The Distribution and effects on Norwegian fjord and coastal ecosystems of polycyclic aromatic hydrocarbons (PAHs) generated by the production of primary aluminium and manganese alloys

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Paper VII, Numerical analyses, page 11, 1st line: 'standardised PCA' should read: 'double-centred PCA'

Paper VII, page 22, text to Fig. 3, 1st line: 'standardised PCA' should read: 'double-centred PCA'

Paper IX, Statistical analyses, page 6, 4th last line: 'standardised PCA' should read 'double-centred PCA'

Paper IX, page 12, text to Fig. 2, 1st line: 'standardised PCA' should read: 'double-centred PCA'

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Abstract

Discharges of polycyclic aromatic hydrocarbons (PAHs) generated by the production of primary aluminium and manganese alloys have been a serious environmental problem in Norwegian fjords and coastal waters. Scientists have been performing environmental assessments in the receiving waters from these production plants for more than twenty-five years, detecting high PAH concentrations in sediments and biota. Most of the assessments have been limited to individual recipient bodies of water. No previous projects have evaluated the results from a holistic point of view, that is, as an integrated analysis within the same ecosystem compartment (for example, inter-fjord sediment to sediment comparison) or between different compartments (e.g. sediments to organisms within and between fjords). Over the past decade, changes in production processes, the installation of scrubbers etc. have drawn attention to the need for a broad-based evaluation of the ecological fate and effects of present and past PAH discharges.

Accordingly, the main objective of this study has been to provide a comprehensive environmental risk assessment of discharges to marine waters of PAHs generated by the production of primary aluminium and manganese alloys. This has been accomplished by studying PAHs in dissolved, colloidal and particulate states in waste and recipient waters, the accumulation of PAHs in bottom sediments and littoral indicator organisms, and the effects of PAHs on benthic fauna at the community and cellular levels. Most of the data have been collected in the course of 25 years of monitoring, although new data have been collected to fill in knowledge gaps. To a large extent, numerical treatment has employed multivariate techniques such as principal component analysis, redundancy analysis and canonical correspondence analysis.

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List of papers

This thesis is based on the papers listed below. They are referred to in the text by their Roman numerals.

I. Næs, K., J. Knutzen and L. Berglind, 1995. Occurence of PAH in marine organisms and sediments from smelter discharge in Norway. *Sci. Total. Environ.*, 163: 93-106

II. Næs, K., T. Bakke and R. Konieczny, 1995. Mobilization of polycyclic aromatic hydrocarbons (PAHs) from polluted seabed and uptake in the blue mussel (*Mytilus edulis*). *J. Mar. Freshwater Res.*, 46: 275-285.

III. Næs, K. and E. Oug, 1997. Multivariate approach to distribution patterns and fate of polycyclic aromatic hydrocarbons in sediments from smelter-affected Norwegian fjords and coastal waters. *Environ. Sci. Technol.*, 31: 1253-1258.

IV. Næs, K. and E. Oug, 1998. The distribution and environmental relationships of polycyclic aromatic hydrocarbons (PAHs) in sediments from Norwegian smelter-affected fjords. *CHEMOSPHERE*, 36: 561-576.

V. Næs, K., E. Oug and J. Knutzen, 1998. Source and species-dependent accumulation of polycyclic aromatic hydrocarbons (PAHs) in littoral indicator organisms from Norwegian smelter-affected marine waters. *Mar. Environ. Res.*, 45: 193-207.

VI. Oug, E., K. Næs and B. Rygg, in prep. Relationships between soft bottom macrofauna and polycyclic aromatic hydrocarbons (PAHs) from smelter discharge in Norwegian fjords and coastal waters. Submitted *Mar. Ecol. Prog.Ser.*

VII. Næs, K., K. Hylland, E. Oug, L. Förlin and G. Ericson, in prep. Accumulation and effects of aluminium-smelter generated PAHs on soft-bottom invertebrates and fish. Submitted *Environ. Toxicol. Chem.*

VIII. Næs, K., J. Axelman, D. Broman and C. Näf, 1998. Role of soot carbon and other carbon matrices in the distribution of PAHs among particles, DOC and the dissolved phase in the effluent and recipient waters of an aluminium reduction plant. *Environ. Sci. Technol.*, 32: 1786-1792.

IX. Axelman, J., K. Næs, D. Broman and C. Näf, in prep. Accumulation of PAHs by SPMDs and caged blue mussels (*Mytilus edulis* L.) and comparison with the PAH distribution in the dissolved, colloidal and particulate size fractions in the water column. Submitted *Environ.Toxicol. Chem.*

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1 Introduction

1.1. Background

Towards the end of the 1900s, Norway developed its hydroelectric power for industrial purposes. The country's topography offered an abundant supply of waterfalls, making hydropower cheap and easily accessible. This paved the way for a flourishing metallurgical industry based on Norwegian mines as well as imported ore. The invention of the Søderberg electrode (Grjotheim and Kvande 1993) in 1917 to 1919 was a milestone for manufacturing. The extensive industrial development earlier in the century has given rise to many of today's local and regional environmental issues. Most manufacturers are situated along fjords or coastal waters, meaning that environmental problems can affect marine ecosystems, fjords in particular. Norwegian fjord ecosystems are of particular importance to the environment for two reasons: first, because they serve as sedimentation basins that trap discharged contaminants, and second, because most of the population of Norway lives along the coast.

Norway currently has about 30 metal works in operation, of which seven are primary aluminium (Al) reduction plants. Three produce manganese (Mn) alloys. For all Al and Mn works, the sea is the main recipient body of water. The most important issues related to the marine environment in these areas revolve around the content of polycyclic aromatic hydrocarbons (PAHs) in the effluents. The PAHs may originate directly from the production processes but, to a great extent in more recent years, they have resulted from the installation of furnace off-gas scrubbers to reduce air emissions (Bjørseth 1979; Thrane 1988; AMS 1994). The lipophilic nature of these compounds render them available for uptake and accumulation by aquatic organisms. This implies that they have a potential for being toxic, carcinogenic and/or mutagenic (Mix 1986; IARC 1987; Baumann 1989; Varanasi et al. 1989; Bucheli and Fent 1995), although these properties vary from one PAH compound to the next.

Environmental assessments of the marine receiving waters have been carried out for the past 25 years, and high PAH concentrations have been detected in sediments and organisms alike (Paper I). In some areas, this has resulted in restrictions on commercial fishing and dietary warnings to consumers. Currently, this situation applies to five fjords. Environmental programmes have mainly been based on concentrations in bottom sediments and indicator organisms (mussels and snails). In many of the fjords, the analyses of the soft-bottom community structure have been included as a measure of effects. Most programmes have been limited to individual recipient bodies of water. No analyses have evaluated the results from a holistic point of view, that is, as an integrated analysis performed within the same ecosystem compartment (for example, inter-fjord sediment to sediment comparison) or between different compartments (for example, sediments to organisms within and between fjords). Apart from the obvious benefits for summarising and comparing data, a holistic approach may also reveal significant patterns impossible to discern from the original tabular data or considered to be background noise in the routine statistics restricted to individual fjords. A holistic approach could thereby enhance man's understanding of the fate of the contaminants in the fjords. Moreover, little attention has been paid to distribution patterns and their correlations with explanatory environmental variables.

Changes in production processes, the installation of cleaning devices, etc., over the past decade have resulted in a strong decline in PAH discharges, more than 90 per cent for some facilities (AMS 1994; Anon. 1994). From an environmental management point of view, further reductions are needed in many plants. However, from the industrial standpoint, some plants may be approaching

the limit of what can realistically be achieved without radical upgrading. Accordingly, based on the improvements already achieved thus far, the need to reduce emissions is not as urgent as it used to be. There is therefore a need for a broad evaluation of the ecological fate and effects of present and past discharges of PAHs, both in order to achieve continuous environmental improvements and as a basis for future developments.

1.2. Objective

The main objective of this study is to provide a comprehensive environmental risk assessment of PAH effluents generated by the production of primary aluminium and manganese alloys. This will be accomplished by addressing the following topics related to fate and effects:

- PAH speciation;
- Transport and sediment incorporation;
- Physical, chemical and biological degradation;
- Accumulation in hard-bottom indicator organisms;
- Effects at the individual and community Slevels.

The assessment will, of necessity, imply an evaluation of the analytical quality as well as the experimental design of programmes performed during 25 years of monitoring smelter recipients. It will subsequently form basis for new revised programmes. It will also provide important background information for further efforts aimed at determining critical PAH loads in marine environments.

2 PAH generation and route of entry into the marine environment

The emission of PAHs is mainly due to the evaporation of volatile organic compounds (VOCs) present in coal tar pitch and petrol coke. Effluent discharges to the sea generally result from the installation of furnace off-gas scrubbers to reduce air emissions. Most of the smelters in Norway are located in the inner reaches of fjords, although two are situated of the coast. While locating plants in enclosed fjord waters may reduce contamination in the open sea, it may have created significant local and regional environmental problems.

2.1. Aluminium production

Aluminium is produced by electrolytic reduction in carbon lined steel cells (pots) containing alumina (Al_2O_3) in an electrolyte of molten cryolite (Na_3AlF_6). The carbon in the bottom and along the sides of the pots serve as cathodes, while carbon electrodes suspended in the electrolyte serve as the anodes. Anode carbon material is consumed during the process, requiring a continuous supply. The temperature in the bath is kept at 950 to 970 °C.

There are two different reduction cell technologies: Søderberg and Prebake. The main difference is the arrangement of the anodes. With Søderberg technology, the anode carbon is supplied as a pasty mix of petrol coke and coal tar pitch in a box-girded frame inside the cell, then baked to solid carbon using surplus heat from the process. During the baking process, the coal tar VOCs evaporate. The prebaked anodes are made from the same mix, but are baked in separate baking furnace and supplied to the cell in the form of prebaked carbon blocks. Hence, the VOCs evaporate even before the anodes enter the reduction plant.

PAH emissions are therefore mainly related to Søderberg technology. PAHs emit from the top of the anode and through cracks in the anode into the primary gas collecting system. Stud pulling also causes PAH emissions. Emissions from Prebake technology are related to the use of collar paste and from tarry glue used in the lower part of the cell.

2.2. Manganese alloy production

Ferromanganese and silicomanganese are produced in closed, steel-lined electrical pots using Søderberg electrodes. An isolating layer of ceramic bricks separates the reaction chamber from the steel lining. The bottom of the pot, and partly also the side walls, are lined with carbon ramming paste. Manganese ore, coke and quartz are the main raw materials. In the process, the mineral is reduced to metal by binding carbon to the oxygen in the mineral, forming carbon monoxide and carbon dioxide gas. All three Norwegian plants use electrodes of calcinated anthracite that use tar pitch as a binder, and all buy their electrodes from the same manufacturer.

Figure 1. Map of Norway with location of aluminium and manganese alloy smelters.

The electrode is baked in an oven at very high temperatures (> 1000 °C). The electrode top is cold (50-60 °C). PAHs are emitted due both to the evaporation of VOCs in the pitch used in the electrodes and from the coke used as a reduction agent. Depending on the type of ore, these contain varying amounts of trace elements such as copper, lead, tin, cadmium, zinc, nickel, chromium, and arsenic, which may cause additional environmental concerns.

2.3. Smelter location and production capacity

Most smelters in Norway are located along or in the inner reaches of fjords. Only two smelters are situated along the coast, see Figure 1 and Table 1.

Currently, approximately 40 per cent of Norway's aluminium production capacity is based on Søderberg technology, while 60 per cent is based on Prebake technology. Of the seven existing aluminium smelters, one is fully Søderberg and one is fully Prebake. The latter is not included in this study. Totally, Norway produces approximately 900,000 metric tonnes of aluminium per year, accounting for 25 per cent of Europe's production capacity and 5 per cent of global production capacity. Altogether, approximately 500,000 metric tonnes of manganese alloy were produced in Norway in 1995, accounting for 30 per cent of European and 10 per cent of global production.

Table 1. Norwegian aluminium and manganese alloy smelters and their approximate production capacities.

Smelter location	Production, established	Production capacity, 1000 metric tons yr.$^{-1}$ (1995)
Årdal	Al (1948)	185
Sunndal	Al (1954)	150
Karmøy	Al (1967)	220
Mosjøen	Al (1958)	120
Lista[1]	Al (1971)	80
Høyanger	Al (1917)	68
Husnes[2]	Al (1965)	86
Sauda	Mn (1923)	210
Øye	Mn (1974)	150
Porsgrunn	Mn (1913)	130

[1] Søderberg only.
[2] Prebake only. Not included in the study.

2.4. Cleaning equipment and discharge arrangements

Waste gases from the aluminium reduction cells and the anode baking plants contain fluorides, dust, sulphur dioxide, carbon monoxide, carbon dioxide and pitch VOCs. Part of the pitch VOCs consists of PAHs. A variety of technologies are employed to reduce air emissions (Anon. 1994). Most of the fluoride, dust and PAHs are removed in dry scrubbers by adding alumina to the waste gases and filtering them through large bag filters. Wet scrubbers are used for SO_2 emissions and residual fluorides, dust and PAHs, by washing waste gases with seawater or, in some cases, caustic soda. Some Søderberg plants and anode plants use electrostatic precipitators to remove dust and pitch VOCs prior to using the dry scrubbers. Most plants differ in their cleaning technologies. Also, within a given plant, measures to reduce discharges may change over time (AMS 1994).

Waste gases from manganese alloy production also contain dust, sulphur dioxide, carbon monoxide and carbon dioxide. During the production process, carbon monoxide gas from the pot rooms passes across seawater scrubbers for particle removal. The "cleaned" CO-gases are then burned and may be used as an energy source. The particle-rich scrubber water passes sedimentation tanks (and may be recycled back to the scrubber) before the bleed is discharged to the sea or, possibly, further cleaned before being discharged to the sea. The same general cleaning technology is used at all three manganese alloy plants.

Since most of the plants use seawater to treat waste gases, scrubber water is discharged into the sea. This may take place via pipelines to surface or subsurface waters. In some plants, the discharges pass through sedimentation basins separated from the sea by sand and gravel dykes. PAH-laden dust from dry cleaning equipment may be deposited in the littoral zone or in deeper waters.

2.5. Recipient waters

The local and regional environmental consequences of industrial activities depend heavily on the geomorphology of any given area. Fjords are particularly important in Norway as they act as sedimentation basins. Fjords are

Table 2. Smelters included in the study and type of recipient waters.

