the moderately contaminated sites in Frierfjord during the GEEP Oslo Workshop (Abdullah, 1988) it was decided to assess this site and an appropriate 'control' as possible study sites.

In practice, the "similar depth" criterion was met fairly easily at both the proposed Hamilton Harbour and Castle Harbour transects, as most of the Bermuda "platform" is at a fairly constant depth of 10 - 15 m. Granulometry may differ from site to site, however, and since this is known to be an important factor in benthic community determination, care would have to be taken in precise location of sites. Insufficient knowledge existed of native populations of biota in either of the two proposed study areas, so pilot surveys were undertaken by divers on 9-10 Sept 1988. The objective of these surveys was (a) to assess the suitability of these transects as habitats of native biota or for transplants; (b) specifically to examine sediment granulometry along these transects and (c) to obtain some grab samples for preliminary examination of macrofaunal and meiofaunal communities.

The preliminary assessment of Castle Harbour by divers was optimistic. A site close to the dump (<50 m offshore) at a depth of approx. 10 m, had a mud/broken shell bottom with evidence of bioturbation and fish were seen

occasionally in the area. A core sample (25 cm^2) taken by divers contained appreciable numbers of nematode species, as revealed in more detailed examination later. At a second (reference) site in Castle Harbour various macroinvertebrates, including holothurians were observed. A grab sample of sediment from that site contained various small bivalves; these too were retained for further analysis. A cage of *Arca zebra* was deployed at this site, with the aim of testing the feasibility of its recovery within two days. Reports from the divers suggested that although fish were not routinely found over the muddy bottoms, they were seen in the adjacent small rocky outcrops ("patch reefs"). In the light of this, a "reference" site (C1) and a "contaminated" site (C2) were chosen, at opposite ends of Castle Harbour, adjacent to patch reefs (see Fig. 2-1).

Due to a combination of factors including ship speed, the closure of a bridge and diving time it was not possible to arrange a survey of both Castle and Hamilton Harbours on the same day. Pilot sampling of Hamilton Harbour was undertaken on Sept. 10, and the first point examined was a potential reference point in Great Sound. This held burrows of the ghost shrimp, and macroinvertebrates (holothurians) were also seen. Sites further towards the inner end of Hamilton Harbour were examined; visibility deteriorated in these (particularly at the innermost site) indicating high turbidity. Ghost shrimp burrows were seen at all sites, though it was not clear if they were occupied. Grab samples and cores were taken at several sites and one cage of mussels was deployed to assess the feasibility of recovery. The efficacy of an "air lift" as a possible sampling device for biota was also assessed.

The preliminary conclusions from the initial surveys of Castle and Hamilton Harbours were that suitable standardized habitats existed at all sampling points for benthic communities (though it was later decided to concentrate the community studies on Hamilton Harbour alone). Coring (by divers) was feasible for meiofaunal sampling. The Shipek grab available at BBS was primarily designed for the collection of chemical samples and was not adequate for obtaining cores of a large enough area to be representative of macrofaunal communities. Instead, it was decided to take 0.05m^2 diameter cores by diver. Transplantation of mussels to any of the sites surveyed seemed feasible. Although fish were scarce over mud bottoms, the patch reefs provided a habitat at which native fish populations might be trapped.

These observations and conclusions were reviewed at a meeting of all Workshop participants on the evening of Sept. 10. The general approach of sampling in two general areas, Castle and Hamilton Harbours, was approved, though with the emphasis placed on Hamilton Harbour where a stronger organic (and organo-metal) gradient was anticipated. The main sampling effort in Castle Harbour took place, at two sites, on Sept. 11 and 22 Sept, and in Hamilton Harbour, at seven sites, on 12, 19, 23 and 24 Sept. The detailed survey closely followed the planned pattern, though the Hamilton Harbour transect was extended by adding two sites, H6 and H7 (Fig. 2-1), principally for greater discrimination of benthic community changes. There were one or two other additions and amendments to answer specific questions arising during the Workshop.

At each of the main sampling sites, systematic collections were made of macrofaunal and meiofaunal benthic communities, sediment samples for chemistry and sediment bioassay, water samples at various depths for chemistry and ciliate bioassay, transplantation and recovery of (caged) mussels, *Arca zebra*, and (where found) reef fish for biochemical analysis and holothurians for lipid and chemical analysis. Full details of the sampling programme are given in Annex III.

3. CHEMICAL ANALYSES

3.1 INTRODUCTION

A basic tenet of GEEP's philosophy is that the assessment of biological effects of pollutants in the marine environment should proceed hand in hand with chemical studies of the levels of relevant contaminants. The Bermuda Workshop therefore followed the pattern of the previous Oslo Workshop in planning for a range of chemical analyses, in particular through collaboration with the IOC/GEMSI group. A substantial part of these analyses, particularly of organic compounds, was carried out under a separate IOC contract to the Bermuda Biological Station; K. Burns and colleagues from BBS took on the responsibility for designing and implementing a detailed strategy for hydrocarbon analysis in sediments and tissues of selected organisms, and also performed some trace metal analyses on sediments. Dr. M. Erhardt (IFM, Kiel) undertook hydrocarbon analyses of the photo-oxidation products of high-volume water samples.

It was also considered important to measure levels of the contaminant tributyl tin (TBT), in view of the large numbers of small boats and frequency of cruise ships in the vicinity of Hamilton Harbour (TBT is a widely used constituent in anti-fouling paints). Dr. G. Bryan (PML, UK) carried out TBT analyses on bottle samples of surface and subsurface waters, and Dr. D. Page (Bowdoin College, Brunswick, Maine) performed TBT analyses of mussel tissues, *A. zebra*, from the transplantation field study.

Trace metal analyses (Cd, Cu, Pb, Zn) for mussel *A. zebra* and holothurian *Isostichopus badionotus* tissues were carried out by S. Soria (Univ. of the Philippines).

3.2 RESULTS

The following table gives a selection of the results of chemical analyses; more detailed descriptions and results can be found in a separate report to IOC (Burns, 1989) and the publications resulting from the Workshop.
Columns in Table 3-1 represent the sampling sites in Hamilton and Castle Harbours, ordered in decreasing distance from the harbour head (thus if a contaminant gradient exists, chemical levels would be expected to be higher for H4-H6 than H1 and H5, with H3 intermediate, and higher for C2 than C1). The rows of Table 3-1 represent the following variables.

1) Ultraviolet fluorescence spectrophotometric (UVF) measurements were made of unfractionated sediment extracts, expressed as equivalents of Arabian Light Crude oil (ALC), µg/g dry wt. (Means based on 3 replicate samples at most sites).

2) Sediment samples were fractionated by high performance liquid chromatography (HPLC) into saturated plus light aromatic and heavy aromatic hydrocarbons, and oxygenated derivatives. (There is particular GEMSI interest in the latter photo-oxidation products.) The level of unresolved hydrocarbons (URE) from a gas chromatographic (GC) analysis of the light aromatic fraction are presented in row 2 (µg/g dry wt); it is a conservative estimate of the amount of petroleum-derived hydrocarbons in the sediment samples.

3) Selected compounds (see Burns, 1989) from the heavier polyaromatic hydrocarbon (PAH) fraction of the sediments were quantified by GC/MS; row 3 gives their total (µg/g dry wt.)

4-6) For whole tissues of mussels, *A. zebra*, subjected to methylene chloride extraction in a Soxhlet apparatus, rows 4-6 of the table give the results of UVF and GC analyses, which broadly parallel those described above for sediments. However, the UVF analyses were more appropriately calibrated with a standard curve for marine diesel oil (MD). Figures are single determinations from pools of c. 10 mussels.

7-8) Holothurian (*I. badionotus*) tissues, subjected to a saponification /hydrolysis extraction procedure, gave the broadly parallel UVF and GC determinations presented in rows 7 and 8. Figures are for single animals at each site.

9) High volume water samples were collected using two SEACHEM samplers, which pump seawater through adsorption columns, allowing "particulate" and "dissolved" phase organics to be extracted separately. Subsequent UVF analysis was calibrated for marine diesel (MD). Row 9 gives the results for the dissolved phase (μg/l).

10) Tributyltin (TBT) levels (μ/g dry wt) were measured in 2 replicate pools of mussel (*A. zebra*) tissue from each transplant site, according to extraction and capillary GC/FID procedures described in Page (1989).

11-12) Water samples (1 l) were taken in Hamilton Harbour of the surface film (0m), using a Garrett Screen, and sub-surface water (0.5m); duplicates were taken for three of the sites, separated by an 11-day period. Organotins were extracted by acidifying with acetic acid and extracting in toluene, quantification being by atomic absorption spectrometry. Results are expressed as concentrations of tin as tributyltin (TBT as ng Sn/l), so should be multiplied by a factor of c. 2.5-2.75 to give concentrations of tributyltin (ng TBT/l).

13-18) Samples of sediment, pooled mussel whole tissue and individual holothurian intestinal tissues were analysed for a suite of heavy metals (Cu, Pb, Cd, Zn). Replication consisted of 8 sediment samples at C2 and 4 replicate mussel pools and 4 holothurians for all sites where material was available. Only the data for Cu and Pb are given in the table (μg/g dry wt).

3.3 DISCUSSION

Organics. Detailed discussion can be found in the report to IOC of Burns (1989), but there is clear evidence of a contamination gradient in Hamilton Harbour in the UVF and GC analyses of sediments (though less pronounced in the lighter aromatic hydrocarbon fraction than for higher molecular weights). *A. zebra* also demonstrate a clear gradient in their uptake of lower molecular weight hydrocarbons, from the Hamilton Harbour transect, over the course of the 11-day transplantation period. Their failure to accumulate clearly differing levels of heavier PAH's across the sites might have been anticipated from the experience of hydrocarbon uptake patterns in the more commonly studied *Mytilus* species.

For Castle Harbour, the data is more equivocal, with no clear evidence of differential organic contamination at the two sites. In general, the holothurian data are too sparse to permit reliable inference.

Table 3-1. Selected chemical levels measured at 7 sites in Hamilton Harbour and 2 sites in Castle Harbour, for water (Wat.), sediment (Sed.), whole tissues of Arca zebra *(A.z.) and intestinal tissues of* Isostichopus badionotus *(I.b.). All units are µg/g dry wt., except water TBT (ng Sn/l) and water UVF MD (µg/l). Where replicate animals/sediment samples were analysed (i.e. not just replicate analytical determinations), the numbers in brackets in the final column represent standard errors of the means in all preceding columns (using a pooled variance estimate).*

	Hamilton Harbour							Castle Harbour		
	H1 (ref)	H5 (ref)	H3	H4	H7	H2	H6	C1 (ref)	C2	(Pooled SE)
Organics										
1 Sed. UVF ALC	-	31	62	741	254	274	128	16	73	(± 46)
2 Sed. GC URE	-	3	7	27	28	37	-	4	7	(± 6)[a]
3 Sed. GC PAH	-	0.1	0.6	11.9	1.5	1.1	-	0.1	0.2	(± 7)[b]
4 *A.z.* UVF MD	5	13	20	42	-	27	-	10	11	-
5 *A.z.* GC URE	8	26	25	58	-	37	-	19	17	-
6 *A.z.* GC PAH	0.5	1.5	0.9	1.1	-	0.7	-	0.5	0.2	-
7 *I.b.* UVF MD	1	16	-	-	5	-	-	3	34	-
8 *I.b.* GC URE	11	38	-	-	-	-	-	1	116	-
9 Wat. UVF MD	-	-	-	2.0	-	-	-	-	0.6	-
Organo-metals										
10 *A.z.* TBT	0.18	0.37	0.56	0.81	-	1.11	-	0.16	0.21	(±0.12)
11 Wat. TBT(0m)	17	35	56	176	34	35	68	-		(± 78)[c]
12 Wat. TBT(½m)	1	3	9	9	22	20	41	-	-	(± 1)[c]
Metals										
13 Sed. Cu	-	10	23	27	70	64	86	1	38	(±2)[d]
14 Sed. Pb	-	58	105	84	157	153	148	3	86	(±8)[d]
15 *A.z.* Cu	7.8	7.9	8.6	9.8	-	9.6	-	6.5	5.7	(±0.7)
16 *A.z.* Pb	3.2	4.9	4.9	6.4	-	7.3	-	1.7	1.8	(±0.6)
17 *I.b.* Cu	6.9	6.4	-	-	5.9	-	-	8.9	6.1	(±1.1)
18 *I.b.* Pb	3.1	6.4	-	-	10.7	-	-	6.0	2.9	(±0.9)

[a] *For C1, C2 and H4 values only, where duplicate samples analysed.*
[b] *Applies to duplicates at H4 only.*
[c] *for H2, H3, H4, where duplicates taken 11 days apart.*
[d] *Applies to C2 values only (8 replicates).*

Organo-metals. TBT is the biocide in many of the current generation of anti-fouling paints, and research in Europe and North America in the last few years has shown it to occur at concentrations toxic to many non-target organisms, resulting in legislation controlling its use. Bermuda does not currently have such legislation and the authorities in Bermuda have requested that the Workshop data be made available to those evaluating the case for control measures.

