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The GEEP Workshop: organic chemical analyses

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ABSTRACT: For the GEEP Workshop, gas chromatographic/mass spectrometric (GC/MS) analyses of selected 2- to 5-ring aromatic hydrocarbons were performed for water, sediments, mussels *Mytilus edulis* and crabs *Carcinus maenas* in the mesocosm experiment at Solbergstrand. The experiment involved dosing of 3 different concentrations of diesel oil and copper to the mesocosm basins, and the chemical analysis showed that elevated abundance of compounds from the diesel oil could be detected in water and organisms but not in sediments. Mussels and crabs sampled along a pollution gradient in Langesundfjord were analysed for selected aromatic hydrocarbons by GC/MS and for selected PCBs by GC. The concentrations formed a clear gradient from the 'cleanest' site in Langesund Bay (Site 1), through increasing levels of contamination (Sites 2 and 3), to the most contaminated site at the head of Langesundfjord, closest to the industrial activity (Site 4). Mussels had the highest concentrations of aromatic hydrocarbons, especially unsubstituted 4- and 5-ring PAHs, while crabs had the highest concentrations of PCBs.

INTRODUCTION

This paper presents the results of the chemical analyses of organic compounds performed for the GEEP 'Biological Effects' Workshop, held in Oslo in August 1986 (see Bayne et al. 1988). The practical work was carried out on field samples from the Frierfjord/Langesundfjord area, which is heavily contaminated by industrial discharges, and in experimental exposures at the mesocosm facility of the Marine Research Station, Solbergstrand.

Evaluation of techniques in biological effects studies requires a good base of information on concentrations of pollutants to which the organisms are likely to have been exposed. Selected aromatic hydrocarbons and polychlorinated biphenyls (PCBs) were therefore analysed. Aromatic hydrocarbons, especially polycyclic aromatic hydrocarbons (PAHs, compounds with 3 or more fused rings) have long been recognized as hazardous environmental chemicals capable of inducing biological responses. They are among the most toxic compounds in diesel oil, and several PAHs are well known chemical carcinogens and/or mutagens (NAS 1972, Arcos & Argus 1975). Because of the lipophilic character of aromatic hydrocarbons, they are readily accumulated in organisms. The concentrations of PAHs in mussels *Mytilus edulis* in Frierfjord/Langesundfjord are high, and these are therefore not recommended for human consumption (Rygg 1985).

Aromatic hydrocarbons have both natural and anthropogenic sources, though the latter are believed to be by far the most important. Major inputs are from incomplete combustion of fossil fuels and discharges of petroleum and petroleum-derived products. Different sources contribute characteristic compounds, and their compositions are often very complex. At present no single method exists for the separation and quantification of all individual compounds found at low concentrations typical of environmental samples; a selection was therefore made (e.g. Appendix 1, Table 1). Aromatic hydrocarbons within the naphthalene (2-ring), phenanthrene and dibenzothiophene (3-ring) homologous series were analysed, including the parent unsubstituted compound and C_1-(methyl), C_2-(dimethyl, ethyl) and C_3-(trimethyl, ethyl/methyl, propyl) alkyl homologues. These are characteristic compounds for petroleum and diesel oil. The 4- and 5-ring unsubstituted PAHs, from fluoranthene to perylene (Appendix 1, Table 1), are only found in minor amounts in petroleum. Their main sources are believed to be from combustion processes though perylene may also originate from natural sources, by rapid transformation of biogenic precursors (Laflamme & Hites 1978, Wakeham et al. 1980). Gas chromatographic/mass spectrometric methods were used for the analysis.

Another important group of compounds is the polychlorinated biphenyls (PCBs), pollutants widely distributed within the environment and a cause of great

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concern because they are highly lipophilic, persistent and readily bioaccumulated. PCBs are industrial chemicals, solely of anthropogenic origin. They differ in degree of chlorination, giving a theoretical total of 209 different congeners. Gas chromatographic methods were used for determination of selected PCB congeners (e.g. Appendix 1, Table 9) and for determination of total PCBs. These analyses were only performed on samples from field sites.