Smelter location	Approx. distances from open coastal waters, km	Recipient area	Primary recipient waters		
			Type	Approx. lenght/ width, km	Sill depth max. depth, m
Årdal	100	Årdal Fjord	Fjord	18/1.5	No sill/650
Sunndal	70	Sunndal Fjord	Fjord	55/2.5	110/320
Karmøy	15	Karmsund	Coastal sound	15/0.5	No sill/100
Vefsn	65	Vefsn Fjord	Fjord	20/4	450/155
Lista	1	Skagerrak	Coastal water	-	-
Sauda	90	Sauda Fjord	Fjord	15/1.5	200/390
Feda	20	Feda Fjord	Fjord	20/1	300/385
Porsgrunn	25	Frier Fjord/ Brevik Fjord	Fjord	10/1-3 8/4	23/100 55/120

high-latitude estuaries excavated or modified by land-based ice (Syvitski et al., 1987). Norway's fjords are predominantly steep, deep and have one or more sills (submarine ridges). Apart from in the brackish surface layer, their temperature regime is controlled by ocean currents (Anon. 1993). In particular, the Gulf Stream plays a major role along the western and northern coasts of Norway. Most of the country's smelters discharge effluents to fjord waters, although two discharge to coastal waters, see Table 2.

Local topographic and hydrodynamic conditions influence contaminant dispersion and entrapment. In general, the fjords along the southern coast of Norway are short and relatively shallow with shallow sills, sometimes causing intermittently or permanently stagnant bottom water. The west-coast fjords are long, narrow, steep-sided, deep, and often have relatively deep sills. They may be up to 1300 m deep and extend up to 200 km inland from the coast. They often contain several flat basins (gradients could be less than 1m km^{-1}) separated by sills and with a sediment cover up to 300 m thick (Syvitski et al., 1987). The fjords further north are somewhat shorter and wider, and often without pronounced sills. The tidal amplitude of the fjords along the southern coast is small (<0.5 m) while the fjords in western and northern Norway have larger tidal amplitudes (0.5 to 2m, increasing northwards). Stagnant bottom water is a rare phenomenon there.

The density stratification in the fjords is important for the dispersal of contaminants. Most freshwater inflow is at the head of the fjord, and brackish water typically flows towards the mouth as a surface plume. In terms of density stratification, the fjords can be schematically divided into upper, intermediate and bottom waters. Upper water circulation is conditioned by the freshwater supply and prevailing onshore-offshore winds. Intermediate and deep water circulation is mainly driven by density fluctuations outside the fjord sill where coastal shelf water lies on top of Atlantic water. Occasionally, nearshore upwelling of the dense Atlantic water may cause spillage over the sill and renewal of basin water (Aure et al. 1997). Basin salinities in Norwegian fjords are close to 35 parts per thousand. Exceptions are those with shallow sills. While the grain size of the bottom sediments varies in shallow waters, in the deeper parts, most sediments are in the silt-clay fraction.

3 Data base

The data used in this study include measurements of PAHs found in the dissolved, colloidal and particulate state in waste and recipient waters, the accumulation of PAHs in bottom sediments and littoral indicator organisms, and the effects of PAHs on benthic fauna at the community and cellular level. Most data have been gathered during 25 years of monitoring, but new data have been collected to fill in knowledge gaps. Numerical treatment has, to a large extent, employed multivariate techniques such as principal component analysis, redundancy analysis and canonical correspondence analysis.

3.1. Existing and new data

This work is largely based on data generated over an extended period of time through fjord monitoring programmes. The sampling strategy in these assessments has not necessarily been consistent throughout the period in question. For example, the selection of species or the exact sampling locations may

Table 3. A. Overview of samples and analyses from the different smelter-affected areas. Asterix denotes new sampling to fill knowledge gaps not covered by the existing monitoring data. Number in parenthesis denote sediment stations for downcore profiling.

Smelter location	Sample type	Analyses	Number of stations
Årdal	Sediments	PAH	30 (2)
	Hard bottom invertebrates	PAH	7
	Soft bottom invertebrates	Community structure	7
Sunndal	Sediments	PAH	31 (1)
	Sediments*	PAH mobilization	4
	Hard bottom invertebrates	PAH	18
	Soft bottom invertebrates*	Community structure	12
	Epibentic invertebrates*	Cellular biomarkers	4
	Fish*	Cellular biomarkers	3
Karmøy	Sediments	PAH	16 (2)
Vefsn	Sediments	PAH	24 (10)
	Hard bottom invertebrates	PAH	18
Lista	Hard bottom invertebrates	PAH	7
	Effluent and receiving waters*	PAH speciation	3
Sauda	Sediments	PAH	45 (6)
	Sediments*	PAH mobilization	3
	Hard bottom invertebrates	PAH	13
	Soft bottom invertebrates	Community strcuture	6
Feda	Sediments	PAH	13 (1)
	Hard bottom invertebrates	PAH	6
	Soft bottom invertebrates	Community strcuture	5
Porsgrunn	Sediments	PAH	40 (19)
	Hard bottom invertebrates	PAH	5

Table 3 B. Total number of samples.

Analyses	Number of samples
PAH speciation in the water masses	27
PAH distribution in sediments	307
PAH distribution in littoral invertebrates	272
Benthic community structure	30
Cellular biomarkers in epibentic invertebrates	59
Cellular biomarkers in fish	83

have changed within a fjord area. In order to determine the sphere of influence of a particular source and to establish background values, the general strategy has been to draw samples along contaminant gradients. Hence, samples have been drawn at a significant number of stations outside the area of primary influence. Further, the stations sampled for chemical analysis were not necessarily the same as those used to assess the effects on benthic fauna. Finally, the suites of PAH compounds quantified in the individual programmes have not necessarily been identical. Consequently, the data sets for the present study had to be optimised, resulting in a reduction in the number of samples compared with the original data base. Nevertheless, a substantial amount of data was extracted from the monitoring programmes, as exemplified in Table 3. New studies were performed to fill in knowledge gaps. This was particularly pronounced in terms of the significance of hot spot sediments (i.e. sediments at the extreme end of the contamination scale) that could be found near effluent outfalls, effects of PAHs at the cellular level and the speciation of PAHs in effluent and recipient waters. Additional sampling was therefore performed to address these issues (Papers II, VII and VIII, IX, respectively), see Table 3.

3.2. Sampling, analysis and quality assurance

Sampling and analytical methods are detailed in Paper I for PAHs in sediments and indicator organisms and in Paper VI for soft-bottom community structure. In general, sediment retrieval for surface and downcore samples has relied on a gravity corer applied with an open 5-cm i.d. liner, occasionally using a Van Veen grab. Care has been taken to avoid the creation of a shock wave in the front of the sampler that might disturb the surface sediment layer (Blomqvist 1985). Accepted cores have always had a well-defined water/sediment interface with water on top of the sediment surface. The cores were divided into sections 1 or 2 cm thick. The same general principles apply to grab samples; the upper one or two centimetres of the sediment have been sampled by a spoon inserted through the inspection hatches of the grab. Ships have been positioned on the sampling locations by leading lines, and more recently by Decca and global positioning systems (GPS). Preference has been given to sampling sediments that were relatively homogenous in terms of texture in the deeper parts of the fjords to reduce patchiness in contaminant concentrations that could seriously influence the conclusions drawn from the assessment studies. To a large extent, this patchiness is a function of grain size variability.

A Van Veen or Petterson grab has been used to retrieve samples for analyses of the soft bottom community structure. The samples were sieved on 1 mm round-holed screens and preserved in a 4 per cent buffered formaldehyde solution. Individual specimens have been identified to species level whenever possible.

Samples of hard-bottom indicator organisms consisted of a homogenate of a number of individuals, generally 50 for blue mussels, 100 for periwinkles, and 5 to 10 for horse mussels and limpets. Blue mussels, periwinkles and limpets were sampled intertidally, while horse mussels were sampled by scuba diving (10 to 15 m depth). If possible, specimens of the same sizes were chosen. Whole soft parts were used, except that the adductor muscles of the mussels were removed.

Sediment and biological samples for PAH analysis have been added internal standards, then Soxhlet-extracted with cyclohexane. The extract has subsequently been analysed using GC/FID or GC/MSD. The PAHs were identified from FID using retention times and from MSD using retention times and SIM (selective ion monitoring), then quantified using the internal standards (Paper I). Detection limits depend on sample amounts, final extract volumes and instrumental conditions. For individual PAHs, they have normally been 0.5 µg/kg dry weight and 0.2 µg/kg wet weight for sediments and biological material,

respectively. For additional information on sampling, analysis and quality assurance concerning PAHs in the water phase and cellular biomarkers, see Papers II, VII and VIII.

Since this study emphasises trends and common patterns in data collected over a long period of time, attention also had to be devoted to analytical performance and the comparability of different sets of data. The same extraction and clean-up method was used throughout the study and the GC was calibrated against certified standards. Further, analytical accuracy was tested against standard reference material, where available. Apart from the analyses in Paper VIII/Paper IX, all analyses were performed by the same laboratory (NIVA), which has frequently participated in international intercalibration exercises during the period in question. Since 1993, the laboratory has been accredited pursuant to NS-EN-45001 and ISO/IEC Guide 25.

3.3. Numerical treatment

Taking a holistic approach to the PAH problem in smelter-affected fjords obviously creates a multivariate data structure. Accordingly, apart from the standard, routine statistical procedures, multivariate techniques have been used extensively, particularly principal component analysis (PCA), redundancy analysis (RDA) and canonical correspondence analysis (CCA).

PCA and RDA (CANOCO v. 3.10 software: ter Braak, 1988; 1990) were applied to the existing assessment data to evaluate trends and common patterns within and across ecosystem compartments. The underlying assumptions for PCA are that if a process acts on a system, it generates a data structure, and that this structure may be extracted from the data by principal component decomposition (Christie 1995). The method employs mathematical transformations of the original data. The aim is to determine a few linear combinations of the original variables which can be used to summarise the data set without losing much information (Meglen 1992). As environmental variables typically show strong correlations, a PCA often results in a small number of uncorrelated components describing most of the overall variability (Vogt 1990; Zitko 1994). The matrix containing the original data is decomposed into the first principal component (PC1) containing a column vector of scorces and a row vector of loadings, plus a residual matrix (Christie 1995). The scorces and loadings vectors are then tuned to minimise the residual matrix. Successive, uncorrelated principal components are extracted by decomposing residual matrices.

RDA is a related analytical technique which includes explanatory variables. Components are decomposed as in PCA, but with an added dimension: scores also are defined as linear combinations of the explanatory variables. Thus, RDA is a constrained PCA designed specifically to detect patterns of variation which correlate with the explanatory variables.

CCA was applied when addressing the effects of PAHs on the soft-bottom community structure. It is a multivariate method designed to elucidate the relationships between biological assemblages of species and their habitats. The technique arranges species and samples along axes which translate into linear combinations of the environmental variables. Hence, the faunal patterns that can be related to the variables are displayed (ter Braak and Verdonschot 1995). The inclusion of covariables in the CCA analysis removes variation that was not part of the primary research question (ter Braak 1995 and Verdonschot).

4 Quantitative and qualitative aspects of PAH discharges

The PAH profile in effluent waters from aluminium plants based on Søderberg technology is dominated by phenanthrene, fluoranthene and pyrene. A large proportion of the low molecular weight compounds are in non-particulate form. The PAHs associated with particles are much more strongly bound than expected from laboratory experiments. This suggests that the bioavailability of smelter-derived PAHs is generally lower than expected.

4.1. The general physico-chemical properties of PAHs

The benzene ring is the building block of PAHs. Strictly speaking, PAHs are restricted to compounds with three or more fused rings, although some dicyclical compounds are often included in the term. PAHs may be considered to be a group of low volatile compounds. The different compounds cover a wide range of physico-chemical properties (Mackay et al. 1992). Properties of particular relevance to the present study are solubility and hydrophobicity, the latter described by the octanol/water partition coefficient. In general, solubility decreases and hydrophobicity increases proportional to molecular weight, see Table 4. Alkylated compounds are less soluble than their parent compounds, as are angular PAHs compared to linear molecules. Of primary environmental importance are those with two to six fused rings, as compounds with higher molecular weights are rather stationary.

4.2. Discharge volumes

Norwegian aluminium and manganese-alloy smelters generally have discharged several tonnes of PAHs per smelter per year to seawater, although some of them have discharged up to several tens of tonnes. Amounts have been drastically reduced in recent years due to the installation of various cleaning devices. Reductions of up to 90 per cent have been achieved by many facilities in the past decade, and even more for some of the smelters.

Actual discharge volumes are uncertain and it is difficult to compare plants due to non-standardised methods for sampling and analysis. The effluent flow may be up to 2 to 3 m^3

Table 4. Physico-chemical properties of selected PAHs. The asterisks denote that a five-carbon ring is included in the molecule.

Compound	No. of fused rings	Mol. weight	Aqueous solubility -log C [a] (25°C), mol m^{-3}	Octanol/water partition coefficient log K_{ow} [a]
Phenanthrene	3	178	2.2	4.57
Fluoranthene	4*	202	2.9	5.22
Pyrene	4	202	3.2	5.18
Benz(a)anthrace	4	228	4.2	5.91
Chrysene/Triphenylene	4	228	5.1	5.86
Benzo(k)fluoranthene	5*	252	5.5	6.0
Benzo(e)pyrene	5	252	4.8	6.2
Benzo(a)pyrene	5	252	4.8	6.2
Indeno(1,2,3-cd)pyrene	6*	276	-	6.5
Benzo(ghi)perylene	6	276	6.0	6.5
Dibenz(a,c/a,h)perylene	6	278	-	-

a) From Mackay et al. 1992

s^{-1}, and the concentration of PAHs in the effluent will vary, depending on conditions such as the efficiency of the scrubbers, the top temperature of the anode, stud pulling, sooting, tapping, etc. Hence, it is difficult to obtain representative numbers for annual discharges. Samples from individual days are often combined into weekly samples which may, in turn, be combined into a monthly integrated sample. The problem with storing samples is that they may lose their more volatile components, changing their original composition.

Some smelters quantify the PAHs in effluent waters by gas chromatographic (GC) analysis. Others define PAHs as 20 per cent toluene-soluble dry matter. Accordingly, there is a strong need to standardise sampling and analysis methods, not only to better estimate discharge quantities, but also to facilitate comparison between PAH profiles in effluent waters and receiving media (pointed out in Papers III and V). Ideally, water samples should be preserved immediately after retrieval by adding extraction medium. Then they should be analysed by GC, as indicated in Paper VIII.

4.3. PAH speciation in effluent and recipient waters

The physico-chemical properties of PAHs are decisive to their distribution and ecotoxicological importance. Although there is a general tendency to bind with particulate matter, previous studies have shown that a significant proportion of the compounds were present in a non-particulate state in air samples from Al plants (Bjørseth 1979; Thrane 1988). Measurements made in connection with the present thesis indicated that this was also the case in the effluent and recipient waters from the Søderberg plant at Lista (Paper VIII). Further, Paper VIII demonstrated that the PAH profile was dominated by phenanthrene, fluoranthene and pyrene. This distribution was recently shown to be generally applicable to Al plants with Søderberg technology (Anon. 1997). At Lista, 60 to 90 per cent of the low molecular weight PAHs and 10 to 30 per cent of the high molecular weight PAHs were not associated with particles (as defined by a GF/F filter).