The water samples show an expected pattern of higher concentrations in surface film than sub-surface, and a clear contamination gradient along the Hamilton Harbour transect; values are comparable with those seen in

contaminated UK marinas before the introduction of legislation. The gradient is particularly clear for sub-surface levels, which also show a remarkably low replication variance in samples taken 11 days apart. By contrast, variability is much higher in surface film concentrations, which are more subject to variations in wind strength and direction.

TBT concentrations in mussel tissues from the transplantation sites in Hamilton Harbour also show a clear contaminant gradient, with high statistical significance (based on the two replicate pools of animals per site), and correlating well with the gradient in the sub-surface water. Not surprisingly, no significant difference was seen for the two Castle Harbour sites, since this area has a higher flushing rate and no major boating or cruise activity.

Metals. Statistically significant tissue metal gradients are apparent in both mussels and holothurians from Hamilton Harbour, though the changes are not marked (concentrations for the metal giving the most clearly delineated gradient, Pb, differ by a factor of 2 to 3 at most). Sediment concentrations also appear to reflect this mild gradient, though lack of replication precludes statistical confirmation.

In Castle Harbour, the difference in sediment metal concentration between the "dump" site C2 and the reference site C1 is much more marked (though the mean levels at C2 are not in excess of those in the inner part of Hamilton Harbour). By contrast, tissue levels for Castle Harbour follow no interpretable pattern.

4. **BIOCHEMICAL STUDIES**

4.1 INTRODUCTION

Two groups of biochemical measurements in fish were assessed: the induction of components of the hepatic mixed function oxidase (MFO) system, and induction of metal-binding proteins (metallothioneins, MT). MFO induction is a potential indicator of the effects of a broad spectrum of chemical compounds related to polycyclic hydrocarbons. MT induction is a known or potential indicator of the impact of certain heavy metals. Neither indicator has been studied to any extent in tropical or sub-tropical fish, although a considerable literature on both indices exists from studies on temperate species; one objective of the work was therefore to evaluate the usefulness of such measurements in sub-tropical biota. A second objective was to assess the feasibility of making such measurements with the minimum of advanced equipment or facilities. The results from the study are summarized as follows.

4.2 MFO INDUCTION MEASUREMENTS

Isolation of MFO enzyme systems. MFOs are localised in the microsomal fraction of liver; this is usually prepared by ultracentrifugation (at up to 100,000 x g) of liver homogenates. This process requires sophisticated equipment. An alternative approach is to isolate microsomes by a combination of precipitation by Ca^{++} accompanied by various low-speed centrifugations. The two approaches were compared in three Bermudian species. In all three, the content of Cytochrome P-450 (a key component of the MFO system) per mg microsomal protein was almost identical in microsomes prepared by either method. The total yield of microsomal protein by the Ca^{++} precipitation method, however, was only 30-50% of that obtained by centrifugation. Since only small amounts of microsomal protein are required for most MFO assays, the Ca++ method should be usable in most field situations, except where only very small amounts of tissue are available.

Species distribution of MFO components. Cyt. P-450 and ethoxyresorufin O-de-ethylase (EROD) were determined in 5 species of local fish. Minor differences were apparent between species, except for the squirrelfish (*Holocentrus rufus*) which had consistently higher (5-fold) Cyt. P-450 contents than the others. Squirrelfish kept in tanks for 10 days had reduced Cyt. P-450 contents, indicating that the high levels were not constitutive but were probably induced by an undetermined dietary component. All of the other species studied had measurable levels of Cyt. P-450 and were suitable for induction studies. The blue grunt *Haemulon sciurus* was chosen for most of the continuing studies because of its availability in the region.

Dose-response relationship of MFO induction to β-naphthoflavone exposure. A dose-response curve was established, relating hepatic MFO induction in the blue grunt to injection of β-naphthoflavone (BNF), a known MFO inducer. Various doses of BNF were administered i.p., and after three days Cyt. P-450 content and EROD activity were measured in liver microsomes. Doses of BNF at 3 mg/kg and above significantly induced Cyt. P-450 and EROD (Fig. 3-1). To complement the spectrophotometric assay of total Cyt. P-450, the content of a specific isozyme (Cyt. P-450e) was assessed by immunoblotting using an antibody against scup *Stenotomus chrysops* Cyt. P-450e. This approach produced almost identical results to the spectrophotometric assays. Thus, "anti-scup P-450e" cross-reacted with some Cyt. P-450 isozymes of all of the tropical species sampled. "Anti-scup P-450e" therefore represents a very specific probe for the Cyt. P-450 isozymes induced by aromatic chemicals in sub-tropical fish. Indeed, the cross-reactivity of this antibody with the

Bermudian species tested was too intense to allow reliable quantitation of the Cyt. P-450e, and precise determinations had to be postponed until liver and microsome samples frozen in liquid nitrogen could be re-analysed at Woods Hole.

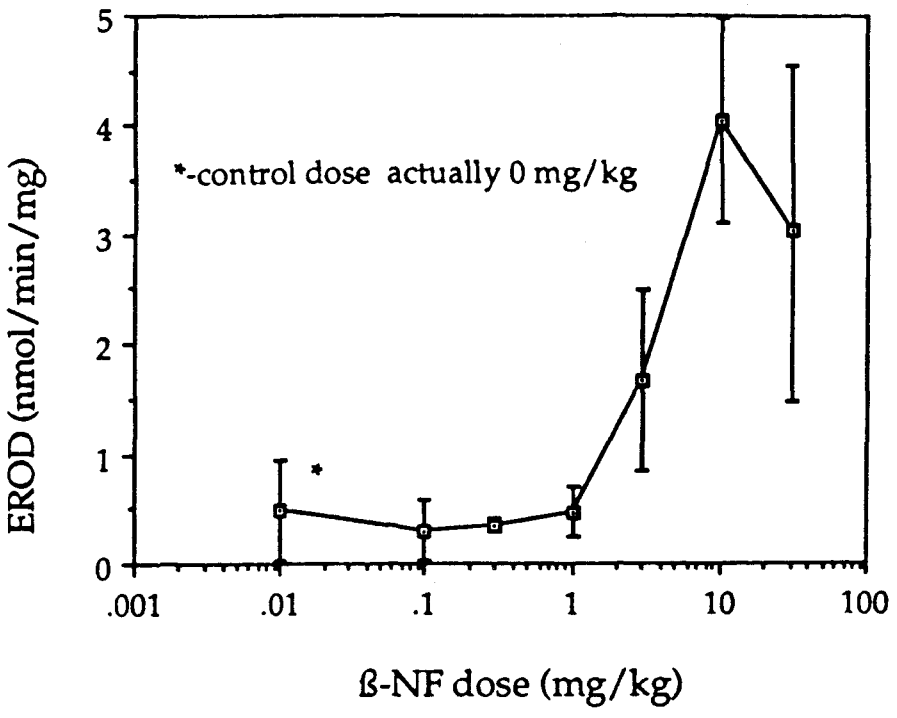

Fig. 4-1. Dose-response for BNF induction of EROD activity. Blue-striped grunt (average weight 98 ± 56g) were given BNF at doses indicated. Error bars indicate ± 1 standard deviation (N = 4 fish at each point).

This technique ('immuno-blotting') therefore provides a very sensitive and specific assay for induced Cyt. P-450e; this would indicate exposure of Bermudian (or other sub-tropical) species to inducing chemicals. The technique requires a minimum of equipment, and a preliminary assessment of induction can be carried out by simple visual comparison to a set of standards of varying concentrations. Precise measurements can be carried out on the same blots using a densitometer in a suitably equipped laboratory.

Time course of MFO induction. The time course of hepatic MFO induction following a single injection of BNF at 10 mg/kg was carried out in blue grunt. Induction could be detected on day 2 using EROD as a indicator, and by day 3 in Cyt. P-450. Induced levels had returned to control values 10 days after injection of the chemical. The time course of this response is significantly accelerated, both in the time to reach maximum induction and in the time to return to normal values, over rates determined in species from more temperate environments.

Evidence for "field" induction of MFO activity. Fish were collected from various test sites and hepatic MFO activity assessed. In blue grunt collected from Hamilton Harbour, Cyt. P-450 and EROD values were elevated in samples from sites H2-4 relative to the control site H1. This elevation was consistent with the higher PAH and PCB levels recorded in sediment and in *A. zebra* from sites H2-4. A similar, and more pronounced induction, was observed in the French grunt sampled from these sites. Although sample size was limited, the clear induction observed in both species confirms their exposure at sites H2-4 to inducing chemicals, and demonstrates the utility of MFO

measurements as a pollution monitoring technique. French grunt and sergeant majors were collected from the Castle Harbour Dump Site (C2) and compared to control fish collected at Ferry Reach. The levels of Cyt. P-450 in the livers of fish from the dump site were elevated over the Ferry Reach values, particularly in the case of sergeant majors, and in both species, Cyt. P-450E (the induced form) was dramatically increased at the dump site. EROD activity was similarly increased. These changes are also consistent with PAH and PCB levels at the C2 site being higher than those from reference sites.

4.3 METALLOTHIONEIN INDUCTION STUDIES

MT isolation and determination. MT was isolated following homogenisation of liver samples in appropriate buffers and centrifugation at 10,000 x g. In blue-striped grunt, MT was detected by a radioimmunoassay procedure involving recognition by an antibody raised towards perch MT; this cross-reactivity indicates that blue-striped grunt and perch MT are structurally similar. MT in two other species (tomtate and squirrelfish) was detected by polarographic methods.

Species distribution of MT. MT was detectable in all the species analysed (blue-striped grunt, tomtate and squirrelfish). As was the case with hepatic MFO components, the squirrelfish contained MT (and associated metals) at concentrations two orders of magnitude higher than in other species, even when sampled from uncontaminated sites. These anomalously high MT and MFO levels remain to be explained.

Dose-response of MT induction. Blue-striped grunt were injected i.p. with increasing doses of $CdCl_2$ and killed eight days later. MT concentration increased consistently with hepatic Cd concentrations.

Time course of MT induction Blue-striped grunt were killed at intervals up to eight days after a single i.p. injection of $CdCl_2$. MT concentrations in the liver reached a maximum at six days and then declined slightly. Overall, the time course of MT induction in blue-striped grunt was much more rapid than that seen in fish from temperate waters, and this undoubtedly reflects increased metabolic rates due to higher environmental temperatures.

Evidence for "field" induction of MT. The possibility that MT was induced by field exposure to ambient metals was examined in blue-striped grunt from Hamilton Harbour and tomtate from Castle Harbour. Blue-striped grunt showed significantly higher MT levels at H7 (a contaminated site) than at Ferry Reach (the reference site); at H1 (Hamilton Harbour reference site) MT was also low, but insufficient samples were obtained for statistical analysis. Hepatic Zn levels showed a similar trend (Fig. 4-2). Tomtate from Castle Harbour also showed significantly elevated MT and Zn from the "dump site" (C2) over those from the reference site (C1). Although Cu was measured in fish from both Hamilton and Castle Harbours, MT levels were better correlated with Zn than with Cu, and given the relative amounts of these metals in various environmental samples, Zn is more probably the main MT inducer in these fish.

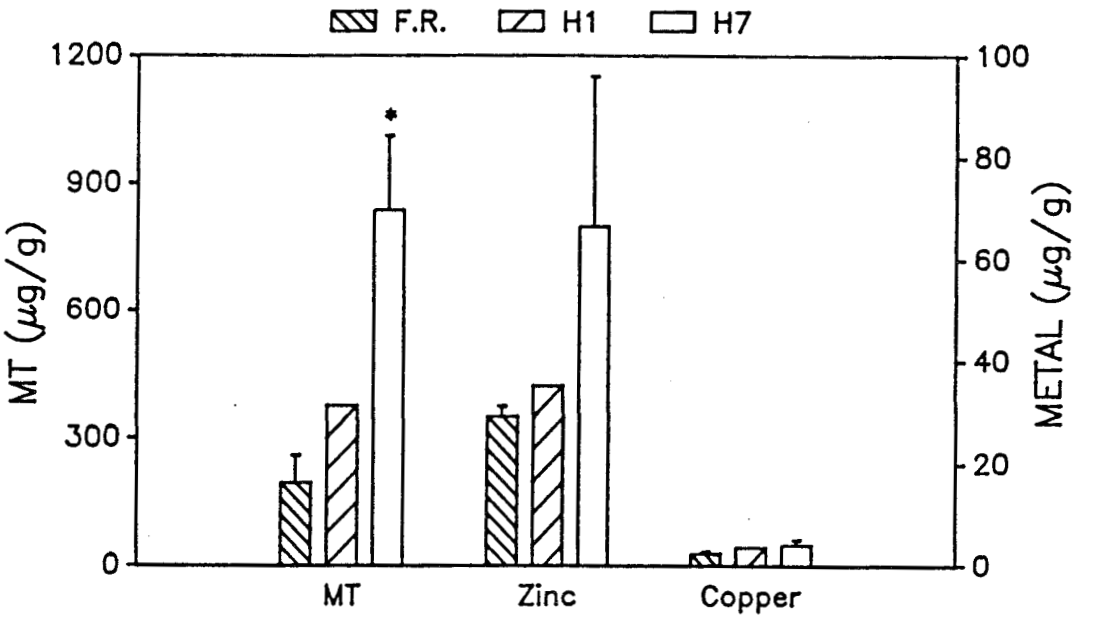

*Fig. 4-2. Hepatic levels of MT, zinc and copper from blue-striped grunt from three sampling sites: Ferry Reach (n=5), H1 (n=2) and H7 (n=6). Bars represent the mean of each group, expressed as µg/g liver (wet wt.), and error bars denote +1 SE. * indicates significant difference ($p<0.05$) compared to Ferry Reach control.*

4.4 CONCLUSIONS

Both MFO and MT induction in a variety of sub-tropical fish species appear to be similar to those in the more extensively studied species from temperate waters. Using relatively simple approaches and equipment, it is possible to induce MFO and MT during experimental studies, and to confirm their induction during "field" exposure to ambient concentrations of organics and metals, respectively.