SAMPLING

In the mesocosm experiment at Solbergstrand, 4 basins were used to dose selected organisms under controlled conditions, over 4 mo. Exposures started on 24 April and terminated during August, after sampling for the workshop had been completed. Target concentrations for water-accommodated fractions (WAF) of diesel oil in basin water were 4, 25 and 100 $\mu g\ l^{-1}$ in the low (L), medium (M) and high (H) dose basins, respectively, with the fourth basin serving as a control (C). (Copper was also dosed at nominal concentrations of 0.8, 5 and 20 $\mu g\ l^{-1}$ in L, M and H basins). Details of the mesocosm experiment are described by Bakke et al. (1988).

The aromatic hydrocarbons in water, sediments and mussels *Mytilus edulis* were analysed on 2 or more occasions during the experimental exposures. Crabs *Carcinus maenas* were sampled for chemical analysis only at the end of the experimental period. Water sample analyses of selected aromatic hydrocarbons were correlated with UV-Fluorescence (UVF) measurements of 'total hydrocarbons' (Bakke et al. 1988), which were performed on a routine basis to control concentration levels of diesel oil in the different basins. Water samples (1 l) were collected in May, June and July in solvent-cleaned water bottles with teflon lined stoppers. Pure diesel oil and diesel oil/water from the mixing unit were also collected for chemical analysis. All samples, except for diesel oil itself, were preserved with 20 ml pure dichloromethane until analysis.

The 5 boxes of sediment transplanted to each of the 4 mesocosm basins (for benthic community studies) were sampled on 3 occasions, in order to monitor any accumulation of hydrocarbons in the sediments. The first samples were taken on 16 May, 2 cores being collected from each of the Boxes 1 to 4 in the control basin. On 13 June, cores were collected from Boxes 1 and 2 in all 4 basins, and on 7 July from Boxes 3 and 4 in all basins. Sediments were sampled using a stainless steel corer (6 cm diameter) inside a core liner of plexiglass placed in the sediment. Core liners were left in the sediment after sampling. First sampling in the control basin was with an 8 cm diameter stainless steel corer without liners. The upper 3 cm of the cores were transferred to glass jars and kept frozen at −20 °C until analysis.

The basins were stocked with mussels on 25 April (Stock 1) and restocked on 7 July (Stock 2), owing to very high mortality of Stock 1 mussels in the high dosage basin after ca 7 wk of exposure to the diesel oil/Cu mixture. Sampling of Stock 1 mussels was performed on 16 May from the control basin, on 13 June from all basins and on 4 August from all except the high dosage basin. Separate samples of 10 individuals were collected from the 2 cages within each basin. Mussels were packed in aluminium foil inside polyethylene bags and immediately frozen until analysis. Stock 2 mussels were sampled from the 4 basins on 4 August, duplicate samples being collected from each basin. Background concentrations in restocked mussels were determined by analysing 2 samples of the stock collected from the vicinity of Solbergstrand on 7 July.

Basins were stocked with crabs on 12 May. Two pools of crabs, each of 4 individuals, were sampled from each basin on 4 August, and stored as described for the mussels. All equipment, corers and aluminium foil, were thoroughly pre-cleaned with solvent or baked at high temperature, in order to avoid contamination during sampling operations and storage.

For the field study in Langesundfjord, mussels and crabs were sampled at 4 sites (1 to 4, Fig. 2 of Follum & Moe 1988) expected to form a gradient of increasing contamination. Duplicate samples were taken at each site; sample size and treatment were the same as described for mesocosm samples.

All samples collected from both mesocosm and field studies were analysed within 2 wk, and the results made available to participants during the last week of the workshop.

ANALYSIS

All solvents used in the analyses were glass distilled and all glassware was baked at 400 °C for several hours prior to use. Anhydrous sodium sulphate was Soxhlet extracted with dichloromethane and dried at 110 °C. Complete procedural blanks were run routinely with every group of samples.

Hydrocarbons in unfiltered water (1 l) from mesocosm basins were extracted with 20+10+10 ml dichloromethane and the combined extracts dried over anhydrous sodium sulphate. Extracts were reduced to near dryness by a rotary evaporator and a gentle stream of pure nitrogen. Care was exercised during this step to avoid extensive losses of the most volatile aromatics, naphthalene and the C_1-naphthalenes. The sample was then redissolved in 200 µl of pentane.