In an aquatic system, PAHs may sorb to numerous phases. Sorption to particles is

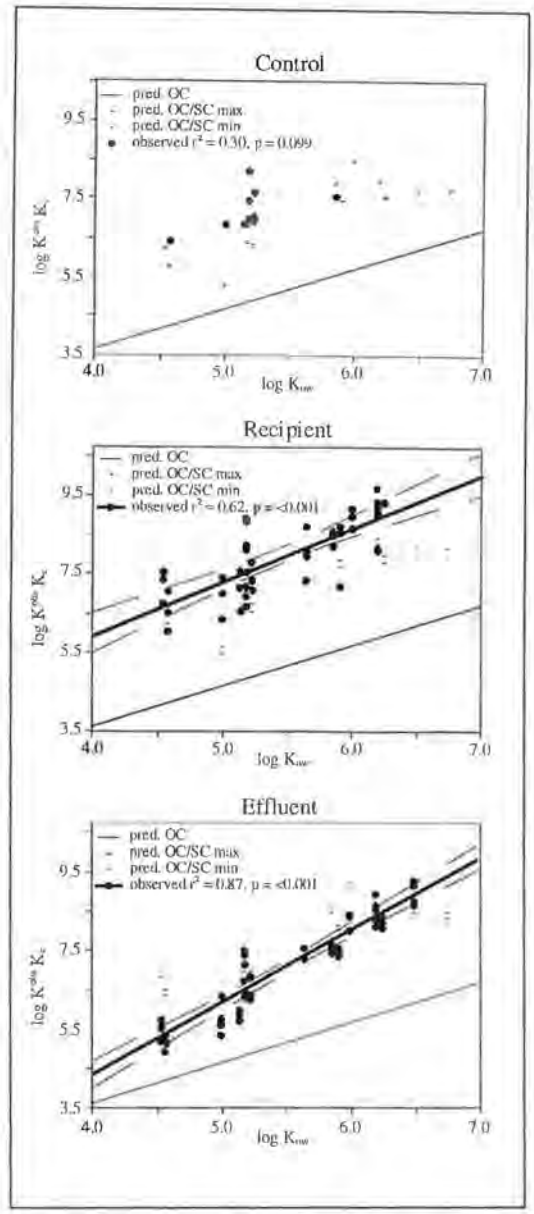

Figure 2. Logaritm of the observed partition coefficients ($\log^{obs} K_c$) of individual PAHs plotted against the respective log K_{ow}. The linear regression lines with 95 % confidence bands have also been plotted. The predicted relationship between log K_{ow} and log K_c based solely on organic carbon partitioning (predicted OC) and based on both soot carbon and organic carbon (predicted SC and OC). The SC and OC prediction is started as a range that takes the uncertainties in particulate soot and organic carbon measurements into account.

described by the particulate-water partition coefficient:

$$K_p = C_p / C_w \qquad (1)$$

where C_p is the mass of the compound per mass of dry weight of particulates, and C_w is the mass in of the compound per mass of water. It has been shown that organic matter is a strong sorbent for hydrophobic compounds (Karickhoff et al. 1979; Gjessing and Berglind 1981). Hence, particulate partitioning may preferably be described as:

$$K_{OC} = K_p / f_{oc} \qquad (2)$$

where K_{OC} = the particulate organic carbon normalised partition coefficient and f_{OC}= the organic carbon fraction of the particulates. Karickhoff (1981) derived an empirical relationship between hydrophobicity (expressed as the octanol-water partition coefficient, K_{ow}) and the normalised organic carbon partition that has been widely used:

$$\log K_{oc} = \log K_{ow} - 0.39 \qquad (3)$$

However, comparing this relationship to the one observed in the effluent and recipient waters of a Søderberg aluminium plant (Paper VIII), the K_{oc} for the individual compounds in the effluent and recipient waters was orders of magnitude higher than predicted from equation 3.

Many of the PAHs discharged from smelters are probably bound to soot-like particles. This association may significantly affect the speciation and fate of PAHs. Measured pore-water concentrations of PAHs from combustion sources have been found to be significantly lower than predicted from calculations based on the organic carbon normalised partition coefficient and attributed to the association of PAHs with soot (Socha and Carpenter 1987; McGroddy and Farrington 1995; McGroddy et al. 1996). Gustafsson and Geschwend (1997) described a method for estimating the partitioning of individual PAHs to soot carbon. By using this and the soot-carbon fraction of the total sorbing matrix, we expanded equation 2 to also include partitioning with soot phases (Paper VIII):

$$K_C = f_{OC} K_{OC} + f_{SC} K_{SC} \qquad (4)$$

where f_{SC} = the soot-carbon fraction of the particulates, and K_{SC} = the normalised soot-carbon partition coefficient. This partitioning was shown to better describe the observed relationships than the partitioning predicted by equation 3, see Figure 2. This means that a matrix with a stronger sorbing capacity than organic carbon controls the partitioning between particles and the dissolved phase in the effluent and recipient waters. Hence, the bioavailability of the smelter-derived PAHs should be restricted (Butler and Crossley 1981). The development of sediment quality criteria is often based on the equilibrium partitioning approach (Di Toro 1991). If this approach is used without taking into account the strong particulate binding that governs smelter-derived PAHs, the possible effects of the contaminants may be overestimated.

5 Transport and sediment incorporation

Most Norwegian fjords affected by effluents from aluminium and manganese-alloy production had a characteristic PAH profile or "fingerprint" in the sediments that was probably indicative of the source. The PAH distribution in the sediments could be well characterised by a simple dispersion model with distance from source as the only explanatory variable. This indicates that, particulate PAHs are severely resistant to transformations during transport and sedimentation, as well as after burial in sediments. The remobilization of PAHs from sediments to higher waters is insignificant compared with today's primary discharges.

5.1. Implications of a significant non-particulate phase

The occurrence of a significant proportion of the PAHs in a non-particulate or dissolved state (as defined by passing through a GF/F filter), at least for low molecular weight compounds released by aluminium Søderberg smelters (Paper VIII), raises questions about the fate and effects of the compounds in this size fraction. As described in Papers III and IV, a shift was detected in the PAH profile proportional to the distance from the smelters. The particulate and non-particulate states will differ as regards sedimentation characteristics. The occurrence of a substantial fraction in the dissolved phase may at least partly account for the general shift towards a sediment PAH profile more dominated by low molecular weight compounds proportional to distance from source. A significant non-particulate fraction also implies that if filtered samples are selected for the screening of discharges, the total emissions in the effluent liquid may be severely underestimated.

Apart from dilution, evaporation and, particularly, the absorption of UV-light with subsequent photo-oxidation will probably be the most important processes for the disappearance rate of non-particulate compounds in the water masses. Disappearance rates, generally expressed as half-lives, vary considerably for a single compound. Not only do they depend on the intrinsic properties of the chemicals involved, but also on the nature of the surrounding environment (Mackay et al. 1992). The higher molecular weight PAHs seem more susceptible to photo-oxidation, although that property may be limited by the low solubility of these compounds (Kochany and Magurie 1994).

Among the reaction products of PAH photolysis are epoxides, quinones and phenols. Common for these products is increased water solubility compared with the parent compounds. Photochemical breakdown could result in enhanced bioavailability and a higher toxic potential. However, it must be borne in mind that photo-induced toxicity is not only a function of the potency of the individual PAH, but also of its propensity to accumulate in an organism (Ankley et al. 1997).

5.2. Transformations of the particulate phase

The most important transformation reactions for particle-bound PAHs during transport, sedimentation and incorporation into the bottom sediments are photochemical, chemical and microbial degradation and desorption into interstitial water. When taking into account that half-lives last from weeks to years (Mackay et al. 1992), and that the residence time of the surface layer in fjords is on the order of days, microbial degradation and porewater partitioning are probably the most important reactions. Both are heavily dependent on the number of fused benzene rings and will affect the PAH profile.

There is a substantial volume of reference literature on microbial degradation of PAHs (Cerniglia 1992). In Paper III, we demonstrated that compound-specific processes operated on the particulate PAHs. However, we also quantified that this was only a minor variance contributor, generally accounting for less than 20 per cent of the total variance in the data

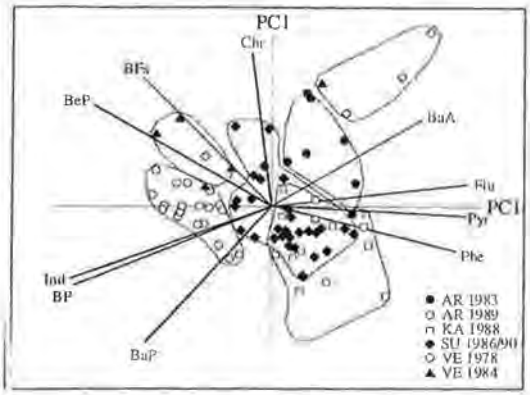

Figure 3. Double-centred PCA for comparison of PAH profiles in fjords with aluminium production.
AR =Årdal, KA = Karmsund, SU = Sunndal, VE = Vefsn

sets (Papers III and IV). The prominent feature was the preservation of the source-specific signals not only during transport and sedimentation, but also after sediment burial. Further, principal component analyses were able to differentiate between the various sources of origin of both surface and subsurface sediment samples, see Figure 3. These results show that smelter-generated PAHs have a severely restricted potential for natural post-deposition transformation reactions. These findings coincide well with recent research on PAH sorption characteristics in sediments (McGroddy and Farrington 1995; McGroddy et al. 1996; Gustafsson and Gschwend 1997). For example, McGroddy et al. (1996), working with in situ and laboratory desorption of PAHs, showed that only 1 to 40 per cent of sedimentary phenanthrene and pyrene appeared to be available for equilibrium partitioning. They concluded that one possible explanation was the slow transport of PAHs through the soot matrix. The resistant nature of smelter-derived PAHs may imply rather low bioavailability to the bottom fauna.

5.3. Explanatory variables for the distribution of PAHs in sediments

The dispersion and sedimentation of PAHs in recipient waters are a function of the particle affinities of the compounds as well as the dynamics of particle transport and deposition. In particular, organic matter has been found to adsorb PAHs in aquatic environments, although this varies from one PAH to the next since the carbon-normalised partition coefficient is linearly related to the hydrophobicity of the contaminants (Karickhoff et al, 1979; Readman et al., 1984). In the various fjord assessments, environmental (explanatory) variables have been measured in an effort to assist in the interpretation of PAH distribution in the fjord. These have mainly been sediment characteristics such as organic carbon, nitrogen and grain size. In Paper IV, the ability of the sediment characteristics and other factors (topographical factors, sediment types, etc.) to explain the variance in the data sets was quantified. The results showed that the most appropriate variables for explaining the variance in total PAH distribution corresponded to the deposition of PAHs, expressed either as a function of distance from source or as the amount of PAH-bearing matter in the sediments. In particular, a simple dispersion model with distance from source as only explanatory variable could generally account for as much as 80 to 90 per cent of the variance. This relationship, represented by the power function:

$$PAH = e^{a} * D^{b} \qquad (5)$$

where D = distance from source, e = basis of the natural logarithm, a = intercept and b = slope of the regression line, is a simple description of the variance in PAH concentration in fjords. The slope of the regression line may be interpreted as the percentage change in PAH concentration per unit change of distance. While other characteristics such as organic carbon were better explanatory variables in some cases, the improvements were not always significant. Distance from source may therefore be sufficient to explain PAH variations.

Although of minor importance in terms of variance, there were profile changes in the fjords (Paper III). For example, the relative amount of lower molecular weight PAHs appeared to increase in direct proportion to distance from source and PAHs with intermediate molecular weights appeared to be more closely associated with organic carbon. How-

ever, those variations could not be adequately explained by the explanatory variables. Further evaluations would require better descriptions of sediment properties and PAH speciation, for instance, by analysing variables such as the different particle types, carbon-containing materials and the dissolved/particulate state of the PAH in question.

5.4. Sediments as a secondary source of PAHs

Accumulated PAHs may remobilize back to the water phase or be buried beneath the layer that is active in sediment-water interaction processes. Generally, a distinction may be made between particle-mediated and diffusive reflux. The magnitude of the reflux depends on processes such as desorption, diffusion, compaction, bioturbation, resuspension by waves and currents, and gas convection.

A diffusive flux of PAHs from the sediments to the overlaying water depends on desorption of the compounds from the solids to the porewater. The presence of soot-like carbon as a sorbing phase has been shown to restrict partitioning into the surrounding porewater (Paper VIII; Gustafsson and Gschwend 1997; McGroddy et al. 1996). Further, the diffusive flux in sediments in which bioturbation is insignificant is often slow and prevented by the build-up of a concentration gradient. According to Schwartzenbach et al. (1993), it is possible to calculate the characteristic transport time for diffusion by:

$$t = x^2/2D_{eff} \qquad (6)$$

where t = time (s), x = distance (cm) and D_{eff} = the effective diffusion coefficient. D_{eff} is dependent on the equilibrium particle partition coefficient, the fraction of organic carbon in the sediment, the density of the sediment solids and the porosity and turtuosity of the sediment. Based on the range of D_{eff} for PAHs (Axelman 1997), it will be on the order of 10 to 1000 years. Since the sediment accumulation rate in smelter-affected fjords is just a few millimetres per year (Skei 1981), it appears that the diffusive flux for unmixed sediments from older underlying sediment layers will be negligible.

The most important factors for the remobilization of PAHs are therefore physical resuspension by waves and currents, and the effects of bioturbation. The former depends on the hydrodynamic conditions on the site. Bioturbation includes the effects of pumping water and particle resuspension. Besides reworking the sediment, bioturbation influences the physical properties of sediments, for example, by loosening them, or promoting resuspension in low velocity currents (Rhoads and Young 1970; Rhoads and Boyer 1982). In Paper II, we sampled fjord bed sections in order to assess remobilization from undisturbed sediments with their original fauna intact. There was a significant correlation between sediment PAH concentration and the remobilization rate. Based on a natural seabed containing 100 µg total PAH g^{-1} d.w., a concentration level often observed in the vicinity of the plants, and resuspension due to bioturbation within an expected natural range, it would be realistic to expect annual remobilization (dissolved and particulate) to the overlaying water of approximately 20 kg PAHs km^{-2}. Bearing in mind that most of the PAHs are associated with particles, most remobilization will take place temporarily and the particles then redeposited. Consequently, compared with the primary discharges, the remobilization of PAHs to the water column is insignificant. Limited remobilization seems to be general for PAHs of pyrogenic origin, contrary to contaminants such as PCBs (Axelman 1997; Baker et al. 1991; McGroddy et al. 1996). Hence, the sediments act mainly as a sink and not as a source.