5. **BIOASSAY STUDIES**

5.1 INTRODUCTION

Whole organism bioassays included the deployment of *A. zebra* in cages at most of the stations for 12 days, and the determination of (a) their scope for growth (SFG), and (b) the analysis of lipid and protein in their tissues. Bioassays were also carried out with samples of sediment using an amphipod (c) and water samples using a ciliate (d).

5.2 PHYSIOLOGICAL ENERGETICS: SCOPE FOR GROWTH

Introduction. Measurement of the energy available for growth and reproduction, termed scope for growth (SFG), provides a rapid and quantitative assessment of the energy status of an animal, as well as insight into the individual components (and mechanisms of toxicity) which affect changes in growth rate.

The primary objective of this study was to use the physiological energetic responses of a native bivalve species (*A. zebra*) to detect and quantify the sublethal effects of contamination in the sub-tropical environment of Bermuda. In this study, SFG was measured in its simplest form, i.e., energy gained from feeding minus the energy lost through metabolic energy expenditure.

Materials and methods. Specimens (50-60 mm shell length) of the Turkey Wing or Bermuda mussel (*A. zebra*) were collected from a sublittoral population in Harrington Sound and transplanted to seven sites; a clean reference site (C1) and a contaminated site (C2) in Castle Harbour, two clean reference sites H5 and H1, respectively at the boundary of Hamilton Harbour and in the lagoon outside Great Sound, and three contaminated sites within Hamilton Harbour (H2, H3 and H4). Three cages (15x15x15cm) each containing 20 mussels were placed at each site at a depth of between 9 and 11 m (12 Sept). After a period of 11 to 12 days exposure, mussels were sampled for physiological and chemical measurement. Sixteen mussels from each site were cleaned of epibionts, numbered and returned to BBS for physiological measurement under standard conditions (30°C, 36°/$_{oo}$ salinity; 0.75 mg seston/l). The clearance rates (i.e. the volume of water cleared of particles/h) were measured in a static system within 9 hours, whereas with only two respirometers the rates of oxygen consumption were measured over a period of c. 24 hours. SFG was calculated on an individual basis by converting clearance rate to energy consumed and absorbed and oxygen consumption to energy expenditure.

Results and discussion. The Castle Harbour reference site (C1) showed the highest recorded clearance rate of 3.2 l/h (Table 5-1). The mean clearance rate measured for mussels from near the "dump" site (C2) was lower than this, at 2.64 l/h (i.e. 83% of C1), but a significant difference between the levels for C1 and C2 could not be established (at the 5% level). There was also no significant difference in the rate of oxygen uptake by mussels from C1 and C2 (Table 5-1). The measured mean SFG of mussels at C2 was 70% of the SFG mean for C1, but a significant difference between the two means was not established (in a 5% level test).

Mussels sampled from Hamilton Harbour (H2,H3,H4) showed a reduction in clearance rate relative to the mussels at reference sites H1 and H5. At site H4 the clearance rate was significantly ($p < 0.05$) lower than at H5. The rate of oxygen consumption (reflecting energy expenditure) by mussels increased at sites in the vicinity of Hamilton Harbour (H2>H4>H3; Table 5-1). The

integration of clearance rate and oxygen uptake in terms of SFG demonstrated a marked and significant ($p < 0.05$) reduction in the energy available for growth and reproduction from 9.8 J/h at H5 and 8.06 J/h at H1, to 3.0 and 3.40 J/h at H4 and H2 respectively, with intermediate values of 5.03 J/h at H3 (Table 5-1, Fig. 5-1a).

These lowered SFG values were accompanied by a significant accumulation of contaminants (hydrocarbons and their polar oxygenated derivatives, PCBs, tributyl tin, dibutyl tin and Pb) in the mussels' tissues. There was a significant negative correlation between the SFG of *A. zebra* and the log concentration of Pb ($r=-0.76$), hydrocarbons ($r=-0.92$), polar oxygenated derivatives ($r=-0.89$), PCBs ($r=-0.95$) and tri- and dibutyl tin ($r=-0.91$) in their tissues. *A. zebra* accumulated hydrocarbons to tissue concentrations that were sufficient to explain the recorded decline in clearance rate, through the mechanism of 'non-specific narcosis' (based on tissue concentration-response relationships established for the mussel, *Mytilus edulis*). Similarly, tributyl tin was accumulated to concentrations that could induce the observed increase in energy expenditure by *A. zebra*, through the mechanism of uncoupling of oxidative phosphorylation.

Toxicological interpretation of the coupled physiological and tissue residue chemistry data therefore indicates that:

a) hydrocarbons and tributyl tin are the major toxic contaminants causing the reduction in SFG of *A. zebra* in Hamilton Harbour;
b) petroleum derived toxicants account for 65% and TBT 35% of this observed decline at H2.

Furthermore, the generally low levels of suspended particulate organic matter in Bermuda waters result in a low SFG, and the additional depression of growth by pollutants is likely to shift the species closer to the limit of growth and reproduction as represented by the zero SFG value (Fig. 5-1a).

Table 5-1. Physiological responses of A. zebra, *mean (SE).*

Site	Clearance rate (l/h)	Respiration rate (ml O_2/h)	Scope for Growth (J/h)
C1	3.18 (0.21)	0.371 (0.015)	8.73 (1.24)
C2	2.64 (0.30)	0.357 (0.015)	6.21 (1.54)
H1	2.90 (0.23)	0.337 (0.024)	8.06 (1.42)
H5	3.13 (0.19)	0.305 (0.009)	9.81 (0.92)
H3	2.41 (0.33)	0.361 (0.021)	5.03 (1.45)
H4	2.08 (0.19)*	0.376 (0.034)	3.00 (1.05)*
H2	2.33 (0.21)	0.420 (0.030)*	3.40 (1.19)*

* Significantly different from "clean reference" site ($p < 0.05$).

5.3 LIPID ANALYSIS

Lipids are an integral constituent of plants and animals. Being a very diverse biochemical group, lipids are important in many physiological processes, including energy transport and storage, cell membrane form and function, and endocrine control of reproduction and metabolism. Recent studies have demonstrated that the exposure of marine invertebrates to various

environmental contaminants can result in reduced triacylglyceride synthesis and decreased mobilization of fatty acids into phospholipids leading to changes in both neutral and polar lipid pools. Ultimately these alterations in lipid pools reduce the ability of a marine organism to adapt to its environment. By measuring changes in the lipid pools of animals exposed to a pollution gradient, the overall impact of the gradient on the organism can be assessed.

A. zebra were collected from Harrington Sound and deployed in cages for 11-12 days as described above at sites C1, C2, and H1 to H5. The soft body tissues from replicate pools of 10 mussels each were homogenized and aliquots were taken for body composition analysis (gross protein, gross lipid, lipid class distribution, dry weight, and ash measurements) as well as for organic contaminant body burdens (Burns, 1989). In addition individual whole *A. zebra* were allocated for heavy metal body burdens (by S. Soria) and were dissected for body composition analysis of specific organ systems (gill, gonad, digestive gland and muscle).

The biochemical composition of *A. zebra* was seen to be similar to that observed in other bivalves that rely on glycogen as their primary energy storage medium. Differences were noted in the lipid content and composition in mussels deployed in the 2 harbours, suggesting that Hamilton Harbour had higher food availability than Castle Harbour; consequently the mussels from Hamilton Harbour were in better physiological condition as indicated by higher lipid levels, primarily the neutral lipids.

Overall, no consistent difference was seen in the gross biochemical or compositional lipid data between the 2 Castle Harbour sites. However, for the gradient of control to impacted sites (H1, H5, H3, H4, H2) in Hamilton Harbour, there was a steady increase in neutral lipids and decrease in phospholipids, giving a significant increase in the neutral:polar lipid ratio by a factor of 2 for the two end-points of the gradient (overall ANOVA, $F = 5.2$, $p<.005$). This suggests a disruption in the mobilization of neutral lipids to phospholipids.

5.4 SEDIMENT BIOASSAY

A standard amphipod sediment bioassay technique was deployed on Hamilton Harbour sediments using a native Bermudian species *Parhyale hawaiensis*. The technique has been successfully deployed with the infaunal Phoxocephalid amphipod, *Rhepoxyniusabronius* and the tube dwelling amphipod, *Ampelisca abdita*. This technique measures mortality and sublethal effects of emergence from sediment after 10 day exposures to test sediments. It is a sensitive and fairly rapid indicator of sediment toxicity, which is inexpensive to set up and requires no elaborate instrumentation.

Due to time constraints and unfamiliarity with general amphipod distribution in Bermuda, an infaunal amphipod was not available for tests. The epibenthic rock-associated amphipod, *P. hawaiensis* was used as a compromise.

Sediments from Hamilton Harbour (sites H2, H3, H4, H5 and H6) plus a negative toxic control sediment from Black Rock Harbor (BRH), Connecticut, USA, and a positive seawater control (SW), were bioassayed by placing 0.5 l of sediment in 3 l plastic containers and adding seawater to approximately 2l. Small flat rocks were added to each test container and 3 replicates per treatment set up. *P. hawaiensis*, collected from Ferry Reach, just north of Long Bird Bridge on Sept. 17 and 18, were randomly sorted into sediment

treatments to obtain 25 individuals per exposure. Containers were maintained static in 29-30°C seawater (36-38°/$_{oo}$ salinity) with continuous aeration. Containers were checked daily for dead amphipods, behavioural observations, plus water level, temperature and salinity. After 10 days the amphipods were removed from the containers and those living, dead and missing were enumerated.

Daily observations revealed that at any time a few *P. hawaiensis* would be on top of the rocks, on the walls of the container, or swimming in the water column. There appeared to be no noticeable differences in this behaviour between Hamilton Harbour sites, but a large increase in avoidance of sediment from BRH was observed. No dead amphipods were observed during the bioassay. After completion of the bioassay, only one dead amphipod was found, in H2 sediment. Missing amphipods were assumed dead when computing mortality. In terms of lowest to highest (assumed) mortality, the mean values were ranked in the order H5, H4, SW, H6, BRH, H3, H2 but no statistically significant difference in percent mortality between sites could be established, and the data must be regarded as failing to demonstrate any biological effects.

5.5 CILIATE BIOASSAY

Population growth in cultures of the ciliate *Euplotes vannus* provide a sensitive index of stress, which was tested during the Workshop as a water quality bioassay, to detect biologically significant differences in the chemistry of water samples taken from the environment. At each site water samples were taken for bioassay just above the bottom (10 cm), at 0.5 m and at the sea surface. Surface microlayer samples were taken with a Garrett Screen.

As it was suspected that tributyl tin (TBT) might occur at as a pollutant in Hamilton Harbour arrangements were made for analysis of samples at Plymouth Marine Laboratory by Dr G.W. Bryan, to supplement the other chemical data. TBT tends to accumulate in the sea surface microlayer, so samples were taken both with the Garrett Screen and at 0.5 m to determine the level of microlayer enhancement. The samples taken by divers just above the bottom were intended to indicate whether there is an input to the water column from the sediments, via the pore water.

Methods. Water samples were membrane filtered (0.45μm) as soon as possible after collection and then stored at 5°C until used. The cultures of the ciliate *E. vannus* were brought from the UK. Although several species of *Euplotes* occur on Bermuda, there was not enough time during the Workshop to isolate and build up dense enough cultures for bioassay experiments. Stock were maintained in 100 ml disposable cell culture flasks and fed twice daily on the marine yeast *Rhodotorula rubra*, but for experiments 60 ml cultures were used and fed on *Saccharomyces*. Use of a non-marine species simplifies the determination of grazing rates as a possible index of stress, because *Saccharomyces* does not reproduce in seawater.

Stock and experimental cultures were kept on the laboratory bench at an ambient temperature of 25°C. Cultures were not maintained axenically. Experimental cultures were initiated with aliquots of 1 ml of ciliates concentrated by filtration on a 5.0 μm Nucleopore filter to give initial densities of 10-40 cells per ml, and sufficient yeast cells (ca 150,000 cells per ml) to ensure that food was never limiting during the 48 h experiments. Under these conditions control populations usually doubled in number every 16 h (specific growth rate R = 1.18 per day) to give densities of 200 per ml after 48 h.