After thawing the sediment samples, large plant particles or animals were removed. Subsamples were taken for hydrocarbon analysis and for determination of dry weights. Sediment samples (50 to 100 g) were saponified under reflux for 2 h with 100 ml methanolic KOH (0.5*N*) and the resulting methanol-water phase extracted with 2 × 30 ml of pentane. The 2 portions were combined and reduced in volume to 200 µl by a rotary evaporator and a gentle stream of nitrogen. Samples of ca 20 g wet weight were dried at 110 °C to constant weight (24 h) to provide the wet weight/dry weight relationship.

Mussels (10 ind. $pool^{-1}$) and crabs (4 ind. $pool^{-1}$) were thawed, opened, the liquid discarded and the soft parts homogenized with a high-speed food blender. Subsamples of ca 5 g wet weight were dried at 110 °C for 24 h to provide a wet weight/dry weight relationship. For extraction of hydrocarbons, samples of wet tissue (10 to 20 g) were saponified and the hydrocarbons partitioned into 2 × 30 ml of pentane. The method used for saponification was identical to that described for sediments.

All extracts for hydrocarbon analysis were subjected to a clean-up procedure prior to final analysis using 50 × 6 mm ID silica gel columns (70 to 230 mesh, 5 % deactivated) slurry packed in pentane. Hydrocarbons were eluted with pentane. After concentrating the samples to near dryness by a gentle stream of nitrogen, 50 to 100 µl of hexane were added.

Total hydrocarbon extracts were analysed for selected 2- to 5-ring aromatic hydrocarbons by gas chromatography/mass spectrometry (GC/MS) in the 'Selected Ion Monitoring' (SIM) mode. The instrument used was a Hewlett Packard model 5987 A equipped with a 30 m × 0.32 mm ID SE-54, 0.17 µm bonded phase fused silica capillary column inserted directly into the ion source. Other conditions were: injector temperature, 280 °C; transfer line, 275 °C; column temperature, 40 to 100 °C at 15 °C min^{-1}, 100 to 270 °C at 6 °C min^{-1}; carrier gas, 1.5 ml He min^{-1}. Electron impact ionization at 70eV was used. Samples (2 µl) were injected by splitless injection.

GC/MS was performed using fully deuterated internal standards: biphenyl for the 2-ring, anthracene for the 3-ring, and pyrene for the 4- and 5-ring aromatic hydrocarbons. These were added to samples prior to extraction/saponification. Response curves for individual compounds were constructed using integrated molecular ion currents obtained by injecting standard mixtures of aromatic hydrocarbons. Complete sets of alkylated compounds were not available as pure reference standards. Response curves for these compounds were obtained by analysing an Ekofisk crude/Arabian light crude reference oil, containing known amounts of all C_1- to C_3-alkyl homologues. Compounds in the oil were originally quantified by gas chromatography, using FID detection, under the assumption that the gas chromatographic response factors were similar to those shown by the structurally-related reference compounds. Gas chromatographic conditions were identical to those described for GC/MS analysis.

Organisms from Langesundfjord were also analysed for content of PCBs using gas chromatographic methods. The first part of the work-up procedure was the same as for the aromatic hydrocarbons. After saponification and extraction into pentane, extracts were divided into 2 equal parts, one for determination of aromatics and one for PCBs. Volumes of the extracts for PCB analysis were adjusted with pentane to 4 ml in 10 ml Sovirel tubes and washed with 4 ml concentrated sulphuric acid. After 1 h, pentane extracts were separated from the acid, evaporated to dryness with pure nitrogen, and redissolved in 100 µl hexane for injection on the gas chromatograph (Hewlett Packard model 5880A with a Ni-63 detector and a 50 m × 0.32 mm ID SE-54 fused silica capillary column, 0.17 µm bonded phase). Gas chromatographic conditions were: injector temperature, 280 °C; detector temperature, 320 °C; column temperature, 100 °C (1 min) to 260 °C at 3 °C min^{-1}; carrier gas, hydrogen at 35 cm s^{-1}; splitless injection of 2 µl with 1 min closing time.

The mixture of congeners used for the quantification of individual compounds and total PCBs are shown in Appendix 1, Table 9. Response factors were calculated relative to IUPAC No. 53, which was used as an internal standard. This was added to the samples prior to saponification. For the determination of total PCBs, peak heights of all the measured congeners, except for No. 53, were added and compared with the same peaks in Phenochlor DP-5.