6 Accumulation and effects on fauna

Organisms living in smelter-affected fjords may accumulate high levels of PAHs in tissues. However, accumulation does not necessarily imply effects which to a great extent is linked to the organism's ability to metabolise PAHs. Generally, only minor effects were observed on bottom fauna and demersal fish from Norwegian fjords affected by effluents from aluminium and manganese-alloy production. On the other hand, some changes associated with infaunal species functioning were detected. These changes appeared before the species and diversity were affected and before detrimental effects became obvious.

The hydrophobic characteristics of PAHs enable them to be adsorbed to biological membranes, due to the high content of organic carbon and lipids in such structures. Uptake occurs either through membranes in the gastrointestinal tract or respiratory system, or through skin contact. Generally, the uptake through the skin is insignificant since transport through these membranes is obstructed by scales or mucous membranes for the majority of aquatic organisms (Boudou and Ribeyre 1989). Hence, accumulation can be expressed as:

$$dC_{organism}/dt = k_1C_{water} + k_2C_{organism} + k_3C_{food} + k_4C_{organism} + k_5C_{organism} \quad (7)$$

where k_1 = the uptake rate from water of the truly dissolved phase, k_2 = the elimination rate constant through the gills, k_3 = the uptake rate constant from food, k_4 = the elimination rate constant by egestion in faeces and k_5 = the elimination rate constant due to metabolic transformations. The magnitude of the different rate constants depends on several factor, such as habitat, feeding mode, metabolic capacity, etc., and vary considerably from one group of organisms to another. In particular, the magnitude of k_5 is linked to the presence of the cytochrome P4501A-system (Bucheli and Fent 1995). This is also important with a view to possible effects since it is the metabolites of the P4501A-system that may have cellular effects on an organism (Halliwell and Gutteridge 1989; Regoli and Orlando 1995).

Molluscs and snails living in the littoral zone have been key organisms for assessing contaminant levels in smelter recipients, in particular the blue mussel *(Mytilus edulis)*, the horse mussel *(Modiolus modiolus)*, the common periwinkle *(Littorina littorea)* and the common limpet *(Patella vulgata)*. In Paper V, we showed that these organisms met the design criteria (Phillips and Segar 1986) for use in monitoring. For example, the results indicating that PAH profile differences between species were associated with habitat preferences and not with the organisms' different feeding modes indicate that the criterion regarding the reflection of ambient PAH was met. The monitoring organisms generally displayed the same trends as were found in the sediments, including the detection of specific PAH profile patterns related to source in the smelter-affected fjords. This implies that the k_5 rate constant in equation 7 must be insignificant also as observed by others (Stegeman and Lech 1991), alternatively that PAHs are not available for metabolic transformations due to their bonding with soot. As for the sediment, a simple dispersion model seemed generally applicable for all four organisms. This model was the same as for describing the distribution of PAHs in sediments (equation 5) and explained 80 to 95 per cent of the variance in individual data sets.

The high concentrations of PAHs observed in sediments in smelter-affected fjords may have adverse effects on benthic fauna (Long et al. 1995). However, in most fjords no correlations have been found between faunal diversity and PAHs (Paper I; Knutzen 1995). Knutzen (1995) suggested that the physical damage done to the habitat due to hypersedimentation, rather than PAHs, was the determining factor for the effects seen in the most

seriously impacted areas. The apparently minor ecological effects contrast with the toxic and sublethal effects reported from laboratory and field studies elsewhere (Long 1992; Long et al. 1995). Most of the smelter-effluent PAHs are associated with soot particles (Paper VIII), which presumably reduce their potential availability to cause effects. Similar conclusions were reached by Paine et al. (1996), that were unable to document toxicity from heavily PAH-contaminated sediments near a primary aluminium smelter in Canada. On the other hand, it is possible that certain biological responses have been overlooked. The ecological effects in Norwegian fjords were mainly assessed on the basis of faunal diversity. Various recent studies (e.g. Olsgard and Gray 1995; Elliott 1994) have shown that measures of diversity are not sufficiently sensitive to describe the full range of effects of contaminants on soft bottom fauna.

In Paper VI, we applied canonical correspondence analysis to the benthic community data from the smelter-affected fjords. Applying a set of environmental (explanatory variables), it was possible to account for 20 to 50 per cent of total species variance. Further, the results showed that there were changes in species composition along a PAH gradient that may be associated with species functioning, se Figure 4. This accounted for 5 to 10 per cent of the total species variance. In particular, the deposit feeding polychaetes decreased in number while carnivores increased proportionate to sediment PAH concentration. These changes took place at rather low PAH levels before the total number of species and diversity were affected and before detrimental effects became obvious. The extent of unexplained variance may imply that some important ecological relationships have not been described, but it may also represent inexplicable "noise" emanating from patchiness and random fluctuations in the biological systems. To that end, in order to achieve a better understanding of the effects of contaminants, there is a need to characterise various natural environmental variables and ecological relationships by monitoring of the effects of contaminants.

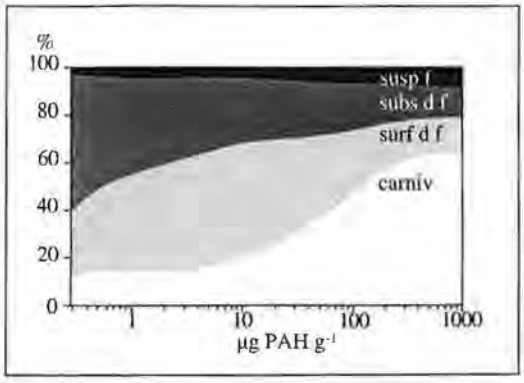

Figure 4 . Estimated tropic composition of species communities along the PAH concentration gradient (sum of 13 compounds). Species are classified according to feeding categories (carnivores, surface deposit feeders, subsurface deposit feeders, suspension feeders).

In the evaluation of soft bottom fauna patterns in relation to PAH gradients (Paper VI), the community changes tended to be more distinct in shallow waters. This could reflect depth-dependent biological features, but it may be speculated whether the effluents have stronger biological effects in shallow waters. According to the discussion in Chapter 5.1., shallow environments are more exposed to dissolved PAHs and their reaction products from photo-oxidation than deep bottom areas receiving PAHs mainly in the particulate fraction. Apart from the known photo-enchanced toxicity (Pelletier et al. 1997; Swartz et al. 1997), the low molecular weight PAHs that are usually in the dissolved state (Paper VIII) may have acute and sub-acute effects (Knutzen 1995 and references therein). It is, however, difficult to be conclusive regarding effects since there are considerable differences in reported toxicity threshold concentrations (Knutzen 1995), and since the speciation of the compound will strongly influence bioavailablity. For example, the truly dissolved fraction is probably crucial to uptake via biological membranes, while the DOC-bound fraction appears to have a reduced bioavailability (Landrum et al. 1987; Kukkonen et al. 1989; Di Toro et al. 1991). However, the DOC-bound fraction was found to be the dominant pool for PAHs with low molecular weight in the recipient waters of the Søderberg aluminium plant at Lista.

Calculation of bioconcentration factors (BCFs) indicated desorption and the uptake of colloidal-bound PAHs in caged blue mussels at this location (Paper IX). More research is therefore needed on the fate and toxicological relevance of the dissolved fraction from the viewpoint of abatement technology and regulatory factors.

To further investigate the possible effects of PAHs on organisms, cellular biomarker responses in epibenthic invertebrates and in fish were studied along with benthic community analyses in Paper VII. PAH contamination in the fjord did not seem to have a serious effect on the benthic biota, although the sediments were classified as "markedly" to "extremely contaminated", according to Norwegian sediment quality criteria (Molvær et al. 1997). These results demonstrate the fact that the presence of a contaminant, even at highly elevated concentrations, is not necessarily synonymous with adverse effects (i.e. contamination is not synonymous with pollution).

One exception to the above conclusions was the sea star *Psilaster andromeda* which appeared to respond to PAH concentrations in the sediments. Some echinoderms, e.g. the sea star are known to metabolise PAHs (den Besten et al. 1994; den Besten et al. 1993), thereby exposing themselves to increased levels of potentially deleterious metabolites. The limited effects on the invertebrates and fish apparently contrast with results from laboratory and field studies conducted elsewhere (Long et al. 1995; Goksøyr et al. 1994), including studies conducted near PAH-generating smelters (Beyer 1996; Ericson et al. in press). However, in the two latter studies the effects were related to PAHs in the water phase and not to sediment-bound compounds. The lack of consistent responses in biomarkers proportionate to sediment PAH levels is probably related to the speciation of the PAHs, the organisms' feeding mode and their ability to metabolise the PAHs, i.e. a significant fifth term in equation 7. As stressed by others (e.g. Chapman 1995; Chapman et al. 1987), it points to the need for linking single-species approaches to measures of effects on higher levels of organisation. Finally, to determine the cause and effect, it is necessary to understand the speciation of the chemical contaminant.

7 Implications on monitoring strategies

The monitoring programmes may serve numerous purposes. However, the different objectives are not necessarily met by the same sampling approach. Needs may even differ radically. This has been given scanty attention by many of the programmes and may, for example, hamper the detection of temporal changes. It points directly back to the need for a strict definition of programme objectives. It is necessary to emphasise this more strongly in future.

Regular monitoring of industrial effluent recipients began in Norway in the early 1970s. The programmes serve multiple purposes. First of all, they can result in general impact assessments by describing contaminant concentration and distribution. Second, they can be used to delineate temporal trends. Third, the food control authorities can use the data for evaluation purposes if there is a need for consumer advice or trade prohibitions. Fourth, some samples may be of interest as they allow scientists to identify previously unknown or new contaminants. Lastly, there has often been an element of basic research in the programmes as they can be used to learn more about contaminant processes. The various programme objectives are not necessarily met by the same sampling approach. They may even differ radically. This points directly back to the need for a strict definition of the objectives of the programme.

Many programmes fail to fully address the prerequisites for detecting changes over time. The success of any monitoring programme in achieving this depends on being able to estimate the variance associated with the measurements. This variance is needed to assess the statistical significance of any observed changes in contaminant concentrations. The total variance of a chemical variable is the sum of the individual variances associated with sampling and analysis (short-term and long-term), and the inherent variability of the sample population under study (Kelly et al. 1994). Population and sampling variance can be combined into "field" variance. In many cases field variance is substantial, exceeding the analytical variance of skilled laboratories. For example, the coefficient of variation (CV) in sediment samples from the inner Årdals Fjord (Paper I) and Sunndals Fjord (Paper VII) ranged from 23 to 102 and 15 to 17 per cent, respectively, while the analytical CV was less than 10 per cent for the various individual PAHs. Further, once analytical and field variances have been determined, they can be used for cost-efficient considerations to achieve an optimal sampling strategy (Kelly et al. 1994).

The final objective of programmes that elucidate temporal changes is generally the statistical verification of differences (at the required resolution) between two or more data sets. This testing is subject to both Type I and Type II errors. Type I refers to the probability of rejecting the null hypothesis (H_0) when it is correct, while Type II error refers to the probability of accepting H_0 when H_0 is false. Type II errors are directly related to the probability (or power) of detecting changes. The power of a monitoring programme determines the type and magnitude of the changes that are likely to be detected (Green 1989; Fairweather 1991; Nicholson and Fryer 1992; Nicholson et al. 1997). Monitoring programmes often fail to devote sufficient attention to Type II error, but an assessment of the power is important from a scientific and an economic point of view because, if specified, changes only have a small chance of being statistically significant, meaning that modifications are required. The power of a monitoring programme is also relevant for the discussion about taking a precautionary approach to contaminant abatement (Buhl-Mortensen 1996; Gray 1996; Gray and Brewers 1996). Given the combination of confidence level, the magnitude of change to be detected, coefficient of variation and acceptable power, it is possible to calculate the sample size.

Consideration must be given to specific issues related to the different parts of the

ecosystem used for monitoring. The value of sediment as a tool for monitoring change over time is critically dependent on local seabed factors such as accumulation rate, mixing depth and mixing intensity (Larsen and Jensen 1989; Jensen and Larsen 1991). Only in those areas where the sediment is not subjected to extensive mixing by hydrographic events, bioturbation or dredging, will a record of the contaminant input be preserved. Given such an environment, the minimum time lag between samplings is estimated by sampling depth (cm) divided by deposition rate (cm/yr). In reality, this frequency needs to be modified to take sediment mixing rates into account.

Programme imperatives also need to be evaluated when using bioindicators. Among other things, these are the metabolic regulation of the pollutant in question, the interaction between different pollutants and the time and space integration capacity of the bioindicators themselves (Phillips and Segar 1986). The most basic premise underlying the use of bioindicators is that they reflect contaminant levels in the ambient environment. This implies that the metabolic regulation of the contaminants should be absent or sufficiently weak to allow minor changes in ambient concentrations to be detected. It is also clear that if the accumulation of a given pollutant is affected by the presence of a second contaminant, the bioindicator concept breaks down. Paper V indicated that the littoral indicator organisms met these requirements. On the other hand, fish have a well-documented ability to metabolise PAHs. Hence, fish fillets are not suitable for temporal or spatial monitoring, but elevated concentrations of parent PAHs or metabolites may be found in fish bile (Paper VII).

The biological effects of PAHs have traditionally been evaluated by assessing the structure of soft bottom communities. Analyses of the data by canonical correspondence analysis (Paper VI) revealed functional changes in infaunal communities along a PAH gradient not previously detected by the traditional measures of diversity. However, the fraction of the species variance which could be explained by the environmental variables in the canonical analyses was rather low, i.e. generally less than 20 per cent. The low percentage indicates that important factors were missing. Some of the unexplained variance may be due to sampling material from separate grabs for chemical and biological analyses. This should be taken into account in future monitoring programmes. The low explained variance clearly points to a need for a better characterisation of natural factors in order to evaluate the consequences of contaminant-induced changes.

Finally, it is necessary to evaluate the magnitude of the variances associated with the different monitoring matrices. This is important because discharges to the sea are decreasing, so the magnitude of the impact changes that ought to be detected will be smaller. Consequently, priority should be given to the monitoring matrices with the smallest variances.