For any treatment there were 3 replicate cultures for each water sample. At the beginning and conclusion of each experiment a 20 ml sample from each replicate was preserved in 1 % Lugol's Iodine. Densities were determined from the means of 3 replicate counts of 1 ml samples in a Sedgewick Rafter counting chamber. Mean counts were then used to calculate specific growth rates (R) and inhibition of R relative to that of control populations was used as the index of stress.

Results and conclusions. Samples for bioassay were taken from 2 Castle Harbour sites (C1 and C2) and 4 Hamilton Harbour sites (H1, H2, H3 and H6). Some additional bioassays were discounted due to the protracted period of storage of water samples (15 days).

Specific growth rates of populations grown in water from Castle Harbour showed that water from the bottom at C2 caused significant inhibition ($p<0.05$). Mean values of R with 95% confidence intervals are shown graphically in Fig. 5-1c. This was to be expected as the chemical data show that both metals and hydrocarbons in sediments from this site are elevated, although the metals alone are unlikely to be responsible.

By contrast, for Hamilton Harbour water samples (H1, H2, H3 and H6), the growth data showed no clear interaction between site and depth, so that the overall pattern of differences between the sites can best be seen by averaging the R values across all 3 depths. There is a strongly significant difference in growth rates between the 4 sites, mainly reflecting a simple drop in mean R from the control site H1. (Fig. 5-1d shows only H1-H3, the mean value for H6 being marginally but not significantly lower than for H3).

5.6 CONCLUSIONS

Bioassay data have been summarised by ranking the responses at each site (Table 5-2). Some broad conclusions can be drawn.

(i) All the bioassay responses suggest that H2 is a polluted site, with significant depressions in scope for growth and ciliate growth rate, and a significant increase in neutral:polar lipid ratio by comparison with the control site(s).

(ii) Scope for growth and lipid class analysis indicate also that H4 is impacted to a similar degree. (Reliable results were not available at H4 for the ciliate bioassay). Differences between H3 and the control site(s) are generally less pronounced, being non-significant for the lipid class data.

(iii) Correlations between the biological responses and the values of potential toxicants are generally high, particularly so for scope for growth. As in the previous IOC/GEEP Oslo Workshop, scope for growth proved itself an especially valuable weapon in the "biological effects armoury".

(iv) The results at Castle Harbour were less conclusive, in suggesting any biological impact at the dump site. Whilst the response of the ciliate bioassay to bottom water from C2 was reflected in a decrease in scope for growth in *A. zebra*, the latter response did not achieve significance at the 5% level. This may be due to transient fluctuations in water quality sampled for the ciliate bioassay, which was not matched by the integrated accumulation of contaminants by the mussels over 12 days exposure. The neutral:polar lipid ratio displayed a contrary trend from control (C1) to potentially impacted site (C2) than that observed for Hamilton Harbour,

though the difference was only barely significant (at the 5% level).

(v) There were indications from the data that responses to environmental samples of water and sediments declined with storage, despite efforts to minimise such changes.

(vi) Choice of species for bioassays is a critical factor. While the native *A. zebra* proved suitable for energetic and lipid studies and the native amphipod *P. hawaiensis* unsuitable for sediment bioassay, the problem was circumvented for the water column bioassay by importing ciliate cultures. More time than was available is necessary to a successful choice in each case.

Table 5-2. Summary of data by ranking the site mean values of the various biological responses considered in this section. Left to right indicates a direction of increasing stress, based on prior assessment for each technique of whether effect of impact is to reduce or increase the response. Sites connected by an underline do not give significantly different responses on pairwise comparisons. Sites not shown were not assayed for that technique.

Response	Hamilton Harbour	Castle Harbour
Scope for growth in *A. zebra*	H5 H1 H3 H2 H4	C1 C2
Neutral:polar lipid in *A. zebra*	H1 H5 H3 H4 H2	C2 C1
Sediment bioassay on *P. hawaiensis*	H5 H4 H6 H3 H2	
Ciliate bioassay on *E. vannus*	H1 H2 H3 H6	C1 C2[a]

[a] *Difference between C1 and C2 for bottom water only (not 0.5m and surface).*

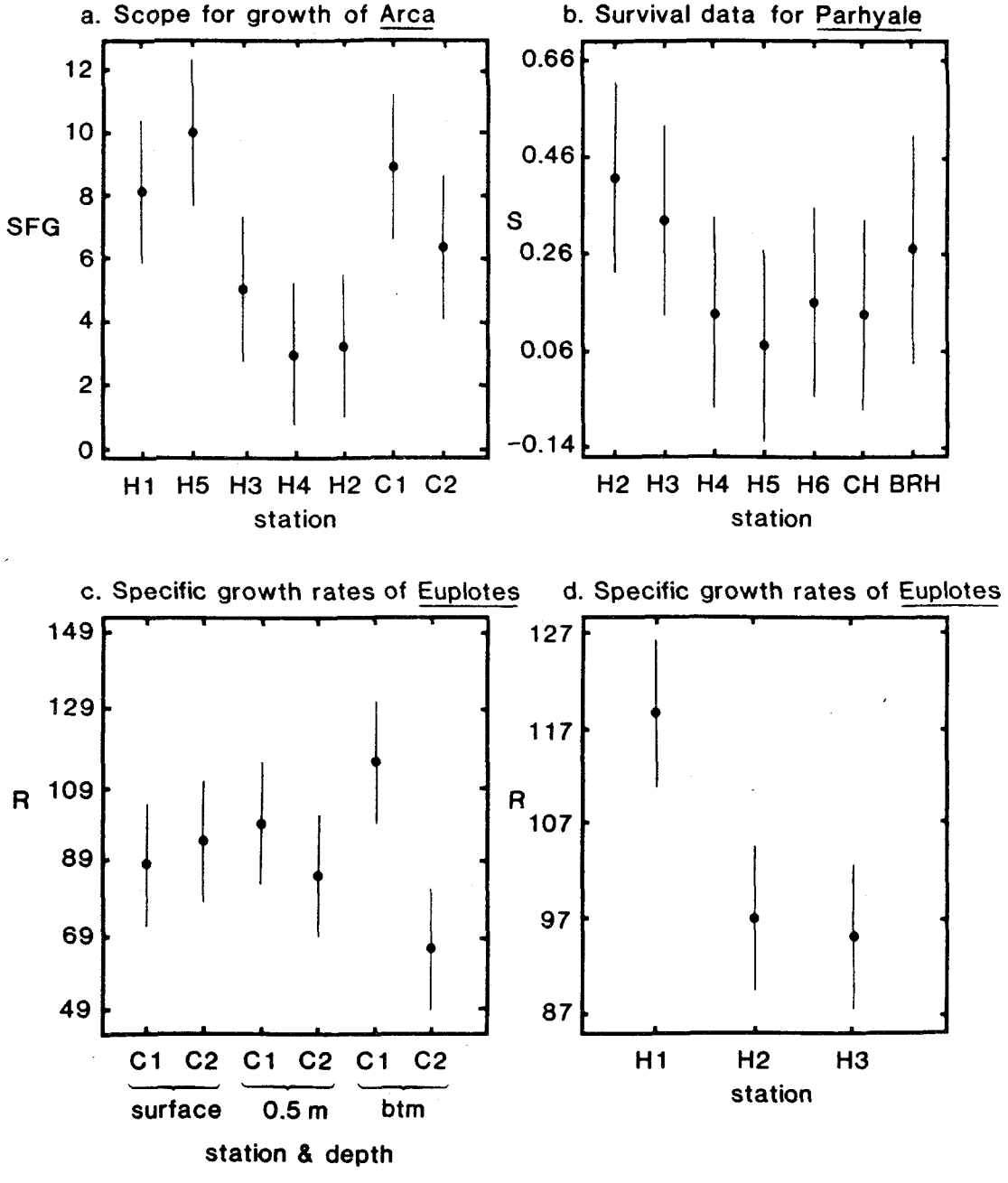

Fig. 5-1. Data summarising whole organism responses. a) Scope for growth (SFG) for Arca zebra *exposed in cages at each site for 12 days. b) Survival data (arcsin transform of numbers surviving) for* Parhyale hawaiensis *exposed for 10 days to sediments collected from each site. c) and d) Specific growth rates (R) of* Euplotes vannus *cultured in water samples collected from the surface film, at 0.5m and just off the bottom at different sites. For Castle Harbour the data are given separately (c), but for Hamilton Harbour data at different depths are pooled (d). All means are given with 95% confidence intervals.*

6. BENTHIC COMMUNITY STUDIES

6.1 INTRODUCTION

Assessing patterns in the structure of benthic communities has several advantages over experimental methods for the detection of anthropogenic disturbance. The benthos can integrate conditions over a period of time rather than reflecting conditions just at the time of sampling. They have advantages over pelagic organisms in that they are immobile and are therefore more useful in assessing local effects. However, benthic communities are extremely complex, comprising a wide size range of organisms and usually a large number of species. Because of this, the benthic community approach to biological effects monitoring has been criticized on the grounds that the sampling, sample analysis and computing involved are so time consuming and labour intensive as not to be a viable cost-effective approach to routine monitoring. Generally, biological effects studies at the community level have involved *either* the macrofauna *or* the meiofauna and the relative advantages of using these two size components of the benthos have been discussed elsewhere. However, there is merit in comparing the response of these two components. Empirical and theoretical considerations suggest that their community structure may be determined by different mechanisms, so a differential response might be indicative of the cause of disturbance.

Working at the species level makes a comprehensive comparative study of this nature impracticable within the financial and time constraints usually imposed on biological impact studies. It is important therefore to assess the 'appropriate taxonomy' required to detect community responses to anthropogenic disturbances, including pollution. There have been recent indications that analysis at taxonomic levels above those of the species may provide an equally clear if not clearer picture of the effects of contaminants.

Methodologies evolved at the GEEP Oslo Workshop (Bayne *et al.*, 1988) constituted an initial attempt to devise more effective protocols for assessing community responses to environmental contaminants. The GEEP Bermuda workshop provided an ideal opportunity to test and further develop these methodologies. In order to test the viability of this approach on a word-wide basis, we attempted to conduct all phases of a benthic survey (field sampling, sample and data analysis, report writing) within a limited timespan, in this case the three-week duration of the GEEP Workshop, and in a sub-tropical environment where the fauna was unfamiliar to the participants. The programme of work was therefore designed to be achievable within this timeframe and with the personnel available.

6.2 METHODS

Sampling. Due to the unavailability of a suitable grab for macrofauna sampling, all samples were collected by SCUBA divers. Six stations (H2-H7) were worked in Hamilton Harbour. As far as possible, the stations were standardized with respect to water depth and sediment type in order to minimise the influence of these "nuisance variables" on the benthic communities.

For macrofauna, four 0.055m^2 core samples were taken at each site and sieved at 0.5mm. Macrofauna were identified to species and the abundance and biomass (wet wt) of each species determined. For meiofauna, four 4.52cm^2 syringe cores were taken to a depth of 5cm at each site, and sieved at 63μm. Abundance of major taxa (Nematoda, Copepoda, Oligochaeta, Polychaeta, Ostracoda, Kinorhyncha, Acari, Priapulida) was determined and the dominant

taxon, Nematoda, was identified to generic level.

Multivariate statistical methods. Multidimensional scaling (MDS) was the chosen method of ordination. This was applied using either standardized or non-standardized data, with various transformations, to the macrofauna abundance and biomass, the meiofauna major taxa and the nematode genera data. Formal significance tests for differences between sites were performed using the ANOSIM randomisation test. Natural and anthropogenic environmental variables were superimposed onto the two dimensional (2D) MDS configurations to provide visual correlations.

Graphical presentations of relative abundance and biomass. *k*-dominance curves were used to visualize diversity profiles, and for the macrofauna the comparison of curves for abundance and biomass (ABC method) was used to assess levels of disturbance. 'Partial dominance curves' were also developed in an attempt to overcome some methodological problems of the ABC technique.

Univariate statistical methods. Various diversity indices were calculated for all faunal components. These were also compared with neutral model predictions in order to assess levels of disturbance.

6.3 RESULTS

Multi-dimensional scaling ordinations. 2D MDS configurations for the macrofauna abundance and biomass data are shown in Fig. 6-1a and b. There is a considerable degree of similarity between these two diagrams. In both cases the site replicates cluster more or less discretely and are arranged in a clear linear sequence (right to left): H6, H7, H2, H3, H4, H5. The ANOSIM test on the standardized root transformed abundance data indicates that all pairs of stations are significantly different from each other except for H6 and H7, and here the significance level is only marginally above 5%. For the standardised root-root transformed biomass data all pairs of sites are significantly different from each other.

MDS configurations for the nematode genera abundance data (Fig. 6-1c) show clear coherence of replicates within stations, which follow a horseshoe-shaped sequence (from right to left): H7, H6+H2, H4, H3, H5. The ANOSIM test indicates that all pairs of stations are significantly different from each other at the 5% level except for H2 and H6.