RESULTS AND DISCUSSION

Results of the chemical analyses in samples of water, sediment, *Mytilus edulis* and *Carcinus maenas* are presented in Appendix 1, Tables 1 and 3 to 10. Blank values have not been subtracted from the results since they were generally negligible. The analytical methods were routinely tested for extraction efficiencies using spiked samples. Absolute recoveries of most of the selected aromatic hydrocarbons and the deuterated standards were 80 ± 20%. For the lower molecular weight compounds, like naphthalene and the C_1-naphthalenes, the recoveries were less, namely 60 ± 20%. This was mainly due to volatilization losses during the concentration steps. Recoveries of the quantified compounds relative to the internal standards were 100 ± 10 %. Absolute recoveries for individual PCBs were 90 ± 10 %. The data presented are not corrected for

differences in recoveries and are precise to only 2 or 3 significant figures.

Appendix 1, Table 1 presents the results for the 3 occasions on which water samples were taken from the mesocosm basins, during the 4 mo of dosing with water accommodated fraction (WAF) of diesel oil and a solution of copper. In the L, M and H dosage basins naphthalene and its C_1- to C_3-alkyl homologues contributed 71 to 95 % of the analysed compounds. Phenanthrene and dibenzothiophene and their alklyl homologues contributed 5 to 17 % and 0 to 13 % respectively. Fluoranthene and pyrene were detected at low concentrations in only 2 samples and the other 4- and 5-ring aromatic hydrocarbons were not detected (detection limit 3 ng l^{-1}). The relative abundance was in good agreement with the composition of compounds in diesel oil. Diesel oil contained 2.6 % of the aromatic hydrocarbons quantified, of which naphthalene and its C_1- to C_3-alkyl homologues made up 79 %, phenanthrene and its C_1- and C_2-alkyl homologues 13 %, and dibenzothiophene and its C_1- to C_3-alkyl homologues 8 %. Fluoranthene, pyrene, benz[a]anthracene and chrysene could also be traced, although at very low concentrations, and the 5-ring aromatic hydrocarbons, benz[b+k]fluoranthene, benz[e+a]pyrene and perylene, were not detected.

There is some uncertainty in the results in 1 sample from the control basin, taken on 22 May, for which the relative abundance of aromatics were totally different from that in the other basins. The concentrations of phenanthrenes and dibenzothiophenes were higher than their concentrations in the low dosage basin. Also, fluoranthene and pyrene levels were higher than in any of the other samples. This suggests that the sample was subject to some form of secondary contamination.

The most volatile compounds quantified, the naphthalenes, were traced in all 3 water samples from the control basin. This may indicate some input of aromatic hydrocarbons to that basin. As discussed later, this suggestion is supported by an increased concentration in the second stock of mussels after 17 d in the control basin. The 4 basins are within the same enclosed building and a flux of the more volatile hydrocarbons from the dosed basins via the atmosphere to the control basin may have taken place.

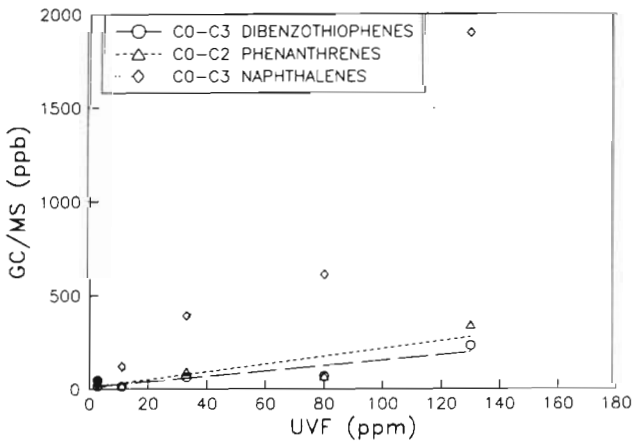

Fig. 1 Relation between selected aromatic hydrocarbons, quantified by GC/MS, and UVF-measurements of 'total hydrocarbons' in water samples from the mesocosm basins

The fluctuations in concentration of the different hydrocarbon components in each of the basins were considerable. In part this may have been caused by erratic fluctuations in the mixing efficiency of diesel oil into water. Such variation was also shown by the UVF measurements of 'total hydrocarbon' concentrations (Bakke et al. 1988). Relations between 'total hydrocarbons' and the sums of naphthalenes, phenanthrenes and dibenzothiophenes are shown in Fig. 1. The correlation coefficients for the 3 groups of compounds were 0.91, 0.77 and 0.83 respectively. The results are based on parallel samples collected on 22 May and 19 June.