8 Conclusions and implications

A substantial amount of data has been gathered throughout twenty-five years of environmental monitoring in bodies of water receiving effluent from Norwegian aluminium and manganese-alloy plants. The present study has applied these data, supplementing them by new surveys to fill knowledge gaps, and taking a holistic approach to providing a comprehensive risk assessment of PAH discharges from the smelters to the marine environment. To summarise, the following conclusions may be drawn (not listed in order of importance):

- A significant proportion of the PAHs released from aluminium reduction plants using the Søderberg technology is in a dissolved form. Studies performed at one of these plants showed that 60 to 90 per cent of low molecular weight PAHs and 10 to 30 per cent of the higher molecular weight compounds were in dissolved and colloidal size fractions in the effluent waters. The fate and effects of the dissolved PAHs should be further addressed.
- The partition coefficients between the particulate and dissolved states were orders of magnitude higher that expected from empirical relationships. The partitioning was to a great extent controlled by a matrix with strong sorbing characteristics, i.e. soot carbon. The strong partitioning of PAHs to soot carbon may call for a reestimation of the toxic potential of smelter-derived PAHs as well as of how sediment quality criteria should be applied.
- The particulate PAHs were little available for biotic or abiotic transformation reactions.
- A simple dispersion model accounted for most of the variance in PAH distribution in the sediments and littoral indicator organisms.
- The PAHs had only minor effects on the diversity of benthic fauna, even when there were high concentrations of PAHs in the sediments.
- There were functional changes in the benthic invertebrate communities in the vicinity of the plants that correlated with the PAH distribution in the sediments. These changes were most clearly seen in shallow waters.
- At the cellular level, there were only limited effects on selected epibenthic invertebrates. Of the four species examined, it was possible to correlate the effects with the PAH load in only one case.
- Based on PAH levels in bile metabolites, fish sampled near the smelters were mildly affected. However, there was no sign of DNA damage in the livers of any of the fish.
- The environmental monitoring programmes should be redesigned, particularly with a view to addressing temporal chances.

Based on the data currently available, one can infer that PAHs generated by the production of primary aluminium, ferromanganese and silicomanganese have small effects on the marine environment. These limited effects must clearly be distinguished from the high concentrations observed in sediments and biota in many of the areas. To that end, it might still be justified that the environmental management may want to reduce the PAH load on marine areas even further in an effort to continue improving the environment and as a precaution against potential effects not yet known.

The effects observed in the present study are primarily caused by discharges accumulated over the past two decades. Bearing in mind the strong abatement initiatives carried out by the plants, the effects of today's emissions to the sea are sure to be even smaller.

Finally, in the introductory section, it was argued that there is a need to take a more holistic approach to the PAH problem in the smelter recipients monitored over the 25-year period. It was implied that such an approach

might reveal significant patterns which were impossible to recognize in the original tabular data or considered to be noise in the routine statistics restricted to individual fjords, thereby enhancing the understanding of the fate of contaminants in the fjords. This has indeed been the case. The benefits of such an approach may be summarised as follows:

The studies have:

- provided quality assurance for PAH data gathered over a considerable period of time;
- shown that a considerable amout of information was "overlooked" in the original monitoring data.
- improved scientists'understanding of the fate and effects of smelter-generated PAHs;
- improved the basis for decisions to be taken by the manufacturers and the administrators of environmental management;
- been important for generating scientific hypotheses.

The benefits from summarising and performing multi-disciplinary analyses are also applicable to other types of environmental data that have been gathered over an extended period of time. This is particularly true because many of today's most salient environmental issues involve high-cost analyses.

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Paper I

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Occurrence of PAH in marine organisms and sediments from smelter discharge in Norway

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Abstract

Norwegian aluminium works have each discharged 1–10 tons or more of PAH per annum directly into fjords, most of which originates from the scrubbing of hall gas in connection with Söderberg technology and from the production of anodes. In recent years, discharges have been considerably reduced, but still > 10 tons per year are discharged from the industry. Environmental assessment studies have systematically been performed from about 1980. Analyses of indicator organisms which accumulate PAH in high concentrations without apparent detrimental effects, have been used to assess the extent of contamination of water masses. The blue mussel (*Mytilus edulis*), and also its close relative the horse mussel (*Modiolus modiolus*), appear to be the most important indicators. The large amounts of waste have resulted in considerable contamination of fjord biota and sediments. PAH concentrations in indicator organisms of > 1000 times the background values have been recorded within 1–2 km of the outfalls. Elevated concentrations have been traced for more than 35–40 km. Surface sediment concentrations in the range of some hundred μg total PAH/g dry wt. have been observed. The concentration gradients vary but are generally steep. In several receiving waters, considerable improvements have been recorded shortly after a reduction in output load, and concentrations in mussels have fallen to 1/100 of previous values in some cases. Remobilization experiments indicate that 'hot spot' accumulations might be of significance.

Keywords: Aluminium smelter discharge; PAH; *Mytilus edulis*; *Modiolus modiolus*; Sediments

1. Introduction

Large amounts of PAH have been, and to some extent still are, discharged into Norwegian fjords and coastal areas. The most important sources have been metal smelters, and among these the aluminium production plants (Fig. 1). Five of the seven existing aluminium works have each discharged in the order of 1–10 tons PAH or more per year to fjord waters [1–3]. Most of this originates from the scrubbing of hall gas in production based on Söderberg technology, but some of it stems from the production of anodes. In recent years, discharges have been considerably reduced [2,3], but > 10 tons per year are still discharged by the industry [3]. About five times as much is presently discharged to air, giving an additional

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Fig. 1. Locations of large smelters in Norway.

unknown load on the water recipients from deposition and run-off from the affected local catchment areas.

Environmental assessment studies including consequences for the utilisation of edible organisms started in the 1970s [4,5]. From about 1980, systematic studies have been performed within the framework of the Norwegian State Pollution Monitoring Programme under the State Pollution Control Authority. Numerous reports from our institute have documented greatly elevated PAH levels, particularly in mussels, snails and sediments and to a lesser extent in crustaceans and fish. Elevated concentrations in edible organisms have resulted in restrictions on commercial fishing and mariculture, and warnings to consumers of seafood within several hundred km^2 of the fjord area (now under revision).

The present paper focuses on a selection of cases which illustrate previous and present situations, i.e. degree of contamination, areal influence and development following measures to reduce loads. Local environmental assessment reports in Norwegian are generally not referred to. However, this documentation is available from the authors (biota: J.K.; sediments: K.N.).

2. Materials and methods

2.1. Sampling

The extent of contamination of water masses has been assessed by analysing indicator organisms which accumulate PAH in high concentrations without apparent detrimental effects. Snails and mussels are most suitable, in particular the brackish-water tolerant blue mussel (*Mytilus edulis*), but also its close relative the horse mussel (*Modiolus modiolus*), the common (edible) periwinkle (*Littorina littorea*) and the common limpet (*Patella vulgata*).

Composite samples of *Littorina* and *Mytilus* have generally consisted of 100 and 50 individuals, respectively. If possible, mussels of a size from (4) 5 to 6 (7) cm have been chosen. Preliminary studies to determine the influence of mussel size or age have shown no systematic variation at high levels of contamination. For limpets and the larger horse mussels, 5–10 individuals have been used as a standard.

Whole soft parts have been used excluding the adductor muscle of *Mytilus / Modiolus*. Cleansing of the intestine before freezing has not been practised. However, in the case of the horse mussel there are indications that the intestine may contain somewhat higher concentrations than the rest of the animal, at least at highly contaminated sites.

Biological samples are kept cold and in a dark place until frozen. At the laboratory, they are later thawed for preparation of composite samples and then stored in a deep-frozen state in glass containers until homogenization and analysis.

Sediment samples have generally been obtained by the use of a corer (mainly gravity [6]) although grabs have been used occasionally. The core samples are immediately sectioned in 2-cm

(or 1-cm) slices after retrieval. If a grab is used, the upper 2-cm layer is sampled using a PTFE-spatula. To ensure sufficient material, replicate core samples are pooled (3–8, dependent on water content). Grain size effects are minimized by primarily sampling silty sediments along the deeper part of the fjord.

Sediment samples are stored according to the same procedure as for biological samples.

2.2. Analysis

Internal standards in cyclohexane are added to 0.1–2 g of freeze-dried sediments. The internal standards are 3,6-dimethylphenanthrene and B,B-Ginaphthyl for analysis with GC/FID, and seven deuterated PAHs for analysis with GC/MSD. The samples are Soxhlet-extracted with cyclohexane for 16 h. The extracts are purified as described by [7] by partitioning with DMF (dimethylformamide)/water. The PAHs are then eluted with cyclohexane from columns filled with 5 g of silica gel deactivated with 15% water. If necessary, sulphur is removed by gel permeation chromatography [8].

A modified version of the method given by [7] is used for extraction and clean-up of PAH in biological material. Internal standards (as for sediments) are added to 10–20 g of sample, which is saponified by refluxing for 8 h with KOH/methanol. PAH are extracted from the solutions with cyclohexane, and the extracts are subsequently washed with methanol/water. The extracts are purified following the same procedure as for sediments with DMF/water partitioning and silica gel chromatography. Subsamples of the homogenized material are taken for the determination of dry weight percentage.

Prior to 1989, PAH were analysed with a Carlo Erba Fractovap 2150 GC with a split/splitless injector, FID and a 25 m × 0.25 mm i.d. DB-5 fused silica column. The GC was connected to a Spectra Physics 4270 integrator. After 1989, this was replaced with a Hewlett-Packard 5890 Series II gas chromatograph with split/splitless injector and FID. The column is a Supelco PTE-5 30 m × 0.25 mm i.d. The data system is a HP 3365 Chemstation (DOS Series). Since 1989, a Hewlett-Packard 5890 Series II GC connected to a 5970B MSD has also been used for PAH analysis. The GC is equipped with a split/splitless injector and a 12 m × 0.2 mm i.d. HP-1 column. The data system is a HP 59940A MS Chemstation (HP-UX series).

Retention times and SIM (selective ion monitoring) are used for identification of PAH. Internal standards are used for quantification of PAH. Prior to 1991, the GC was calibrated using the Supelco standard containing 16 individual PAH. From 1991, SRM 1491 from NIST has been used for GC-calibration and the relative accuracies for sediments and mussels have been controlled against SRM 1941 for sediments and SRM 1974 for mussels, respectively. In general, the individual PAHs given in Table 1 have been quantified. The detection limits depend on sample amounts, final extract volumes and instrument conditions. Normally they are (for individual PAHs) 0.5 μg/kg dry weight and 0.2 μg/kg wet weight for sediments and biological material, respectively.

3. Contamination in organisms

3.1. Background levels

The natural background concentrations derived from forest fires, volcanic eruptions, etc. are further increased by the diffuse loading from a variety of anthropogenic sources. These may originate via atmospheric transport, run-off from land and oceanic currents. In order to estimate the areal influence of point sources, it is necessary to establish reference values. As such 'high diffuse background concentrations' are chosen, meaning the upper part of the interval of concentrations commonly occurring over large areas and outside any obvious influence from point sources. This is an imprecise definition, varying with the distance to urban and industrial centres and also with localisation in relation to the main transport routes from these centres. For littoral organisms, another uncertainty is the episodic influence from marine traffic.

Due to previous analytical difficulties, many data obtained in the past from sites with apparently little contamination (for example [9] and references) must be used with care. Consequently, the former view of 'high background

Table 1
Examples of typical PAH profiles in scrubber waste water and indicator organisms from recipients of aluminium smelters

Compound	Lista, 1991		Vefsnfjord, 1991	
	Effluent ng/l	Periwinkle μg/kg wet wt.	Effluent ng/l	Blue mussel μg/kg wet wt.
Phenanthrene	500	914	70	504
Anthracene	100	146	21	12
Fluoranthene	600	2149	905	743
Pyrene	400	3132	963	478
Benz[*a*]anthracene[a]	300	700	215	416
Triphenylene/chrysene	1300	3595	760	278
Benzo[*b*]fluoranthene[a]	1100	1521	846[d]	283[d]
Benzo[*j,k*]fluoranthene[a]	600	385	—	—
Benzo[*e*]pyrene	1100	995	355	166
Benzo[*a*]pyrene[a]	600	244	236	46
Dibenzopyrenes[a]	—	—	54	—
Indeno[1,2,3-*cd*]pyrene[a]	500	137	150	4
Dibenz[*a,c* and/or *a,h*]anthracene[b]	100	36	36	2
Benzo[*g,h,i*]perylene	600	145	212	6
Total PAH[c]	7900	14510	4841	2996
CPAH	3200	3023	1537	749

CPAH, sum of these substances.
[a]These PAHs are probably (group 2A) or possibly (group 2B) carcinogenic to humans according to IARC [10].
[b]Only the *a,h*-isomer potentially carcinogenic.
[c]Includes minor contributions from various dicyclics/heterocyclics.
[d]Including benzo[*j,k*]fluoranthene.

concentrations' in organisms from the Norwegian coast has been revised. For the blue mussel, it may be tentatively concluded to be 50, 20 and 1 μg/kg wet wt. or about 300, 100 and 5 μg/kg dry wt. for total PAH, carcinogenic PAH (CPAH) and benzo[*a*]pyrene (B(a)P), respectively. CPAH is defined as the sum of substances in the groups 1 (as yet none), 2A and 2B of IARC [10]. In remote areas with little traffic, the values may be considerably lower: < 10 μg/kg wet wt. total PAH [11] and perhaps of the order of 0.1–0.2 μg/kg wet wt. B(a)P [11–14].

Less data are available for the horse mussel, the common periwinkle and the common limpet, but for practical purposes, it appears that the high background levels may be regarded as similar to or somewhat lower than in *Mytilus*.

3.2. Overview of registrations

Table 1 shows some typical PAH profiles of gas scrubber waste water and indicator organisms. In addition to the listed compounds, 10–15 others (naphthalenes, other dicyclic/heterocyclic, methylated naphthalenes/phenanthrene/anthracene, perylene) occasionally occur in minor amounts, i.e. 1–5% of the total PAH. Usually fluoranthene, pyrene, chrysene/triphenylene and the potentially carcinogenic benzofluoranthenes are predominant.

However, the relative importance of the different compounds may vary considerably over time both in waste and organisms. Consequently, the tracing of smelter effects may be difficult outside clearly affected areas; the more so because of the similarities in PAH profiles between smelter waste and the general background of combustion-derived PAH.