MDS configurations for the meiofauna major taxa (Fig. 6-1d) show no clear trend or pattern. There is some indication that replicates at H3 and H5 form coherent clusters, but at the other stations replicates are intermingled showing no obvious differences between them. However, there is some indication of the horseshoe effect detected in the nematode MDS (Fig. 6-1c) being repeated in the meiofauna groups, and the majority of pairwise comparisons in the ANOSIM test indicate significant differences around the 5% level, except for H2, H6 and H7.

Relationships with environmental data. In Fig. 6-2, symbols representing the values of median grain size of the sediment, water depth, lead concentration in the sediment, tributyl tin (TBT) in the water at 0.5m depth and total sediment hydrocarbons are superimposed on the 2D MDS configuration for standardized root-transformed macrofauna abundance. From this it is clear that there is no good relationship between the configuration and the natural environmental variables (grain size and water depth). There is a correlation with lead concentration, with the highest concentrations on the right side of the configuration (the innermost stations H6, H2 and H7) grading to the lowest on the left. Relationships with the concentrations of zinc and copper were

essentially the same as those for lead. However, the range of variation from highest to lowest is rather small. For TBT, the trend is similar but the range of variation is much more pronounced. PAH shows a poor correlation, with the the highest concentration at station H4.

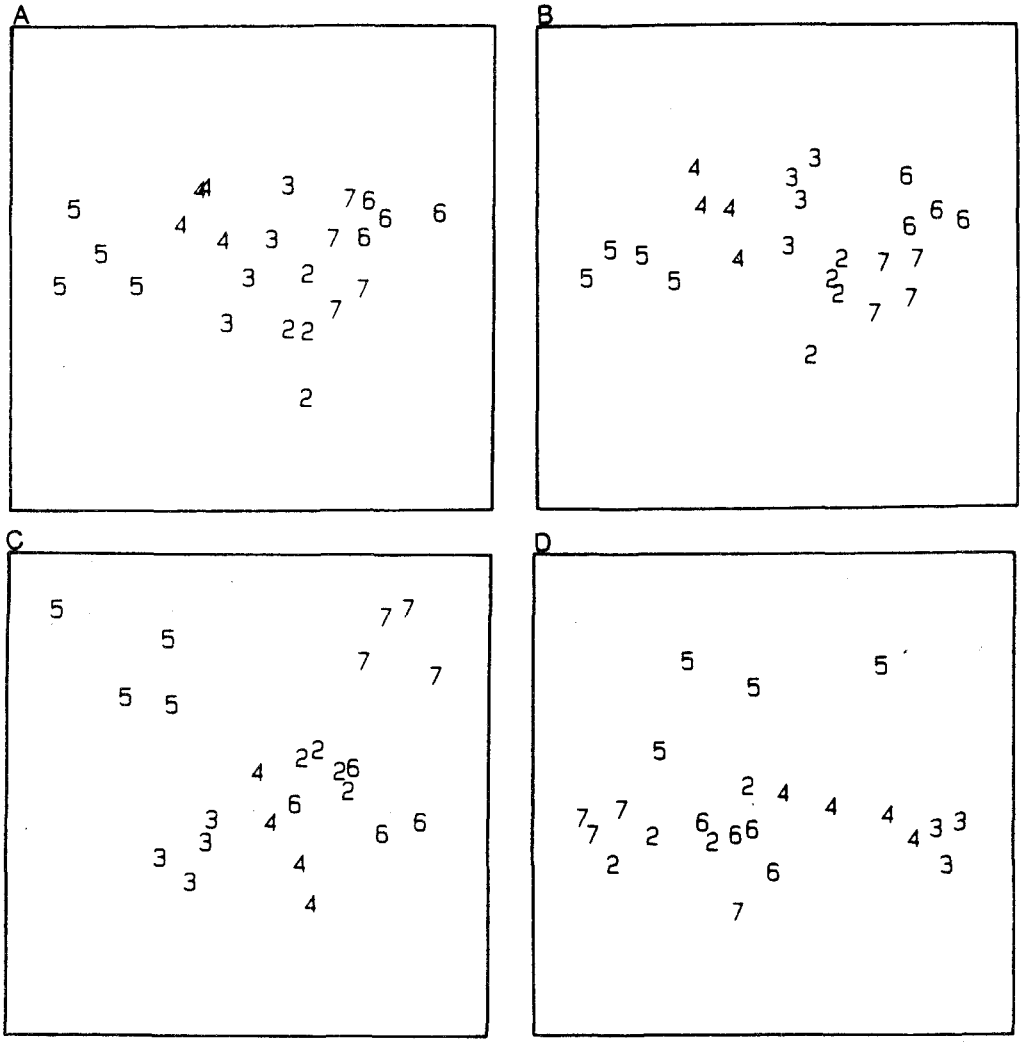

Fig. 6-1. 2-d MDS configurations for: (a) macrofauna standardized root-transformed abundance (stress = 0.15); (b) macrofauna standardized double root-transformed biomass (stress = 0.14); (c) nematode root-transformed abundance (stress = 0.17); (d) meiofauna groups root-transformed abundance (stress = 0.10).

The same plots for the root-transformed nematode data showed somewhat similar relationships to those for macrofauna, though with a slightly less well-defined relation between the 2-d configuration and the organo-tin and metal concentrations.

Distributional graphs. *k*-dominance curves for the macrofauna abundance (Fig. 6-3a) essentially show two groups of curves: H2, H5 and H7 are below H3, H4 and H6, indicating higher diversity and lower dominance. For the macrofauna biomass data (Fig. 6-3b) the curves for H3, H4 and H5 cross one another with a more even distribution than for the other stations. These are followed in increasing order of dominance by H2, H7 and H6.

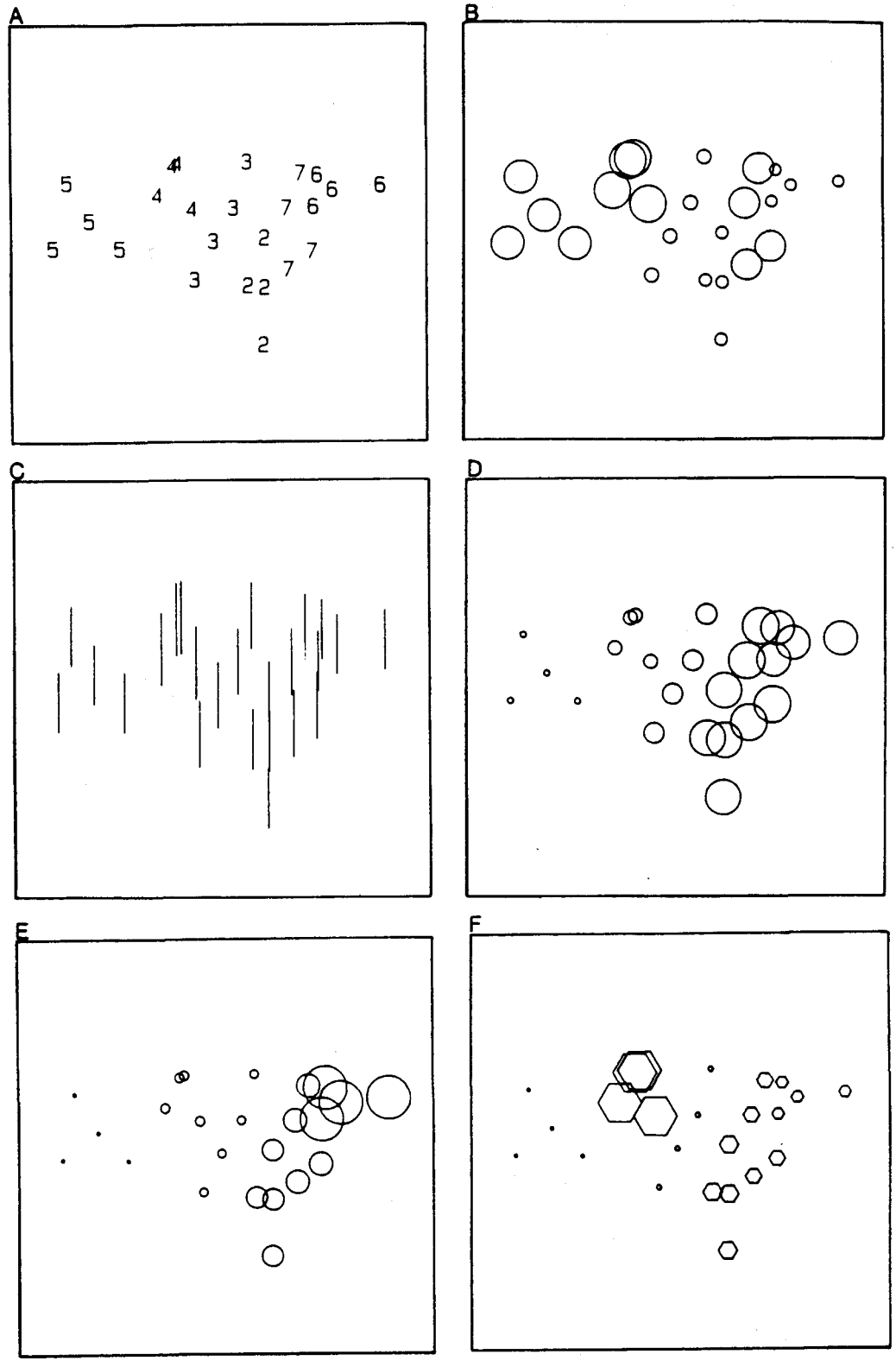

Fig. 6-2. (a) 2-d MDS configuration for macrofauna standardized root-transformed abundance (as Fig. 6-1a). (b-f) Same configuration with symbols representing values of environmental variables superimposed: (b) circles represent median grain size of sediment; (c) vertical lines represent water depth; (d) circles represent sediment lead concentration; (e) circles represent water TBT concentration; (f) hexagons represent sediment PAH concentration.

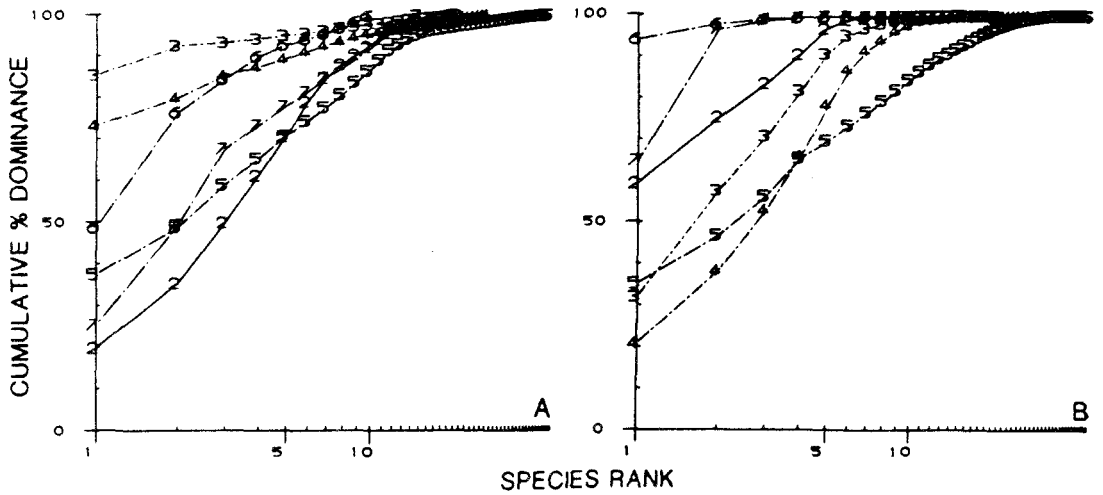

Fig. 6-3. (a) k-dominance curves for macrofauna abundance data (sum of four replicates at each station). (b) As (a) but for biomass.

Combining these data into ABC curves for each station (Fig. 6-4) produces clearly different patterns. Stations H6, H2 and H7 have the undisturbed configuration with the biomass curve above the abundance curve throughout its length. Stations H4 and H3 show a moderately/grossly disturbed configuration with the abundance curve above the biomass curve for most of its length but crossing it for the rarer species. Station H5 shows the moderately disturbed situation, with the abundance and biomass curves closely coincident.

For the nematode abundance data *k*-dominance curves based on the totals for all four replicates are rather similar for each station, but the curves for H3 and H5 are below the remainder, indicating higher diversity at these stations.

Diversity indices. Diversity (H'), using $\log_e$ in the calculation, was determined for the macrofauna, nematodes and meiofauna groups. For the macrofauna there were significant differences in diversity between stations, in sequence (lowest to highest): H3, H4, H6, H7, H2, H5. For the nematodes, differences in diversity between stations were less marked but H5 and H3 had higher diversity than the remainder. For meiofauna groups there were no significant differences in diversity between stations.

V statistics calculated from Caswell's neutral model are given for the macrofauna and nematode data in Table 6-1. Essentially, a value of zero indicates neutrality, positive values indicate greater equitability than predicted and negative values lower equitability. Values greater than +2 or less than -2 indicate a significant departure from neutrality.