Concentrations of aromatic hydrocarbons in silt/clay sediments transplanted into the mesocosm basins are given in Appendix 1, Table 3 (detection limit 1 ng g^{-1} dry weight); they indicate weak contamination in all basins. The ubiquity of aromatic hydrocarbons in marine sediments has been well established, especially the presence of PAHs (e.g. Youngblood & Blumer 1975, Laflamme & Hites 1978). PAHs have low aqueous solubilities and large distribution coefficients, which strongly favours their binding to particles in aquatic environments, leading to an accumulation in sediments. Two sediment cores from each of 4 boxes from the control basin were sampled on 16 May. Although the boxes had been in the control basin for nearly 1 mo before sampling, these results should give an indication of the original concentrations in these sediments from Bjørnhodet Bay (see Fig. 1 of Follum & Moe 1988). Broadly, the 2- and 3-ring aromatics spanned similar concentration ranges, with the exception of phenanthrenes, for which the concentrations were a little higher. Four-ring compounds were more abundant still and the highest levels were seen for 5-ring aromatics. The higher concentrations of unsubstituted PAHs, compared to the 2-ring naphthalenes and 3-ring alkyl-substituted dibenzothiophenes, indicate that the major source of aromatic hydrocarbons is from combustion or pyrolytic processes rather than direct input of non-combustion aromatics (e.g. fossil fuels, diesel oil). In comparison with duplicate measurements within each box, some significant variation can be seen between boxes in the control basin, particularly in sediments for Box 2. However, a sediment core collected on 13 June from the same box did not show a similarly high abundance of 5-ring aromatics. Such differences in concentration could be caused by inhomogeneity in the origi-