Table 2 gives summary information on past and more recent data for organisms from near-source sites in selected coastal waters. Compared with the previously mentioned high background levels, 0.3 and 0.005 mg/kg dry wt. for total PAH and

Table 2
Examples of previous and present near-source levels of PAH, CPAH (carcinogenic PAH after IARC [10]) and benzo[*a*]pyrene (mg/kg dry wt.) in mussels and snails from Norwegian PAH discharges

Locality/species	Year	PAH	CPAH	B(a)P	% Dry wt.
Lista[a]					
Common limpet	1978–1986	37–176	2.2–10.0	0.10–0.90	≈ 20
Common periwinkle	1985–1991	43–290	5.9–22.8	0.56–2.20	≈ 20
Common mussel	1989	296	75	11.5	15.3
Karmøy[a]					
Common periwinkle	1988	127	22.8	3.1	20.2
Inner Årdalsfjord[a]					
Horse mussel	1983	531	196	28.9	17.5
Horse mussel	1992	9	4.0	0.50	14.7
Inner Sunndalsfjord[a]					
Common periwinkle	1987	61	12	1.1	16.5
Common periwinkle	1991	1.2	0.22	≈ 0.01	22.1
Horse mussel	1987	130	42	3.0	16.9
Horse mussel	1991–1992[b]	1.4	0.42	≈ 0.03	9.7
Inner Vefsnfjord[a]					
Common mussel	1984	65	24	1.5	16.9
Common mussel	1990	9.3[b]	1.1	0.02	14.6
Inner Ranfjord					
Common mussel	1981	936	350	52	—
Common mussel	1989	401	195	28	11.6
Common mussel	1990	11	2.3	≈ 0.05	20.0
Horse mussel	1989	333	198	48	11.4
Horse mussel	1990	4.3	2.5	0.15	22.1

[a]Aluminium smelter discharge.
[b]Mean of five samples.

B(a)P, respectively, it is evident that in extreme cases, mussels and snails collected within 1–2 km from the scrubber outfalls have exceeded background values by as much as 3000–5000 times.

In several of the recipients, considerable improvement has been recorded shortly after measures have been implemented to reduce load. This is consistent with the observations of half-lives of days to a few weeks for various PAHs in bivalves [15–17]. However, even after the large reductions of PAH load, concentrations in mussels from the most polluted parts of the Saudafjord, the Årdalsfjord, the Sunndalsfjord, the Vefsnfjord and the Ranfjord still exceed the assumed background values by more than one order of magnitude.

3.3. Examples of areal influence

The discharges of PAH in scrubber water have had far-reaching effects on contamination of benthic organisms, as illustrated in Figs. 2–4. In each case the source is situated at the head of the fjord, and all or a significant part of the waste is discharged to shallow water. At the time of observation, PAH loads were approx. 1 (uncertain), 5 and 8 tons PAH per year for the Vefsnfjord, the Sunndalsfjord and the Saudafjord, respectively. However, these numbers should be regarded as

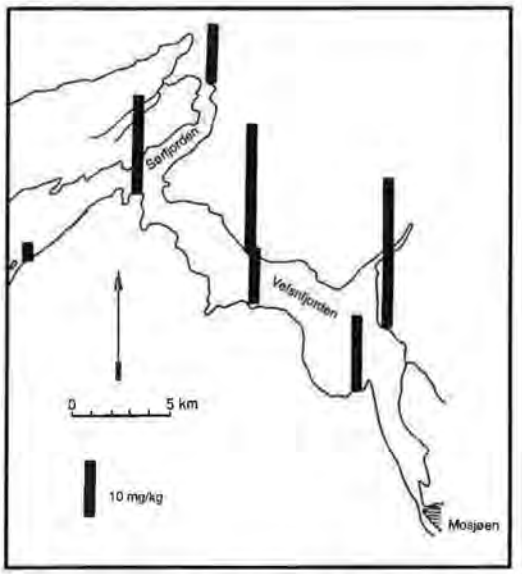

Fig. 2. PAH concentrations (mg/kg dry wt.) in blue mussels from the Vefsnfjord, 1989.

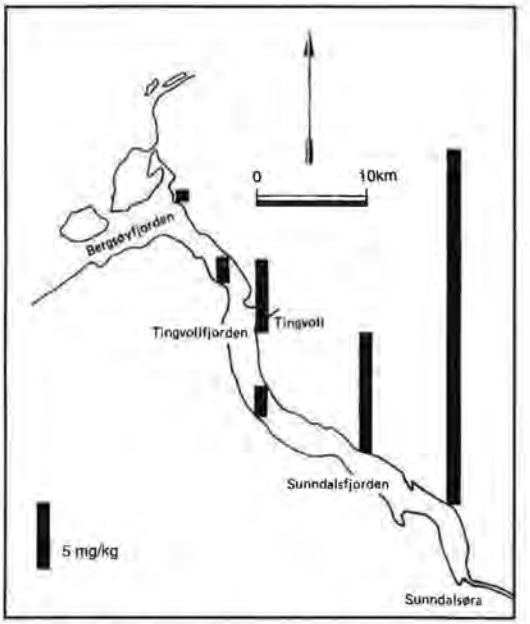

Fig. 3. PAH concentrations (mg/kg dry wt.) in blue mussels from the Sunndalsfjord, 1987. Pretreatment situation.

approximate estimates, since representative sampling of scrubber effluents is difficult, particularly at aluminium works with Söderberg electrodes, and with waste flows which may exceed 2 m^3/s.

In the three cases illustrated in Figs. 2–4, elevated concentrations have been traced for more than 35–40 km from the source. Consequently, extensive transport of the small soot particles characteristic of scrubber waste has been documented. The Saudafjord case with a shallow water outfall illustrates that such spreading usually will be most efficient in the surface layer, as indicated by the consistently higher concentrations in the blue mussel, as compared with the horse mussel (Fig. 4). The former is a littoral or slightly sublittoral species, whereas the horse mussel is found below the brackish surface water of this fjord.

The Årdalsfjord represents a somewhat different case since the waste consists of dust from anode production mixed with scrubber waste. The resulting slurry is discharged at a depth of 35 m in the inner part of the fjord. Fig. 5 shows how PAH levels in the horse mussel decrease with increasing distance from the source. Even after a load reduction of approx. 90%, slightly elevated PAH contents in mussels are observed at the mouth of the fjord. (Also in this case, the load estimate is uncertain, being recalculated from analysis of 'tar').

It should be added that the extent of areal influence, and particularly the distribution pattern of PAH contamination in littoral indicator organisms, may show considerable variation even under (as far as known) constant load conditions. Over one year (1989–1990), concentrations in 6–7 samples from the same stations in the Vefsnfjord, varied by a factor of three and five for *Modiolus* and *Mytilus*, respectively. Similar changes have been observed in blue mussels from the Frierfjord area. Such variations may be due to biological factors (e.g. spawning), variations in waste discharges and fluctuating current patterns in the receiving waters. For reliable descriptions of areal influence and detection of moderate changes over time, more frequent sampling is required than hitherto practiced in the monitoring of most Norwegian fjords.

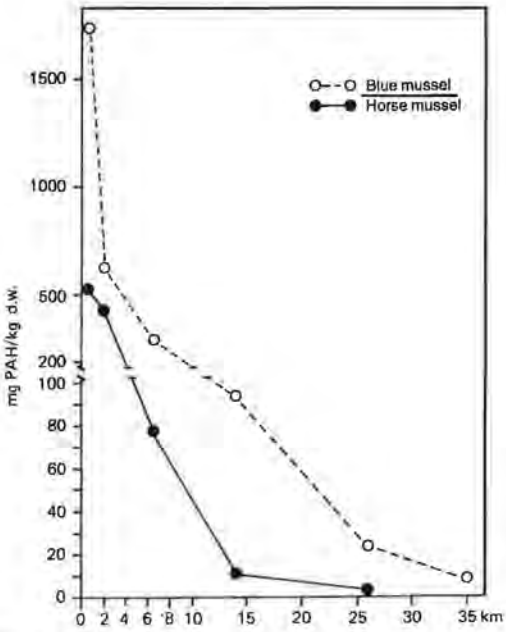

Fig. 4. PAH concentrations (mg/kg dry wt.) in mussels from the Saudafjord, 1986. Improved conditions in later years, compare with Table 4; note broken scale.

For the Karmøy aluminium smelter, exceptionally steep gradients were observed (Fig. 6). Most likely, the explanation is two waste sedimentation ponds significantly reducing spreading. (The slight increase seen at northern stations may be caused by local ship traffic or other sources in this urbanized area).

3.4. Examples of trends in relation to load

The main results of load reductions are presented in Table 2 for the most polluted parts of selected recipients. Regular yearly monitoring has only been carried out at Lista and in the Frierfjord (Fig. 1). In the other fjords, studies have been performed on a more ad hoc basis in order to update information on recipient conditions following changes in production volumes, introduction of new technology, or treatment of waste water. Due to the previous inadequate basis for estimating the discharges, it is only meaningful to relate load to concentrations in organisms in a few cases.

The Lista smelter has no treatment of the scrubber effluent of approx. 2.5 m^3/s and has had no major changes in production volume over the last 15 years. Thus, PAH concentrations in snails (observed once a year) merely have shown fluctuations as indicated in Table 2.

In the Frierfjord, the effects of successive load reductions from a ferromanganese smelter have been observed since 1986. Table 3 gives summary information on the load and PAH concentrations in the blue mussel for the period 1980–1991. Due to lack of mussels in the low salinity surface water, samples have been collected just outside the mouth of the fjord, about 10 km from the source. The mean PAH concentration for the years 1980–1986 was still about 100 times the background value. The calculated mean load in Table 3 for the period before treatment should be regarded as approximate due to inadequate sampling frequency. Nevertheless, a load reduction of more than 90% is in good agreement overall with the mussel data. It is also seen from Table 3 that the levels of CPAH and B(a)P in mussels decreased approximately proportionally with total PAH, contributing 25–30 and 2–3% of the total, respectively.

Another recipient for effluents from a ferromanganese smelter is the Saudafjord, which in recent years has experienced a reduction in PAH loads from perhaps as much as about 8 tons per year in 1985–1986 to 2.5–3 tons/year in 1989, 0.4 tons/year in 1990 and 0.1 tons/year in 1991/1992. The load reduction of possibly 99% for the period has resulted in a similar decrease in PAH concentrations in the blue mussel, but somewhat less in the horse mussel (Table 4). The discrepancy between the two species is probably due to their different habitats. The PAH burden in the blue mussel will mostly be a function of the direct discharge to the surface layer, whereas the horse mussel lives half buried in the persistently polluted sediment.

3.5. Comparison between indicator species

As molluscs in general appear to have low cytochrome P450 mono-oxygenase activity [18], all

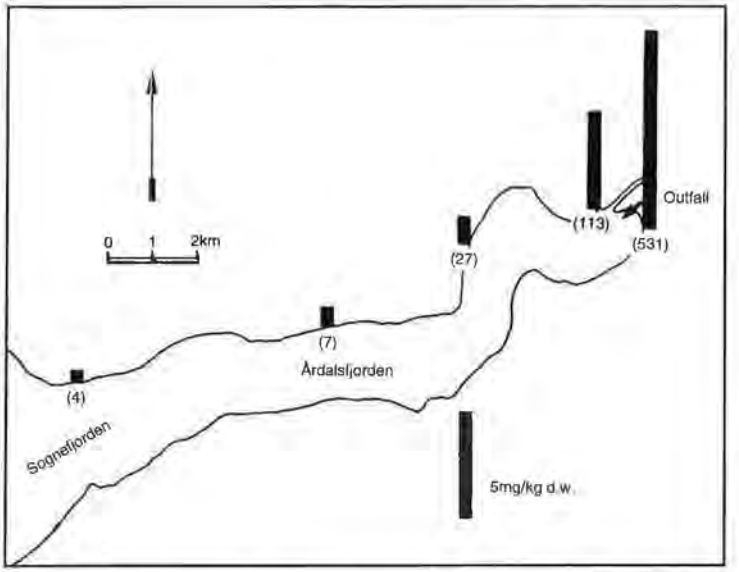

Fig. 5. PAH concentrations (mg/kg dry wt.) in the horse mussels from the Årdalsfjord, 1992; figures in parentheses below columns denote data from 1983, i.e. before abatement measures.

species within the group should be useful as indicators. Nevertheless, the results show that there may be differences which should be taken into account, in particular with respect to the accumulation of potentially carcinogenic compounds, and hence regarding the question of utilisation for food.

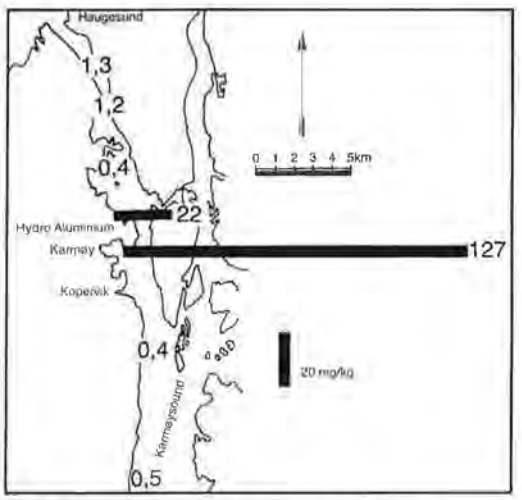

Fig. 6. PAH concentrations (mg/kg dry wt.) in the common periwinkle from Karmøy, 1988.

The difference in the PAH profiles of various species exposed to the same source is illustrated in Table 5, comparing the percentage contribution to total PAH from a group of relatively soluble PAHs (phenanthrene (Ph), fluoranthene (F) and pyrene (P)) with two groups of less soluble substances: benzofluoranthenes (BF) plus benzopyrenes (BP) and CPAH (which partly overlap with BF + BP).

Periwinkles and limpets tend to underestimate CPAH in the effluent and accumulate relatively more of the fraction with low molecular weight and relatively high solubility. For both species, mean CPAH percentage was significantly different from CPAH in the effluent ($P < 0.001$, t-test). Apparently, periwinkles retained CPAH to a somewhat larger extent than limpets ($P < 0.05$).

A less than proportional accumulation of CPAH is also seen in the blue mussels from the Vefsnfjord, but not from the Saudafjord. The possible

Table 3
Yearly load of PAH to the Frierfjord and results of monitoring in mussels (*Mytilus edulis*), 1980–1991

Year	Approx. load tonne/year	*N*	Mean concentration, mg/kg dry wt.		
			PAH	CPAH	B(a)P
1980–1986	4.3	33	29.4	—	—
1987	2.1	3	20.6	6.0	0.47
1988	0.8	3	11.9	3.4	0.34
1989	0.5	3	2.8	1.0	0.06
1990	0.35	5	1.18	0.39	0.041
1991	0.25	6	1.34	0.34	0.028

N, number of mussels analyses.

explanation is that mussels from the latter fjord live in less exposed (below the halocline) habitats and thus are relatively more affected by contaminated sediments. Horse mussels seem to accumulate CPAH and other heavy PAHs effectively.

Usually the different indicator species have been chosen according to occurrence and availability in the recipients. Thus there are a scarcity of cases in which two species have been sampled at the same time and place. Where the blue mussel and the horse mussel have been sampled together in various fjords, the latter had a higher percentage of CPAH in 20 out of 23 cases. The difference between means (35 and 50%, respectively) was significant at $P < 0.001$.