For the macrobenthos, equitability at H4 and H3 is significantly lower than neutral model predictions, suggesting a severely disturbed condition. At H5 diversity is also depressed but this does not quite achieve statistical significance. For the nematodes, all sites are close to neutrality.

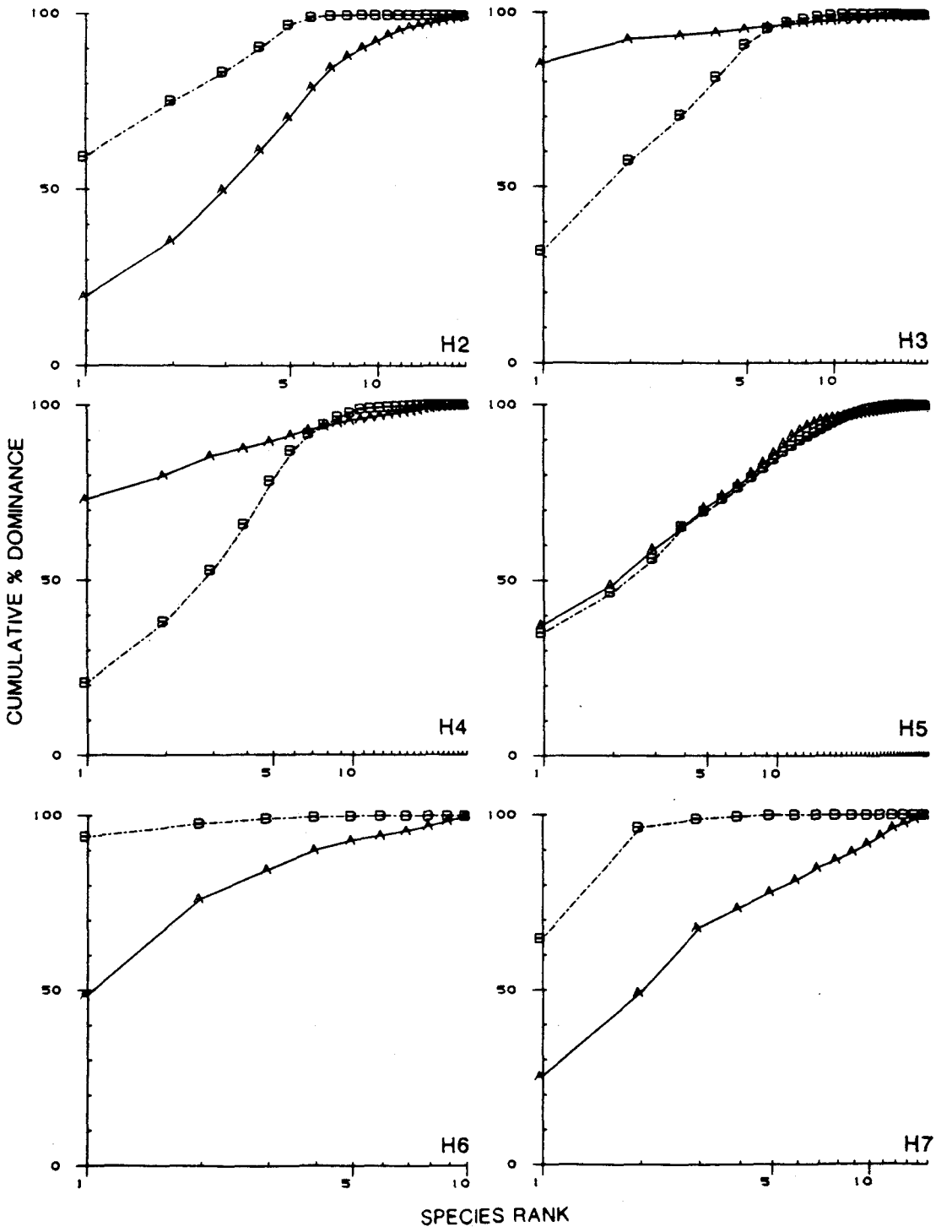

Fig. 6-4. ABC curves for the macrofauna (sum of four replicates at each station); A = abundance, B = biomass.

Table 6-1. V-statistics for summed replicates at each station, derived from Caswell's neutral model.

Site	Macrobenthos	Nematodes
H6	-1.3	-0.4
H2	+0.5	-0.1
H7	-0.2	-0.4
H4	-4.5	-0.5
H3	-5.4	+0.4
H5	-1.9	0.0

Taxonomic reductions. The MDS and ABC analyses were re-run with the macrofauna species and nematode genera data aggregated to higher taxonomic levels. The macrofauna MDS ordinations at family and phylum levels (Fig. 6-5) show little reduction of information. At the family level, ANOSIM indicates that the discrimination is exactly the same as at the species level. At the phylum level, although stations H3 and H4 cannot now be separated, the remaining groups are more distinct (cf. Figs. 6-1a and 6-5b). However, the nematode MDS ordinations sustained substantial loss of discrimination at family level and more severe loss above that.

The ABC plots for the macrofauna at family level gave essentially the same picture as the species plots.

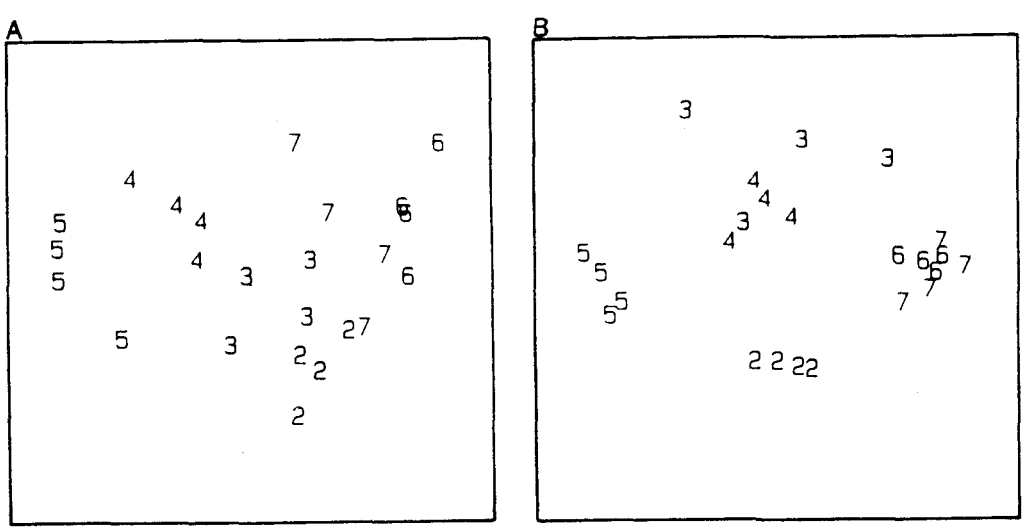

Fig. 6-5. 2-d MDS configurations for standardized root-transformed macrofauna abundance data with species aggregated into: (a) families (stress = 0.17); (b) phyla (stress = 0.09). Compare with Fig. 6-1a for comparable species level analysis.

6.4 DISCUSSION AND CONCLUSIONS

The macrobenthic and nematode taxonomic composition differs significantly between sites and there are indications of a similar trend in the distribution of major meiofaunal taxa. However, these differences do not correlate with differences in the natural environmental variables normally considered to influence community composition, i.e. water depth and sediment granulometry. These distributions do, however, correlate with the concentrations of metals in the sediment and particularly well with TBT in the water column, but not with hydrocarbon concentrations. All stress indices (ABC curves, diversity profiles and departures in equitability from neutrality) indicate severe disturbance of the macrofauna at H4 and H3, and also to a lesser degree at H5. This is not reflected in the nematodes or in the meiofauna groups which have similar diversity profiles at all stations and no departures from neutrality. The most disturbed sites with respect to the macrofauna are not those which have the highest pollutant concentrations. Furthermore, the fact that the meiofauna appear to be undisturbed at these sites is not commensurate with pollution being the cause and we must therefore seek other explanations.

It is unlikely that the correlations between the heavy metals and the MDS configurations reflect a causal relationship because of the small range of variation in concentration between sites and the low absolute values. It is more probable that these relatively low levels of contamination are covariant with a natural environmental gradient which is affecting the fauna, such as organic matter. The sediment at the two innermost stations (H2 and H6) contained considerable quantities of organic material which was clearly land-derived (e.g. the cones of the conifer *Casuarina* were prevalent). However, the strong correlation with TBT concentrations cannot be dismissed so easily and may at least partially be responsible for the differences in faunal composition between sites, as evidenced by the experimental studies on individual organisms at these sites (see section 5 of this report). There is evidence, therefore, that differences in community composition which relate to pollutant effects can be detected by multivariate analyses at contaminant concentrations which are below those for which univariate community measures indicate a stress response. In order to improve the sensitivity of the benthic community approach to biological effects determination, we therefore perceive a real need for the development of new stress measures which retain this multivariate information.

It is known that mechanisms for diversity maintenance differ between the meiofauna and the macrofauna. Most meiofauna are motile forms actively seeking out food particles of the preferred size, shape and quality, thus probably maintaining diversity by narrowly specialized feeding behaviour and partitioning of food resources. Their diversity is therefore likely to be relatively unaffected by the stability of the sediment. Macrofauna, on the other hand, are relatively unselective in their food requirements and depend on spatial partitioning of the habitat to maintain diversity. Thus diversity may be markedly depressed by sediment instability. Physical instability of the sediment is thus a possible cause for the disturbance of the macrofauna. Stations H4 and H3 are very close to the major shipping lane for large cruise liners which frequently enter and leave Hamilton and are very likely to be regularly disturbed, relative to the more sheltered areas of the inner harbour. It is these two sites which showed the most disturbance. Station H5 is not close to the major shipping lane but the situation here was complicated by the presence of *Cladophora* on the sediment surface, which may have resulted in the presence of several small species not typical of the sediment itself, e.g. Amphipoda, Isopoda and Leptostraca, which were not present at any other station. This situation may well confound the ABC curves and diversity measures in comparison with the other stations. These findings demonstrate

the utility of comparing macrofauna and meiofauna community structure in the same exercise, as this may provide useful insights into the the causes of disturbance.

The experiments with taxonomic aggregation showed that, for the macrofauna, the use of operational taxonomic units (OTUs) as high as the family level resulted in very little loss of discrimination compared with the analysis of species data. For the nematodes, however, there was considerable loss of information above the genus level. This suggests that the genus represents the optimum taxonomic level for most efficient discrimination and possibly approximates the operational ecological units (OEUs), although in this work we could not compare the results with an analysis at the species level.

Finally it has been demonstrated that, using an appropriate degree of taxonomic discrimination above the species level, a meaningful study of this kind can be relatively cost-effective and economical on time. The whole study (planning and executing the survey, sample analysis, data analysis and report writing) was completed by four people plus the assistance of one statistician in three weeks.

7. ACKNOWLEDGEMENTS

The operation of a Workshop of the sort described here requires a great deal of support and co-operation from many agencies and individuals. The principal financial support came from the Intergovernmental Oceanographic Commission through the GIPME Programme, on behalf of its GEEP and GEMSI groups of experts, but acknowledgement should also be made to the Director and staff of the Bermuda Biological Station for Research, for a grant-in-aid to support ship time and for their professional support and co-operation, and to the Bedford Institute of the Department of Fisheries and Oceans (Canada), the Plymouth Marine Laboratory of the Natural Environment Research Council (UK), the Woods Hole Oceanographic Institution (USA) and the Institute of Marine Affairs, Trinidad, for financial and logistical support.

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ANNEX I

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ANNEX II

LECTURES PRESENTED AT THE GEEP BERMUDA WORKSHOP

R.F. Addison: Hepatic mono-oxygenase enzyme activity in fish as an indicator of pollution.

J.J. Stegeman: Cytochrome P-450 isozymes: their regulation and use as indicators of marine pollution.

K.W. Renton: Molecular biological approaches to pollution monitoring.

C. Hogstrand: Metallothionein as an indicator of elevated heavy metal levels in water.

B. Bradley: Protein synthesis and gene regulation in bioassay.

K.J. Scott: Sediment bioassays: opportunities and limitations.

J. Widdows: Application of Scope for Growth for the assessment of marine environmental quality.

A.R.D. Stebbing: A ciliate bioassay: origins and prospects.

D. Leavitt: Lipid partitioning in marine invertebrates.

K.R. Clarke, H.M. Platt and R.M. Warwick:

Determination of pollution-induced disturbance by analysis of benthic community structure.

M. Ehrhardt: What becomes of oil pollution in the sea?

ANNEX III

DETAILS OF FIELD SAMPLING PROGRAMME

The following collections/deployments were carried out at each site, except where later indicated otherwise.