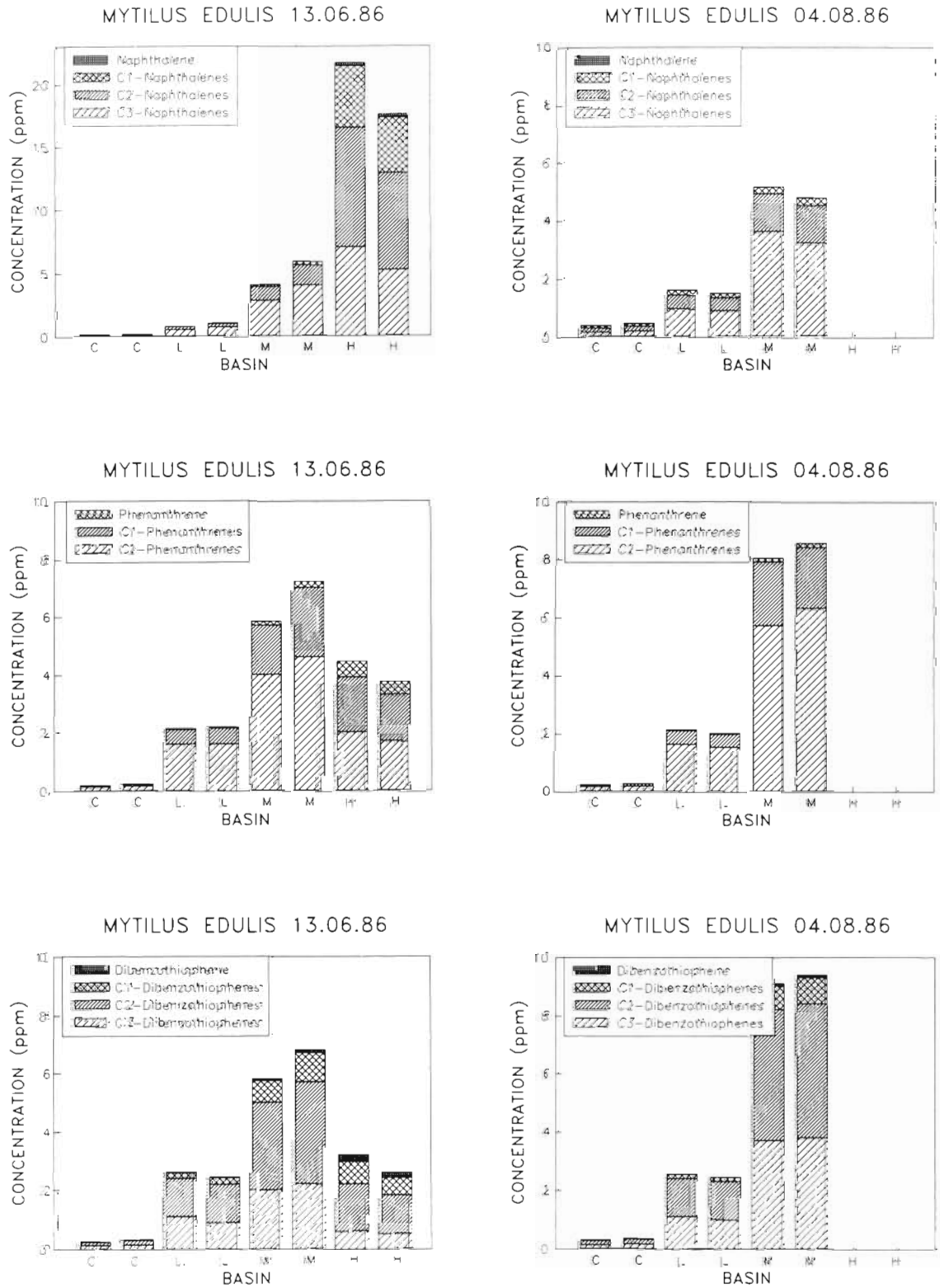

Fig. 2. *Mytilus edulis*. Concentrations (μg g^{-1} dry wt) of selected 2- and 3-ring aromatic hydrocarbons in whole tissues of mussels put into the basins on 25 April and sampled on 13 June and 4 August (2 replicates, each of 10 individuals; C: control, L: low, M: medium, H: high dosage)

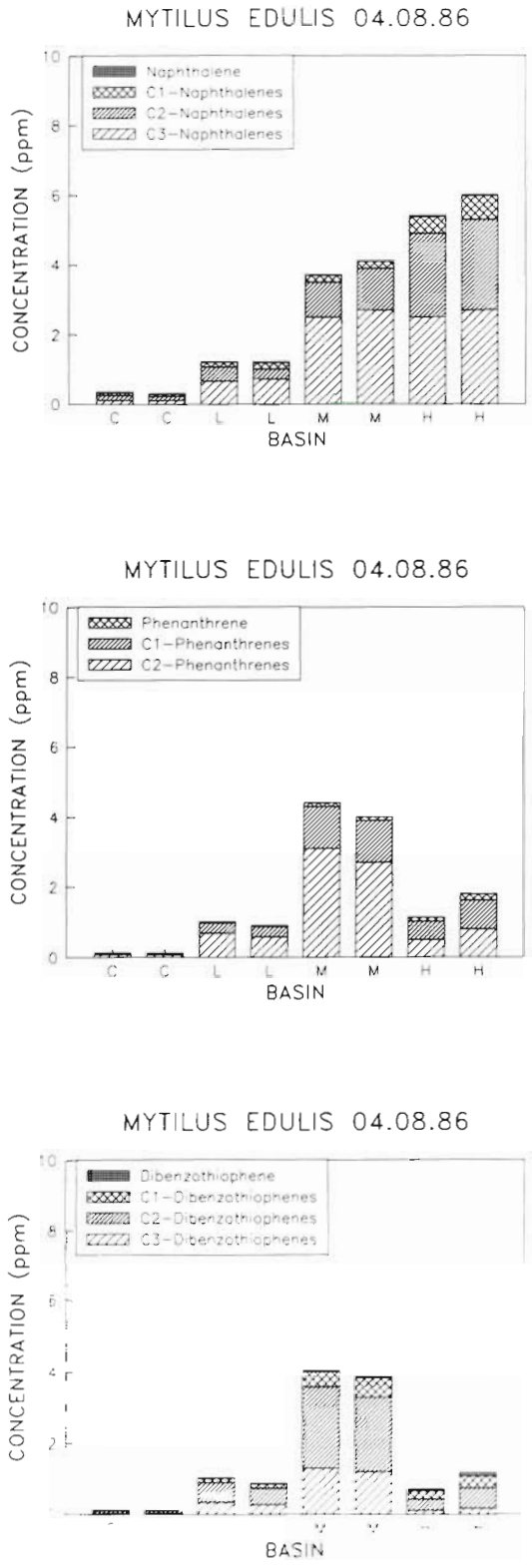

Fig. 3. *Mytilus edulis*. Concentrations (µg g^{-1} dry wt) of selected 2- and 3-ring aromatic hydrocarbons in whole tissues of mussels put into the basins on 18 July and sampled on 4 August