The mean percentages of B(a)P in the effluents listed in Table 5 were approx. 6, 9 and 3% at Lista, in the Vefsnfjord and in the Saudafjord,

Table 4
PAH, CPAH and B(a)P (mg/kg dry wt.) in mussels from the inner Saudafjord, 1986–1992

Species	Year	PAH	CPAH	B(a)P
Blue mussel	1986	1729	780	146
	1990	27.1	15.9	2.1
	1991	11.3	3.6	0.62
	1992	8.3	2.7	0.47
Horse mussel	1986	523	281	62
	1990	204	96	17.5
	1991	46.1	20.8	3.3
	1992	33.0	12.9	2.2

Mean of two near-source sampling stations (< 2 km from outfall).

respectively, but with a variation over more than one order of magnitude. Similar variation intervals have been observed in organisms. With this reservation, characteristic percentages of B(a)P have been 1–3% in periwinkles, 2–5% in the blue mussel and 3–10% in the horse mussel.

Analysis of the brown algae bladder wrack and knotted wrack at Lista resulted in approximately the same accumulations of PAH, CPAH and B(a)P as in the common limpet, and consequently subrepresentation of high-molecular PAH compared with the composition of the effluent. In the Ranfjord, total PAH accumulation in algae was lower than that in blue mussels by a factor of 10, and the same was observed versus horse mussels in the Årdalsfjord. However, in the latter case, exposure via different water masses may have played a more decisive role than differences in accumulation properties.

In conclusion, mussels appear to be the most important indicators, in particular giving a better reflection of potentially carcinogenic PAH than snails or algae.

4. Distribution in sediments

4.1. Background levels

As pointed out earlier, PAH occur naturally in the environment. Thus, preindustrial values are found in down-core samples. However, for practical purposes, it may be preferable to establish background or reference values for samples from recent deposits, i.e. representative concentrations from areas with only a diffuse input of PAH.

Table 5
Percentage contribution to total PAH from different groups of compounds in waste water, snails and mussels

Locality, medium	N	Ph/F/P		BF/BP		CPAH	
Lista, 1979–1991							
Effluent	21	24 ± 17	6–74	33 ± 12	9–51	36 ± 13	8–57
Limpet, 1980–86	7	67 ± 9	53–75	6 ± 2	4–8	8 ± 6	4–20
Periwinkle, 1985–91	7	63 ± 12	43–78	12 ± 6	3–22	15 ± 7	10–25
Vefsnfjord, 1989–90							
Effluent	17	24 ± 14	7–53	42 ± 11	26–63	43 ± 9	24–56
Blue mussel	7	61 ± 17	40–81	16 ± 11	3–30	15 ± 8	7–27
Horse mussel	7	21 ± 5	16–31	58 ± 7	49–70	43 ± 6	32–49
Saudafjord, 1990							
Effluent	12	47 ± 21	7–74	16 ± 10	2–28	18 ± 11	3–31
Blue mussel	6	30 ± 26	7–67	23 ± 13	10–44	40 ± 21	10–66
Horse mussel	6	19 ± 10	5–26	57 ± 11	41–68	45 ± 19	23–65

Figures presented are mean ± S.D. and range; Ph, phenanthrene; F, fluoranthene; P, pyrene; BF, benzofluoranthenes; BP, benzopyrenes.

Reference values are dependent on sediment type and characteristics. The main factors are grain-size distribution, organic quantity and composition, redox condition and biological activity. In evaluating PAH results from a number of Norwegian fjords, background concentrations in fjord sediments of < 0.3 $\mu g/g$ dry wt. and < 0.01 $\mu g/g$ dry wt. are expected for total PAH and benzo[*a*]pyrene, respectively.

4.2. Concentration levels in different areas

The large amounts of PAH discharged from the Al-smelters have resulted in pronounced sediment contamination. An overview of surface (0–2 or 0–1 cm) concentrations from different fjord recipients is given in Table 6.

Compared to the background concentration, it is evident that contamination exceeding natural values by 100–> 1000 times is observed in the close vicinity of the aluminium plants.

The PAH profile is often characterized by high concentrations of fluoranthene, chrysene/triphenylene, benzofluoranthenes, benzopyrenes and benzoperylene. Further, a CPAH contribution of 35–50% of total PAH is generally observed. Fig. 7 shows a typical PAH profile from the Sunndalsfjord.

4.3. Transport, areal influence and inventory

Very high concentrations are observed close to the outfalls, and elevated levels can be observed in areas some tens of kilometres away from the

Table 6
Concentrations of PAH ($\mu g/g$ dry wt.) in surface sediments from different aluminium smelter discharges

Site	Total PAH	CPAH	Benzo[*a*]pyrene	Sampling locations (km from smelter)
Årdalsfjord, 1983/1989	45–790	23–390	4–83	0.5–1.5
Sunndalsfjord, 1986	1.1–770	0.5–253	0.07–34	0.05–50
Karmsund, 1988	8.8–90	3.3–37	0.6–6.7	0.5–10
Vefsnfjord, 1989	1.1–34	0.5–15	0.005–2.4	0.1–25
Høyangerfjord, 1988	0.3–103	0.2–44	0.008–2.9	0.15–7

CPAH, sum of probably or possibly carcinogenic PAH after IARC [10].

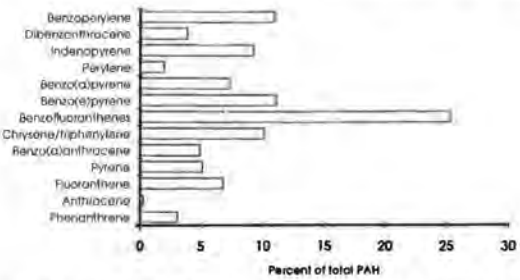

Fig. 7. PAH profile of surface sediment from the Sunndalsfjord, 1986 (conc. of total PAH of 25 μg/g dry wt.).

factories. However, as shown in Fig. 8 for the Sunndalsfjord, the spatial gradients for surface sediments are often steep.

The gradient slope will vary with fjord bottom topography (for example, silled or not silled), water-mass movements and discharge characteristics. Estimates based on data from Table 6 indicate that a 100-km^2 area of fjord bottom adjacent to the Al-smelters must be classified as heavily contaminated. The total seabed area classified as

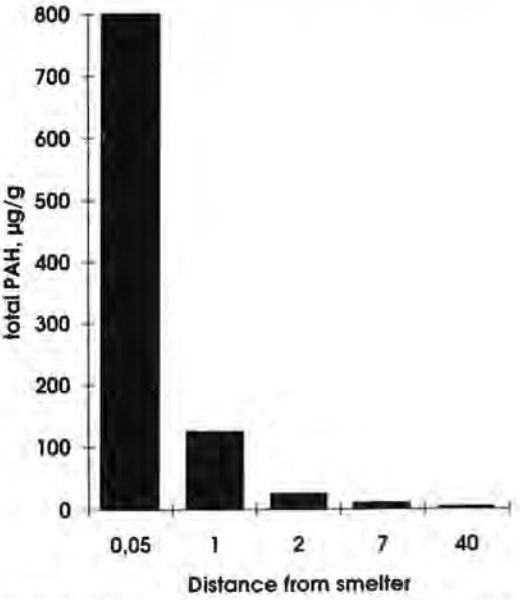

Fig. 8. Surface sediment concentration of total PAH (μg/g dry wt.) from the Sunndalsfjord.

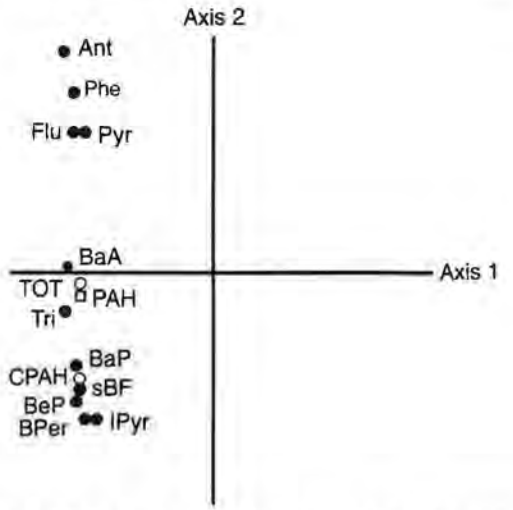

Fig. 9. PCA ordination of PAH compounds in sediments from 11 Norwegian fjords (160 stations); Phe, phenanthrene; Ant, antracene; Flu, fluoranthene; Pyr, pyrene; BaA, benz[*a*]anthracene; Tri, triphenylene/chrysene; sBF, benzo[*b,j,k*]fluoranthene; BeP, benzo[*e*]pyrene; BaP, benzo[*a*]pyrene; Per, perylene; IPyr, indeno(1,2,3-cd)pyrene; BPer, benzo[*ghi*]perylene; TOT, total PAH including di-/heterocyclic compounds; PAH, total PAH with three or more rings; CPAH, sum carcinogenic PAH.

heavily contaminated by PAH from smelters is even larger because concentration levels similar to those with Al-smelter recipients are recorded in fjords with ferroalloy, iron and coke production works.

Earlier investigations have demonstrated that PAH profiles may change with distance from the source [5]. These changes may be related to the behaviour of the individual compounds, and may be caused by selective transport and various mechanisms for incorporation into the sediments. However, little information is available on the distribution pattern (apart from differences in concentrations) of individual PAH compounds. Even fewer studies have been conducted in order to relate distribution patterns to measured environmental factors.

Fig. 9 shows the results of 'principal components analysis' (PCA) comprising 160 surface samples (0–1 or 0–2 cm, log-transformed concen-

trations) from 11 fjords (CANOCO program, [19]). Preliminary results [20] show that in general more than 90% of the variance in the sediment data are accounted for by principal component 1 (PC 1) which is a 'concentration' (probably to a great extent, distance from a point source) axis. An interesting trend appears on the vertical axis (PC 2). Here the individual (or groups of) PAHs are in general ordered in a sequence from low to high molecular weight. Naes et al. [21] showed that the PCA axis 2 coordinates were linearly correlated to molecular weight of individual PAHs, thus indicating the significance of physical/chemical properties for the variance described by PC 2.

Few studies have addressed the PAH inventories of fjord sediments and compared those with discharge history. This is due to the fact that monitoring has emphasized surface sediment; thus little information based on down-core samples is available. Discharge data are also of poor quality. The total PAH stored in the upper 10 cm of the sediments in the Frierfjord, a 17-km^2, 90-m deep silled (23 m) fjord acting as a recipient from a ferroalloy smelter (Fig. 1) has been calculated to be 13 tons. On the basis of sediment dating and discharge information, this was estimated to account for 30% of the accumulated input to the fjord. The Frierfjord is part of a larger fjord system of approximately 40 km^2 extending 20 km from the source. If the entire area is considered, 40% of the discharge could be accounted for. It must be stressed that the figures are uncertain. The discrepancies between discharge and inventory are, apart for uncertain input data, a result of transport and transformation processes, the latter will include photo-oxidation, chemical oxidation and metabolization by aquatic bacteria, fungi, algae and animals [22–24].

4.4. Trends in relation to load

Total discharges have been considerably reduced over the past few years. Reductions in sedimentary concentrations might therefore be expected. However, the sensitivity of sediments for trend monitoring is a function of the interactions between the change in the net accumulation rate of the contaminant, the accumulation rate of the sediment, the depth and intensity of mixing of the bottom sediment, and 'statistical' factors like time span between samplings, thickness of analysed sample, analytical precision and sediment concentration variance at the sampling site ('concentration patchiness') [25]. These factors are often not properly addressed in monitoring programmes. The variance between duplicate samples at the same station can be considerable. In a survey of the inner Årdalsfjord, the following concentrations (mean $\pm$ S.D.) in total PAH from three different stations were detected: 135 ± 83 μg/g dry wt. ($n = 5$), 310 ± 320 μg/g dry wt. ($n = 5$), and 271 ± 91 μg/g dry wt. ($n = 5$). This considerable variance (up to a 20-fold difference) at the same station was ascribed to variations in sedimentation of PAH containing carbon particles. The variability in surface sediment concentration in the inner Årdalsfjord is unusually high, but considerable variations have also been observed in other smelter recipients. Thus, the importance of properly designed programmes when performing time trend monitoring is illustrated.

The discharge history may be established by down-core analysis, preferrably accompanied by dating (analysis of unsupported lead-210). An example is given in Fig. 10 with results from a sediment core from the Kristiansandsfjord. The reduced surface concentrations compared with the subsurface maximum reflect production change and discharge reduction.

Down-core concentration profiles are a function of both discharge history and possible elimi-

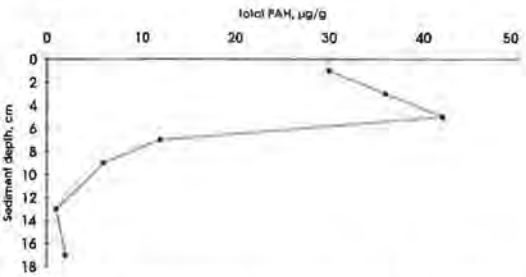

Fig. 10. PAH concentrations (μg/g dry wt.) in a sediment core from the Kristiansandsfjord, 1991.

nation reactions of PAH in the sediment. There are numerous observations of microbial degradation of mono- and polycyclic aromatic hydrocarbons ([23,26–28] and references therein). A general inspection of down-core PAH results from different Norwegian smelter recipients shows little or random variation in relative occurrence of individual PAH compounds. However, data analyses using advanced statistical tools have not yet been performed.

High loads have resulted in areas with very high PAH concentrations near the outfalls. The contaminants might be recycled back to the water phase or buried beneath the layer which is active in sediment-water interaction processes. Remobilization experiments with 'hot spot' sediments indicate that these accumulations might become significant as a potential contaminant source as the smelters are now implementing measures to clean up and reduce their direct discharges [29].

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Paper II

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Mobilization of PAH from Polluted Seabed and Uptake in the Blue Mussel (*Mytilus edulis* L.)

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Abstract. In several Norwegian fjords that have received effluents from smelters, there are significant accumulations of polycyclic aromatic hydrocarbons (PAH) in the sediments; near the outfalls, concentrations may reach several hundred μg total PAH g^{-1}. As the smelters are now implementing measures to clean up and reduce discharges, the accumulations from previous discharges could assume greater relative importance as a potential source. In order to give an assessment of the significance of sedimentary PAH 'hot spots' in Norwegian smelter-affected fjords, an experiment was performed in which seabed sediment sections (0·5×0·5 m, 1·3 to 543 μg total PAH g^{-1}) were collected from the Oslo-, Sauda- and Sunndalsfjords and placed in an experimental set-up in which Oslofjord water taken from a depth of 40 m was passed over the sediment surfaces. The mobilization experiments indicated that a natural seabed containing 100 μg PAH g^{-1}, and with a resuspension (due to bioturbation) within an expected natural range, might give an annual contribution to the overlying water of the order of 20 kg PAH km^{-2}. Mussels continuously exposed to the water from the test sediments accumulated PAH in a clear response to concentrations in sediments and water and to the degree of resuspension. PAH from the Saudafjord were apparently more bioavailable than those from the Sunndalsfjord, and it is argued that a more rapid accumulation in the former case was stimulated by an elevated organic carbon content in the sediment.