Benthic faunal community:
4 macrofauna cores (internal diameter 26.4 cm, area 0.0547 m^2, to depth c. 25 cm, collected by diver)
5 meiofauna cores (internal diameter 2.4 cm, area 4.52 cm^2, to depth c. 5 cm, collected by diver)
1 core for sediment granulometry (exactly as for meiofauna core)

Sediment bioassay:
c. 1500 cm^3 of surface sediment (to depth approx 5 cm, collected in a 7 cm diameter by 50 cm core tube, by diver) for use with bioassay on an infaunal species

Water bioassay (ciliate) and chemistry (TBT):
Bottle (1 l) collected at surface (using surface film sampler)
Bottle (1 l) collected at c. 0.5 m below surface
Bottle at c. 10 cm from bottom (collected by diver)
Further replicate of surface and 0.5m samples for TBT analysis (only) taken on separate sampling day.

Sediment chemistry:
1 jar (250 ml) of surface sediment (to c. 5cm) for organic and inorganic chemistry, collected by diver
Replicated on separate sampling occasion by 2 further cores (area 4.1 cm^2, depth 5cm) collected for inorganic and 1 for inorganic chemistry, collected by diver

Mussel (*Arca zebra*) deployment:
3 cages, each containing 20 animals, cage legs placed in sediment by diver (cage bottom > 10cm off sediment), tied to building block. Separate block holds buoy 6 ft (+/- 3 ft) below surface. Recovered by diver after 10/11 days, for energetics and lipid analyses, and PAH chemistry of tissues.

Fish (several species) collection:
Up to 10 usable fish (i.e. species for which some baseline lab data available) collected for biochemistry at each of 4 field sites (by trap and line).

Holothurian (*Isostichopus badionotus*) collection:
4 animals collected (adventitiously, by diver) at some sites (for lipid analysis and chemistry)

CASTLE HARBOUR

SITE C1 ("HARBOUR REFERENCE")

300m N. of Wallers Bay, W. of entrance to Castle Harbour.
Lat: 32° 20' 19"; Long: 64° 40' 51".
Water depth 11m, mud sediment (but with some shell/coral debris)

9 Sept 9.30-10.30: Pilot survey. Sediment cores and fish collected; divers looked (unsuccessfully) for native bivalve populations. Attempt (also unsuccessful) to get small benthic grab working. Test mussel cage deployment made.
11 Sept 14.15-15.45: Collections for benthos, sediment and water bioassays, sediment chemistry. Mussels deployed. (Recovered test deployment of mussel cage from 9 Sept).
22 Sept 9.30-10.30: Collected further chemistry sediment cores, holothurians, 5 fish and recovered mussels.

SITE C2 (DUMPSITE)

Off W. end of St. David's Island (airport), 50m S. of dumpsite (principally scrapped vehicles).
Lat: 32° 21' 35"; Long: 64° 41' 53".
Water depth 11m, mud sediment (but with high cover of the green algae, *Cladophora*).

9 Sept 10.45-11.30: Pilot survey, sediment cores and fish collected.
11 Sept 16.00-17.30: Collections for benthos, sediment and water bioassays, sediment chemistry. Mussels deployed.
22 Sept 10.45-11.45: Collected further chemistry sediment cores, holothurians, 6 fish and recovered mussels.

HAMILTON HARBOUR

SITE H1 ("LAGOON REFERENCE" SITE)

c. 20m S. of channel marker no. 30, N. of Spanish Point, outside the entrance to Great Sound.
Lat: 32° 19' 04"; Long: 64° 48' 39".
Near-surface coral reef patches on sand at c. 10m depth

12 Sept 10-10.30: Mussel cages deployed and water samples collected (bottom, 0.5m and surface) - no other sampling. Caged mussels attached to blocks on the bottom with sub-surface buoy attached to separate block, as usual.
24 Sept 1.45-2.15: Further water samples taken, mussels recovered, and 6 holothurians and 6 fish collected.

SITE H2 (INNER HAMILTON HARBOUR)

Off Hamilton in the Inner Hamilton Harbour. 250m E. of White's Island, 100m off Lower Ferry on Paget (to S.), 300m off city quay (to N.).
Lat: 32° 17' 18"; Long: 64° 46' 48".
Water depth 9m, fine mud sediment.

10 Sept 14.30-15.30: Pilot survey. Sediment cores collected, test mussel cage deployed, test of Shipek grab (proved unsuitable for benthic sampling).

12 Sept 11-12.30: Collections for benthos, sediment and water bioassays, sediment chemistry. Mussels deployed, with sub-surface buoy at 6ft depth (high tide).
23 Sept 10.30-11.30: Collected further sediment chemistry cores and water samples. Mussels recovered. 5 fish caught by line off Albuoy Point quay - mid-way between H2 and H4.

SITE H3 (SPECTACLE ISLAND)

Near entrance to Hamilton Harbour. Just S. of channel between Saltus Island and Spectacle Island (100m N. from Spectacle Island).
Lat: 32° 17' 12"; Long: 64° 47' 58".
Water depth 10m, mud sediment.

10 Sept 15.30-16.15: Pilot survey. Sediment core taken.
12 Sept 13.00-14.45: Collections for benthos, sediment and water bioassays, sediment chemistry. Mussels deployed (only 2 rather than 3 cages, 25 animals in each).
23 Sept 12.30-12.45: Mussels recovered.
24 Sept 11.40-12.00: Further sediment chemistry cores and water samples.

SITE H4 (PRINCESS HOTEL)

Immediately off E. end of Princess Hotel (50m) in Hamilton Harbour.
Lat: 32° 17' 18"; Long: 64° 47' 25".
Water depth 11m, mud sediment.

12 Sept 15.00-16.45: Collections for benthos, sediment and water bioassays, sediment chemistry. Mussels deployed.
23 Sept 11.30-12.30: Further sediment chemistry cores and water samples.
24 Sept 13.30-13.45: Mussels recovered.

SITE H5 ("HARBOUR REFERENCE")

Darrell Island. 200m N. of E. end of Darrell Island, on the edge of the Granaway Deep.
Lat: 32° 16' 36"; Long: 64° 48' 54".
Benthic sampling and sediment chemistry cores taken 450m nearer Paget shore (Lat: 32° 16' 21"; Long: 64° 48' 52") in water depth 10m, mud sediment but with partial (light) *Cladophora* cover.

12 Sept 17.00-17.15: Mussel cages deployed.
19 Sept 11.45-13.45: Collections for benthos, sediment chemistry and sediment bioassay.
24 Sept 12.00-13.30: Further collection for sediment chemistry and water samples. Mussels recovered. 4 fish trapped. 6 Holothurians collected.

SITE H6 (NR. RED HOLE, INNER HARBOUR)

Towards Red Hole, further into the Inner Harbour than H2. 60m off Paget shore and opposite Harbour Master's Office on Hamilton quay (180m), amongst moored yachts.
Lat: 32° 17' 22"; Long: 64° 46' 36".
Water depth 9m, fine mud sediment.

19 Sept 9.30-11.30: Benthos and sediment chemistry sampling.
23 Sept 9.30-10.30: Further collection for sediment chemistry and water samples (this time bottom as well as 0.5m and surface water). 1 fish trapped.

SITE H7 (BLUE HOLE, OFF WHITE'S ISLAND)

Between W. tip of White's Island (240m) and Hodson's Ferry on Paget shore (90m).
Lat: 32° 17' 5"; Long: 64° 47' 2"
Water depth 10m, mud sediment.

24 Sept 9.40-11.40: Benthic sampling. Sediment chemistry and water sampling. 13 fish trapped and caught by line off W. end of White's Island 60m S. of channel marker no. 53, 250m from H7. 4 Holothurians collected.

ANNEX IV

ABSTRACTS OF SCIENTIFIC PAPERS RESULTING FROM THE WORKSHOP

The following papers will be published as a Special Issue of the Journal of Experimental Marine Biology and Ecology, early in 1990. These papers are currently being refereed, in the normal way for publication in the open literature, so the final structure of the Special Issue may change from that given below.

CHEMISTRY

The IOC/GEEP Bermuda workshop: organic and trace metal contaminants in sediments, seawater and organisms

K.A. Burns, M.G. Ehrhardt, J. MacPherson, J.A. Tierney, G. Kananen and D. Connelly

This paper presents the results of chemical analyses of sediments, seawater and bioindicator organisms collected in support of the Second Workshop by the IOC-IMO-UNEP Group of Experts on the Effects of Pollutants (GEEP) held in Bermuda in October 1988. The data show gradients for the trace metals Cu, Pb and Zn in sediments and petroleum hydrocarbons in sediments and organisms related to point and non-point source inputs to the local harbour waters. Evidence for local sources of PAH combustion products and PCBs is presented although no obvious point sources could be identified. Methods of organic analyses were expanded to provide an estimate of the relative abundance of hydrocarbon oxidation products compared to the parent hydrocarbon contaminants. Water samples confirmed that the harbours suffer inputs of relatively low boiling fuel products in addition to deposition of fossil fuel combustion products. However, in the island's subtropical environment processes of evaporation, tidal flushing and photo-degradation are rapid enough to prevent significant accumulation of light hydrocarbons in the calcareous sand sediments. Hydrocarbons in sediments were medium and high molecular weight residues, while the bivalve and holothurian bioindicator organisms concentrated the more soluble lighter hydrocarbon and PCB components. The *Arca zebra* bivalves displayed bioaccumulation patterns for organic contaminants similar to those known for *Mytilus edulis* and *Perna viridis*. The data base was too small to determine the full potential of the benthic feeding holothurians as bioindicators, although they did accumulate some organic contaminants. Significant proportions of the hydrocarbon residues in all samples were present as oxygenated reaction products, highlighting the need for further research on the bioaccumulation of relative bioactivities of the oxidation products.

Lipophilic dissolved organic material concentrated from inshore waters during the 1988 GEEP Workshop in Bermuda. I. Aromatic hydrocarbons and alkyl derivatives

K.A. Burns and M.G. Ehrhardt

In conjunction with the second IOC/GEEP workshop, lipophilic dissolved organic material was concentrated from Bermuda inshore waters by liquid-solid adsorption on Amberlite XAD-2. SEASTAR water samplers modified for the use of glass adsorption columns were moored at depths between 1 and 2 metres on the reef platform and in inland seawater basins (Hamilton Harbour, Castle Harbour). Sample volumes were from 221 to 435 litres. The adsorbed material was eluted from the resin with refluxing aqueous acetone employing an apparatus specially designed for that purpose. Fractions containing aliphatic and aromatic hydrocarbons were obtained by liquid chromatography on silica gel. Single compounds were identified and quantified by capillary GC/MS. Alkylated PAH with minor contributions of unsubstituted parent compounds were detected in the basins, strongly suggesting a fossil fuel origin.

Concentrations diminishing steeply over a relatively short distance between contaminated harbour water and a control station suggests, for unsubstituted PAH, a source of limited spatial extension. An even steeper concentration gradient of alkyl substituted PAH is interpreted as reflecting higher reactivity with respect to environmental alteration. Thus, predominance of unsubstituted over substituted PAH in environmental samples could, in extension of current opinion, have two reasons: a significant contribution of pyrolysis products or easier conversion/degradability of substituted PAH.

Lipophilic dissolved organic material concentrated from inshore waters during the 1988 GEEP Workshop in Bermuda. II. Alkylbenzenes and their photo-oxidation products

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Individual alkylbenzenes were quantified by GC/MS in lipophilic concentrates of Bermuda inshore waters, as were photochemically generated hydroxyl and carbonyl derivatives. Concentrations of photochemically generated hydroxyl derivatives exceeded those of carbonyl derivatives in harbour water with a relatively high load of dissolved organic material, as indicated by total lipid weight and UV fluorescence measurements. In waters with low organic carbon load concentrations of carbonyl derivatives remained essentially unchanged, but concentrations of hydroxyl derivatives dropped below the detection limit. Photochemical stability of a hydroxyl derivative observed in model experiments suggests that their decreasing concentrations are not the result of photochemical oxidation. We propose that the total concentration of dissolved organic material in the water influences the degree of oxidation and thus the composition of products formed.

Lumped concentrations of specific photo-oxidation products such as alkylacetophenones, alkylbenzaldehydes, quinones, alkylbenzyl alcohols, alkylphenylethanols were, depending upon locality, up to more than 7 times higher than those of the parent hydrocarbons, indicating rapid conversion rates in this subtropical environment. The results support the stated need for further assessment of the relative toxicities of the reaction products in addition to the parent hydrocarbon contaminants in marine ecosystems.