nal sediment or by perturbation of sediment during transfer.

Sediment cores were collected from all basins on 13 June and 7 July. Somewhat unexpectedly, sediments from the dosed basins did not show any increase over time in concentrations of hydrocarbons associated with diesel oil (see Gray et al. 1988 for statistical analysis of these data). The aromatic hydrocarbons in the water are partly dissolved/dispersed and, because of their lipophilic character, partly bound to the suspended particulate material. These results indicate low deposition of suspended particulate material and a low transfer of dissolved/dispersed hydrocarbons from water to sediment.

Concentrations of aromatic hydrocarbons in Stock 1 and Stock 2 mussels (transferred into the basins on 25 April and 18 July) are given in Appendix 1, Tables 6 & 7 respectively. The variability from duplicate cages within each basin is relatively small, indicating that the analytical method used has fairly good precision. The background concentrations of aromatic hydrocarbons in the mussels were relatively low, as shown both by the results from the control basin and by the concentrations in the 2 samples collected from the stock population on 7 July. As discussed earlier, the concentrations of most 2- and 3-ring aromatics increased in Stock 2 mussels after 17 d in the control basin, reaching nearly the same levels as in Stock 1 mussels. The concentrations of individual compounds in Stock 1 control mussels were relatively stable over the experimental period, the exception being a slight increase in the naphthalenes on the last sampling date, 4 August.

Results from basins exposed to WAF diesel oil showed that mussels had accumulated 2- and 3-ring aromatic hydrocarbons. Figs. 2 & 3 demonstrate this accumulation in Stock 1 and Stock 2 mussels respectively. Concentrations of 4- and 5-ring PAHs were low and differed little from the control basin. Diesel oil contains only traces of these compounds. In mussels from the same stock sampled at the same time, there was increased accumulation of the naphthalenes across the L, M and H basins, though for Stock 2 (sampled on 4 Aug) levels of C_3-naphthalenes were similar in the M and H basins. The only remaining Stock 1 mussels from the H basin were sampled on 13 June (after 7 wk exposure), and contained very high concentrations of alkylated naphthalenes; mussels in the high dosage basins were clearly intoxicated by the added diesel oil/Cu mixture.

Though an increasing trend in concentrations across L, M and H basins was also seen for other compounds (phenanthrene and dibenzothiophene), levels in M and H mussels were sometimes comparable (C_1-phenanthrenes and C_1-dibenzothiophenes) and sometimes reversed the trend (C_2-phenanthrenes and C_2- to C_3-

dibenzothiophenes). Intoxication of the high dose mussels is likely to have been a factor in this reversal. The high abundance of naphthalenes and low abundance of the alkyl homologues of phenanthrene and dibenzothiophene can be explained if it is assumed that there have been alterations in both uptake and elimination rates of aromatics. Major routes of uptake are through the gut and the gills. If mussels from the high dose basin were in such a bad condition that they had stopped filtering, there would have been decreased uptake and this could explain the lower concentrations of the 3-ring aromatics in H dose mussels than in the M basin. Since higher concentrations of naphthalenes were accumulated in H than M there must also have been a decrease in the elimination rates for naphthalenes, for example by decreased metabolism of the compounds.

Concentrations of aromatic hydrocarbons in crabs (whole tissues) in the mesocosm basins are given in Appendix 1, Table 8. The dosing period was 84 d and, though the concentrations of 2- and 3-ring aromatics increased steadily with increasing dose, the accumulations were lower than in mussels. The major increase was in the naphthalenes. Concentrations of the 4- and 5-ring PAHs were below the detection limit (1 ng g^{-1} dry weight) in nearly all samples.