BIOCHEMISTRY

Experimental and environmental induction of cytochrome P-450E in fish from Bermuda waters

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The chemical induction of liver microsomal cytochrome P-450 in fish from Bermuda was evaluated by examining the rates of ethoxyresorufin O-deethylase (EROD) activity and the levels of protein recognized in Western blot by monoclonal antibody 1-12-3 to scup cytochrome P-450E (P-450E). Scup P-450E is the EROD catalyst and a teleost representative of the hydrocarbon-inducible P-450-IA gene family in vertebrates. Treatment of blue-striped grunt (*Haemulon sciurus*) with β-naphthoflavone (BNF), an aromatic hydrocarbon-type inducer of P-450E, resulted in a strong induction of total P-450, EROD activity and the immunodetected P-450E homologue, at doses greater than 1 mg/kg. The induction peaked at about 3 days, declining progressively after that. A P-450E counterpart was also induced by BNF in squirrelfish (*Holocentrus rufus*). Analysis of freshly caught blue-striped grunt, squirrelfish, and four additional fish species, from sites in Hamilton Harbour, Castle Harbour and Ferry Reach, Bermuda, revealed appreciable levels of EROD activity and P-450E in most of these fish. Two species, squirrelfish and tomtate (*Haemulon aurolineatum*) had unusually high levels of total microsomal P-450 (up to 1.5 nmol/mg), of unknown isozymic composition. The levels of both EROD activity and P-450E homologue in some species, notably blue-striped grunt, French grunt (*Haemulon flavolineatum*) and sergeant major (*Abudefduf saxatilis*) were significantly higher in fish taken at sites characterized by higher levels of sedimentary hydrocarbons and/or polychlorinated biphenyls. Association of EROD or P-450E content with contaminant residues was strongest when comparison was with the levels of bioavailable PCBs, i.e. PCB content in the bivalve *Arca zebra* from the sampling sites. The results indicate that induction of P-450E by environmental chemicals is occurring in many fish in Bermuda waters; the origin of these chemicals is not known. The results further support the utility of P-450 induction as an indicator of chemical contamination in aquatic systems.

Metallothionein as an indicator of heavy metal exposure in two subtropical fish species

C. Hogstrand and C. Haux

Induction of metallothionein (MT) and levels of cadmium, zinc and copper in the liver of the subtropical fish, blue-striped grunt (*Haemulon sciurus*) were studied after cadmium injection. Hepatic levels of MT, zinc and copper were also analyzed in blue-striped grunt and tomtate (*H. aurolineatum*) caught at heavy metal polluted areas of Hamilton Harbour and Castle Harbour, respectively. In addition, a comparison of the hepatic content of MT, cadmium, zinc and copper was made between blue-striped grunt, tomtate and squirrelfish (*Holocentrus rufus*), caught at unpolluted locations. Hepatic MT was assayed by a RIA, using an antiserum raised against MT from perch (*Perca fluviatilis*) and partially purified MT from blue-striped grunt as standard. Hepatic MT was significantly ($p<0.001$) included in a dose-dependent manner in response to a single intraperitoneal injection of $CdCl_2$. The doses were 2200, 740, 250, 82, 27 and 0 μg Cd/kg body weight, respectively. Significantly elevated hepatic MT levels ($p<0.01$) were found two days after injection of 2200 μg Cd/kg body weight and maximum content of MT in liver was reached after six days. MT values in fish six days after treatment were increased five fold

compared to the control group. Significantly increased MT levels ($p<0.05$) were also found in livers of blue-striped grunt and tomtate caught at contaminated sites compared to control fish. The increased MT levels were in good accordance with the hepatic zinc and copper levels in these fish. When levels of MT, cadmium, zinc and copper in liver from three subtropical fish species were compared, it was found that blue-striped grunt and tomtate resembled other fish species, such as rainbow trout (*Oncorhyncus mykiss*) and perch (*Perca fluviatilis*), in these respects, and that squirrelfish had high values.

BIOASSAY

Measurement of physiological energetics (scope for growth) and chemical contaminants in mussels (*Arca zebra*) transplanted along a pollution gradient in Bermuda

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Mussels (*Arca zebra*) were transplanted to two sites in Castle Harbour and five sites along a 'pollution gradient' in Hamilton Harbour, Bermuda. After 11-12 days mussels were sampled for measurement of physiological responses (such as feeding rate, food absorption and respiration rate) and analysis of chemical contaminants in their body tissues (metals, alkyltins, hydrocarbons and their polar oxygenated derivatives, and polychlorinated biphenyls). Physiological responses were integrated by means of the energy balance equation and performance was assessed in terms of 'scope for growth'. Mussels sampled 50m from the Castle Harbour 'dump site' showed a slight, but not significant ($P>0.05$), decline in scope for growth and a slight increase in accumulated contaminants (tributyltin, hydrocarbons and PCBs) in comparison with the Castle Harbour 'reference site'. In contrast, mussels sampled from sites along the length of Hamilton Harbour showed a marked decline in scope for growth ($P<0.05$), due to a reduction in feeding rate and an increase in metabolic energy expenditure. This was accompanied by a significant accumulation of contaminants (Pb, tri-and di-butyltin, petroleum hydrocarbons and their polar oxygenated derivatives and PCBs). There was a significant negative correlation between the scope for growth of *Arca* and the concentrations of Pb ($r=-0.76$), TBT ($r=-0.91$), hydrocarbons ($r=-0.92$) and PCBs ($r=-0.95$) in their tissues. *Arca* accumulated hydrocarbons to tissue concentrations that were sufficient to explain the recorded decline in feeding rate, through the mechanism of 'non-specific narcosis' (based on relationships established for the mussel *Mytilus edulis*). Similarly, tributyltin was accumulated to concentrations that could induce the observed increase in energy expenditure, through the mechanism of uncoupling of oxidative phosphorylation. Toxicological interpretation of the coupled physiological and tissue residue chemistry data therefore indicates that hydrocarbons and tributyltin are the major toxic contaminants causing the reduction in scope for growth of *Arca* in Hamilton Harbour.

Water quality bioassays in two Bermudan harbours using the ciliate *Euplotes vannus*, in relation to tributyltin distribution.

A.R.D. Stebbing, S. Soria, G.W. Bryan and J.J. Cleary

Laboratory cultures of the ciliate *Euplotes vannus* were used to bioassay water samples taken from the sea surface, 0.5m and near bottom at stations

along contamination gradients in Castle Harbour and Hamilton Harbour on Bermuda. Inhibition of population growth rate was used as an index of exposure to toxic contaminants. Significant differences in growth rates ($P<0.005$) were observed between responses to near-bottom water samples taken at control (C1) and contaminated (C2) stations in Castle Harbour, which were reflected in the elevated concentrations of metals (Cu, Pb, Zn) and petroleum hydrocarbons (expressed in terms of Arabian Light Crude and chrysene equivalents) in sediments at C2. Bioassays of four Hamilton Harbour samples indicated most significant differences ($P<0.005$) between the control site (H1) and the other three (H2, H3 and H6) when results from all three depths are pooled. Some bioassays were discounted due to the protracted period of storage of water samples (15 days). Highest concentrations of tributyltin (TBT) occurred in the surface microlayer (307 ng TBT/l at H4), although not invariably so, due to their susceptibility to sea state and wind speed and direction. At 0.5m concentrations showed a steady decline in concentration from the head of Hamilton Harbour (41 ng TBT/l) seawards to the control site (H1) outside the entrance to Great Sound (0.9 ng TBT/l). Dibutyl tin (DBT), the less toxic degradation product of TBT, occurred in higher concentrations at all stations. The data indicated TBT inputs related to the distribution of boating activity, and the high degradation rates from DBT to TBT and limited exchange of contaminated water with water from the open sea. The TBT and DBT data for analyses of water from Hamilton Harbour related in a coherent way with the tissue residue data for *Arca zebra* deployed during the workshop at the same sites. While it is known that the concentrations of TBT found in Hamilton Harbour are high enough to be toxic to some species, TBT alone could not have accounted for the depression of ciliate growth rates in the bioassay experiments.

Changes in the biochemical composition of a subtropical bivalve, *Arca zebra*, in response to contaminant gradients near Bermuda

D.F. Leavitt, B.A. Lancaster, A.S. Lancaster and J. McDowell Capuzzo

To quantify the biochemical composition of a subtropical bivalve and to examine the changes in biochemical composition in relation to contaminant gradients, the turkey wing mussel, *Arca zebra*, was deployed in cages along two contaminant gradients (Castle Harbour and Hamilton Harbour) in Bermuda for 12 days. Following exposure to the gradients, pooled homogenized samples of the mussel were analyzed for protein, ash, total lipid and lipid class composition. The resulting data indicated that the biochemical composition of *A. zebra* was similar to that observed in other bivalves that rely on glycogen as their primary energy storage medium. Differences were noted in the lipid content and composition in mussels deployed in the two harbours. These differences suggested that Hamilton Harbour had higher food availability than Castle Harbour and consequently the *A. zebra* from Hamilton Harbour were in better physiological condition as indicated by higher lipid levels, primarily the neutral lipids. In evaluating the contaminant gradients, the biochemical composition of the mussels suggests that Castle Harbour is marginally impacted by the dump site. Hamilton Harbour, on the other hand, demonstrates a classic response of bivalves impacted by anthropogenic inputs to the ecosystem. The *A. zebra* deployed in Hamilton Harbour had significantly increased levels of neutral lipids and significantly depressed levels of polar lipids, suggesting a disruption in the mobilization of neutral lipids to phospholipids. The relationship of sterol ester to sterol varied consistently along the contaminant gradients. Changes in biochemical composition of *A. zebra* are responsive to gradients of anthropogenic inputs into the ecosystem.

COMMUNITY STUDIES

Analysis of macrobenthic and meiobenthic community structure in relation to disturbance in Hamilton Harbour, Bermuda

R.M. Warwick, H.M. Platt, K.R. Clarke, J. Agard and J. Gobin

A comparison of the community structure of the macrobenthos and meiobenthos at six stations in Hamilton Harbour, Bermuda showed that the two components were affected differently by environmental disturbance. Univariate statistical analysis of the macrofauna species data gave clear indications of disturbance at two stations, which did not relate to levels of pollutants. The meiofauna were apparently undisturbed at all localities investigated. The explanation for this differential response was most likely to be physical disturbance of the sediment by the passage of large cruise liners. Multivariate statistical analyses indicated that differences in faunal composition between stations for both macrobenthos and meiobenthos were not determined by differences in water depth or sediment type. However, the faunal composition did correlate with certain anthropogenic variables, particularly the tributyl tin concentrations in the water column. Thus, multivariate analyses detected differences in community composition which could be related to the pollution gradient at contaminant concentrations below those at which univariate measures could detect any stress-response. For macrobenthos, taxonomic aggregation of the species data to family level resulted in little loss of information both in univariate and multivariate analyses but for the nematode component of the meiofauna aggregation from genus to family level resulted in a substantial loss of information.

Comparisons of dominance curves

K.R. Clarke

In the study of community structure, a widely-used technique for graphical representation of species abundance (or biomass) patterns in a sample is the dominance curve, in which species are ranked by abundance and the percentage of the total number of individuals belonging to each species is plotted against (log) species rank. Alternatively, these percentages are cumulated, as in "*k*-dominance" curves, or separate *k*-dominance curves for abundance and biomass are superimposed, giving "Abundance-Biomass Comparison" (ABC) curves. When such curves are replicated for samples from a number of sites, times or treatments, questions of statistical significance of apparent differences arise. A framework for such tests is described, and illustrated with benthic community data from the IOC/GEEP Bermuda Workshop, and other pollution impact studies. Also discussed is an approximately linearising transformation for the y-axis of a *k*-dominance plot, designed to improve clarity of presentation. In addition, a new descriptive tool is proposed for displaying "partial dominance" patterns in community data, and it is suggested that this may mitigate a recent criticism of the ABC method, namely its over-dependence on the single most dominant species.

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
37 Suppl.	Papers submitted to the 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	IOC, Unesco Place de Fontenoy 75700 Paris, France	English
40 Suppl.	IOC Workshop on the Technical Aspects of Tsunami Analyses, Prediction and Communications *Submitted Papers* Sidney, B.C., Canada, 29-31 July 1985	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 (out of stock) Spanish
44 Suppl.	IOC/FAO Workshop on Recruitment in Tropical Coastal Demersal Communities - *Submitted Papers* Ciudad del Carmen, Campeche, Mexico, 21-25 April 1986	IOC, Unesco Place de Fontenoy 75700 Paris, France	English
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	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	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
52	SCOR-IOC-Unesco Symposium on Vertical Motion in the Equatorial Upper Ocean and its Effects upon Living Resources and the Atmosphere Paris, 6-10 May 1985	IOC, Unesco Place de Fontenoy 75700 Paris, France	English
53	IOC Workshop on the Biological Effects of Pollutants Oslo, 11-29 August 1986	IOC, Unesco Place de Fontenoy 75700 Paris, France	English
54	Workshop on Sea-level Measurements in Hostile Conditions Bidston, UK, 28-31 March 1988	IOC, Unesco Place de Fontenoy 75700 Paris, France	English
55	IBCCA Workshop on Data Sources and Compilation Boulder, Colorado, 18-19 July 1988	IOC, Unesco Place de Fontenoy 75700 Paris, France	English
56	IOC/FAO Workshop on Recruitment of Penaeid Prawns in the Indo-West Pacific Region (PREP) Cleveland, Australia, 24-30 July 1988	IOC, Unesco Place de Fontenoy 75700 Paris, France	English
57	IOC Workshop on International Co-operation in the Study of Red Tides and Ocean Blooms Takamatsu, Japan, 16-17 November 1987	IOC, Unesco Place de Fontenoy 75700 Paris, France	English