For the Field sites 1 to 4 in Langesundfjord, concentrations of aromatic hydrocarbons in mussel whole tissues are given in Appendix 1, Table 4. Results show that mussels were contaminated with aromatic hydrocarbons, unsubstituted PAHs being present at relatively high concentrations. PAH contamination in Frierfjord/Langesundfjord originates mainly from the electrometallurgical industry in the area. These concentrations increased in a clear gradient through Sites 1 to 4 (Fig. 4), as expected from their locations relative to the contaminant source. Results were in fairly good accordance with earlier investigations at the same locations (Rygg 1981). Concentrations of the naphthalenes were approximately the same at all 4 sampling sites, whereas the phenanthrenes and dibenzothiophenes followed the same trend as for the 4- and 5-ring aromatics, though in a less pronounced fashion.

Concentrations of aromatic hydrocarbons in crabs from Langesundfjord Sites 1 to 4 (Appendix 1, Table 8) were much lower than in mussels from the same sites. Different feeding regimes for the 2 species are a possible reason for the lower concentrations in the crabs. Crustaceans are also able to eliminate PAHs relatively rapidly by metabolism and excretion (Neff 1979). The typical petroleum hydrocarbons, naphthalene and its alkyl homologues, were detected, but the dibenzothiophenes were below the detection limit (1 ng g^{-1} dry wt) in most crab samples. Different concentrations of aromatic hydrocarbons were accumulated from the

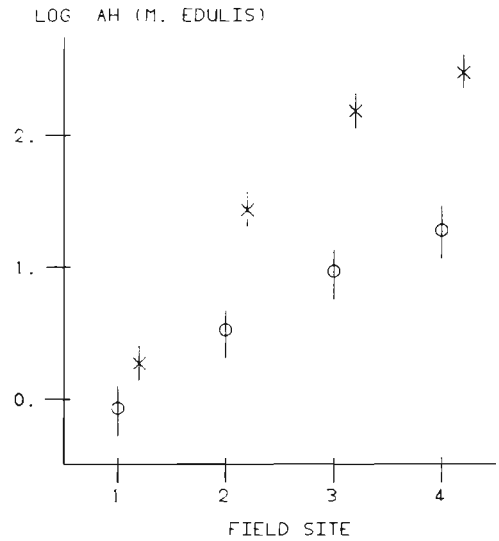

Fig. 4. *Mytilus edulis*. Total concentrations (µg g^{-1} dry wt) of selected aromatic hydrocarbons in whole tissues of mussels from the Langesundfjord Sites 1 to 4; 2- and 3-ring compounds (○), and 4- and 5-ring compounds (×) from Appendix 1, Table 4. Values are: mean ± 95 % CI, based on pooled SE from all 4 sites; $\log_e$ scales were used for the computations and the figure

4 sampling sites, with the same trend of increasing concentrations closer to the discharge sources as observed for mussel tissues.

Sediments from sampling sites for benthic community studies in Frierfjord/Langesundfjord (Sites A to G, Fig. 2 of Follum & Moe 1988) were analysed for selected aromatic hydrocarbons by the Norwegian Institute for Water Research (NIVA), using similar GC/MS techniques. Results can be found in Appendix 1, Table 2.

Concentrations of PCBs in the samples of mussels and crabs from epibenthic sampling sites in Langesundfjord (Sites 1 to 4) were also determined (Appendix 1, Tables 9 and 10). The individual PCB congeners analysed are often among those predominating in environmental samples and here they constituted 49 to 64 % of the total PCBs in the organisms. The variability of duplicate analyses within sites is low, indicating that the analytical method has fairly good precision. The relative abundance of the individual PCB congeners was much the same for mussels and crabs, with the exception of the very high concentration of compound No. 52 in the mussels. Because of overlap in the chromatograms between this peak and a big unknown peak, the concentration of No. 52 is most probably too high. PCB congeners No. 101, 118, 153 and 138 had high relative abundance both in crabs and mussels. Three of these compounds do not possess vicinal H-atoms and are therefore difficult to metabolize by the organisms. Absolute concentrations

of total PCBs and individual PCB congeners were relatively low both in mussels and crabs (with crabs having the higher concentrations). Nonetheless, a clear contaminant gradient was observed from Site 1 to Site 4, in line with the trend for aromatic hydrocarbons.

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