Introduction

Large amounts of PAH have been discharged to Norwegian fjords and coastal areas. The most important sources have been metal smelters. Today there are 11 PAH-generating production works in operation. The discharges have been of the order of up to 10, in some cases possibly 20, tons PAH or more per year from each source to fjord waters (Knutzen 1986; Leinum 1992; Naes *et al.* 1995). Most of this originates from the scrubbing of hall gas (Al works) and pot fume (Fe/Si-Mn works) in production based on Söderberg technology, but some of it stems from the production of anodes. In later years discharges have been considerably reduced, up to 99% for some of the smelters (Leinum 1992; Anon. 1994; Naes *et al.* 1995).

Owing to the nearness of waterfalls for hydroelectric power production, most of the smelters are located in the inner part of long, deep fjords. These fjords, which may have one or more sills, tend to entrap the pollutants relatively efficiently (Skei 1981). This situation is benefical to the North Sea but imposes great contaminant input to the local receiving sea water. Considerable pollution of fjord biota and sediments has been observed, with PAH concentrations in indicator organisms of more than 1000 times the background values within 1–2 km from the outfalls. Elevated concentrations have been traced for more than 35–40 km. High concentrations in biota have led to advices against consumption of mussels, and in some cases fish, in several of the fjords. Surface sediment concentrations in the range of some hundred μg total PAH g^{-1} d.w. (dry weight) have been recorded, especially near the outfalls (Naes *et al.* 1995).

The smelters are now carrying out initiatives to clean up and reduce discharges. The accumulations from previous discharges could thus become of greater relative importance as a potential source of pollution and counteract a further improvement of the fjord. If so, it might be more cost-effective to carry out remedial action on the 'hot spot' areas instead of imposing stronger regulations on the direct discharges from the smelters (Skei 1992).

The main aims of this study have therefore been to quantify (orders of magnitude) the role of 'hot spot' sediments as a contaminant source, addressing the extent of mobilization, the bioavailability of mobilized PAH, and possible differences in the PAH profile between sediments, water and mussels. The mechanisms of mobilization are not addressed in this paper.

Materials and Methods

Sampling and Experimental Design

Sediment samples were collected from the Sunndalsfjord, the Saudafjord and the Oslofjord (Fig. 1) during August and Septem-

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ber 1990. The waters of the Sunndalsfjord and the Saudafjord receive PAH-containing discharges from aluminium and ferromanganese/silicomanganese smelters, respectively, whereas the Oslofjord serves as a reference for areas with general human activity but no point sources. An USNEL box corer (Rosfelder and Marshall 1967) taking 50×50 cm sections down to approximately 40 cm sediment depth was used. The corer had been modified to host an acrylic liner that ensured retrieval and transport of undisturbed samples from the fjord bottom to the experimental site (Berge *et al.* 1986). From each of the smelter-affected fjords, 10 cores were taken at different distances from the contaminant source at a water depth of 55 to 178 m. Three cores were taken from the Oslofjord (200 m water depth). Accepted cores were undisturbed, with the original infauna intact, and had a sediment volume of approximately 75 L.

Fig. 1. Sampling locations.

The samples were transported in their core liners to the Norwegian Institute for Water Research Marine Research Station Solbergstrand (MRS) in the outer Oslofjord and installed in a flow-through system (Fig. 2). The cores were sealed with a lid and provided with sea water pumped from 40 m depth and filtered successively through three CUNU Micro-clean III filters of 75, 25 and 1 μm pore sizes. The water was delivered continuously at a rate of approximately 40 mL min^{-1} and passed the sediment surface in a laminar fashion. Seawater volume was 35–40 L (approximately 50×50×15 cm). The flow rate was low enough to avoid physical resuspension but high enough to fully ensure oxygen saturation. The water temperature and salinity varied from 6·0 to 12·2°C and from 33·0 to 34·7, respectively, during the whole experiment.

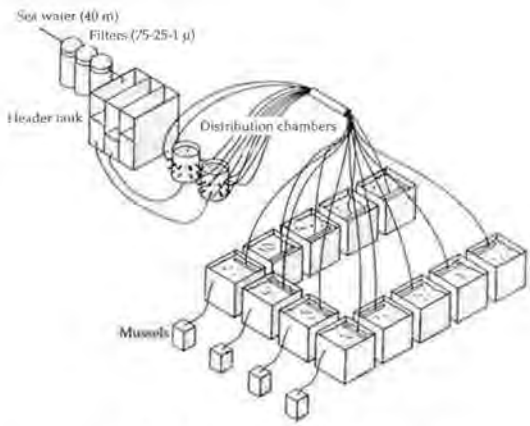

Fig. 2. Experimental design.

By the addition of a specific number of equal-size shore shrimps (15 and 30 individuals of *Palaemon* sp. per core), selected sediments were subjected to a small continuous disturbance.

Downstream of each of the cores was placed a 25 L glass aquarium with 200 mussels (*Mytilus edulis* L.) of size 2–5 cm shell length (average biomass 1·5 g w.w. (wet weight) $individual^{-1}$). The test population was large enough to ensure a sufficient number of individuals for the required sampling and small enough to ensure that oxygen was not depleted in the aquaria (calculated total average oxygen consumption 14 mL O_2 h^{-1}, calculated oxygen supply rate 17–19 mL O_2 h^{-1}). The mussels were sampled from a clean shoreline section adjacent to MRS. Twice a week the aquaria were gently rinsed and 1 L of a culture of the microalga *Phaeadactylum tricornutum* (approximately 5–10×10^9 cells, or 350–700 mg d.w. L^{-1}) grown in clean Oslofjord water was added as extra food in addition to the natural suspended particles from the test boxes.

The experiment was run for six months, from September 1990 to May 1991. Effluent water from the sediment cores was sampled and analysed for PAH (3–7 rings) on five occasions (one day, one week, one month, three months and six months after start of the experiment), mussels after one month, three months and six months of exposure, and sediments after the end of the experiment. Sediment analysis also included metals, organic matter and grain size. Suspended particulate matter (SPM) in the effluent water was sampled every second week, oxygen concentration was measured twice a week, and temperature and salinity were monitored continuously.

Sediment Analysis

PAH concentrations in the sediments were determined by gas chromatography with mass selective detector (GC–MSD). Addition of seven deuterated internal PAH standards in cyclohexane to 0·1–2 g of freeze-dried sediments was used for quantification, and retention

times and SIM (selective ion monitoring) were used for identification of PAH. The samples were Soxhlet-extracted with cyclohexane for 16 h and purified as described by Grimmer and Bøhnke (1975) by partitioning with DMF (dimethylformamide):water. The PAH were then eluted with cyclohexane from columns filled with 5 g of silica gel deactivated with 15% water. If necessary, sulfur was removed by gel permeation chromatography (Anon. 1991). Standard reference material (SRM) 1491 from NIST (US National Institute of Standards and Technology) was used for GC calibration, and the relative accuracies were controlled against SRM 1941.

Total metal concentrations were determined from a freeze-dried sample (0·2 g) by the atomic absorption (AAS) technique described by Loring and Rantala (1992) after microwave digestion with a combination of HF and aqua regia in a Teflon bomb (Rantala and Loring 1989).

Total organic carbon and total nitrogen were determined with a Carlo–Erba CHN analyser. Carbonates were removed by acid fume. Grain size distribution was determined by means of a Coulter counter.

Water Analysis

PAH in effluent water from the cores were determined by adsorption to XAD-8. The resin (grain size of 20–60 mesh) was rinsed three times over three days with 0·1 M NaOH, thereafter one day each in a Soxhlet apparatus with methanol, acetonitrile, dichloromethane and finally methanol. After rinsing with deionized water, the resin was placed in glass columns (1·9 cm i.d.) to a resin volume of 17 mL (bed volume) and coupled to the core liners with a 0·5-m-long silicon tube. An effluent flow of approximately 40 mL min^{-1} corresponds to 2·4 bed volumes min^{-1}, which is in agreement with the recommendation by Kunte and Pfeiffer (1985). After extraction of 5 L of sample, the PAH were eluted with 50 ml Rathburn glass-distilled acetone, thereafter with 150 mL Rathburn glass-distilled dichloromethane (Kunte and Pfeiffer 1985). Finally, the PAH were analysed by GC–MSD.

XAD-8 does not adsorb PAH from the water with 100% efficiency (Junk *et al.* 1976; Shinohara *et al.* 1977; Van Rossum and Webb 1978; Kunte and Pfeiffer 1985). Tests of the adsorption efficiency of the resin on three occasions (total PAH 17–1220 ng L^{-1}) during the experiment showed an adsorption of 30–80% for individual PAH with a mean efficiency of 52% for total PAH over the three tests. There was a decreasing recovery of individual PAH with increasing number of rings in the molecule, with approximately 50% for benzo(a)pyrene. The findings are comparable to adsorption efficiencies of 36–40% and 59% given by Kunte and Pfeiffer (1985) and Junk *et al.* (1976), respectively.

SPM in the effluent water was determined gravimetrically after filtration onto a GF/C filter.

Mussel Analysis

Samples of approximately 50 individuals were taken from each aquarium at each date. The sampling should preferably have been done with replacement to keep the total biomass in the aquaria constant, but since the main aim of the experiment was a comparison of the relative uptakes in response to the various treatments, this was not done. Replacement would also have introduced new individuals from a wild population in the midst of the experiment, which by itself would introduce interpretation uncertainties.

The mussels were rinsed on the outside and frozen. Prior to analysis, the mussels were opened with a scalpel and left for a short time on blotting paper to remove the water in the mantle cavity before the complete soft tissues from the 50 individuals were removed and combined into one pooled sample.

A modified version of the method given by Grimmer and Bøhnke (1975) was used for extraction and clean-up of PAH in mussel tissues. Internal standards (as for sediments) were added to 10–20 g of sample, which were saponified by refluxing for 8 h with KOH/methanol. PAH were extracted from the solutions with cyclohexane, and the extracts were subsequently washed with methanol:water. The extracts were purified by following the same procedure as for sediments with DMF:water partitioning and silica gel chromatography. The same SRM as for sediments was used for GC calibration, and relative accuracies were controlled against SRM 1974.

The term 'total PAH' is used for the sum of the individual PAH quantified. These were:

Phenanthrene	Benzo(j,k)fluoranthene*
Anthracene	Benzo(e)pyrene
2-Methylanthracene	Benzo(a)pyrene*
9-Methylanthracene	Perylene
1-Methylphenanthrene	Indeno(1,2,3-cd)pyrene*
Fluoranthene	Dibenz(a,h)anthracene*
Pyrene	Benzo(ghi)perylene
Benz(a)anthracene*	Dibenzopyrenes*
Chrysene	Coronene
Benzo(b)fluoranthene*	

PAH marked with asterisks are probably (Group 2A) or possibly (Group 2B) carcinogenic to humans (CPAH) according to Anon. (1987).

The similarities in concentration of the individual PAH above (the PAH profile) in mussels and waters were analysed by cluster analysis and multivariate ordination (multidimensional scaling) on basis of Bray–Curtis similarity index values (Bray and Curtis 1957) between pairs of samples. Analysis was done on non-transformed percentage values (the concentration of each component expressed as a percentage of the total PAH of the sample).

The Pearson rank test was applied to test for significant correlations. Paired and grouped *t*-tests on log-transformed values were used to test for differences between means.

Results

Concentrations of PAH in Sediments

The Sunndalsfjord and the Saudafjord have for two to three decades received smelter-generated PAH. Over the past few years the discharges have been greatly reduced (Table 1). Summary information of concentrations in the experimental sediments is given in Table 2.

The test sediments from the smelter-affected fjords were heavily to extremely polluted with PAH according to the sediment quality criteria (SQC) (Table 3) for Norwegian fjord sediments (Rygg and Thélin 1993). The Oslofjord sediments are classified as moderately polluted. The CPAH content was within the range often observed in Norwegian fjords affected by smelter-generated PAH (30–50% of total PAH) (Naes *et al.* 1995). The Oslofjord and Sunndalsfjord sediments were unaffected to moderately affected by trace metals, whereas the Saudafjord samples were markedly to extremely polluted according to the SQC. High concentrations of Mn were also detected in sediments from the Saudafjord, which receives effluent water from the ferro/silico-manganese smelter.

The C/N ratio indicates that the organic material in the Sunndal- and Saudafjord sediments is largely

Table 1. Direct annual discharges (t) from 1987 to 1993 of smelter-generated PAH to the Sunndalsfjord (Anon. 1994) and the Saudafjord (Knutzen and Berglind 1993; J. Rob, Elkem Mangan Sauda, personal communication)

The estimates are uncertain and must be treated as indications

Fjord	1987	1988	1989	1990	1991	1992	1993
Sunndal	6	4·5	2	2	1	0·5	0·5
Sauda	8	3	2·5	0·5	0·1	0·05	0·05

Table 2. Concentrations of Al, Mn, Fe, Cd, Pb, Zn and Cu, total organic carbon (TOC), organic carbon to nitrogen ratio (C/N), total PAH, and PAH probably or possibly carcinogenic to humans according to Anon. (1987) (CPAH) in the 0–2 cm section of the sediments at the end of the experiment

Oslofjord, 15 pooled subsamples from 3 different sediment samples (except PAH); Saudafjord, 5 pooled subsamples from each of 10 sediment samples; Sunndalsfjord, 5 pooled subsamples from each of 2–6 sediment samples

		Oslofjord	Saudafjord	Sunndalsfjord
Al	(mg g^{-1})	53	10–53	54–84
Mn	(mg g^{-1})	5·4	8–106	0·6–0·8
Fe	(mg g^{-1})	42	15–39	36–47
Cd	(μg g^{-1})	0·1	3·4 to 249	0·08–0·2
Pb	(μg g^{-1})	73	79–1180	23–29
Zn	(μg g^{-1})	200	370–9950	100–130
Cu	(μg g^{-1})	40	39–306	47–51
TOC	(mg g^{-1})	21	17–49	10–22
C/N		9·1	14–27	>11 to 22
PAH	(μg g^{-1})	1·3–1·5	9–543	7–67
CPAH	(%)	27–31	41–50	31–52

Table 3. Norwegian sediment quality criteria (SQC) for total PAH, in μg g^{-1} (Rygg and Thélin 1993)

Not/slightly polluted	Moderately polluted	Markedly polluted	Severely polluted	Extremely polluted
<0·3	0·3 to 2	2–6	6–20	>20

terrestrial-derived and transported to the fjord by general runoff and riverine input. The organic matter in the Oslofjord sediments is probably mainly derived from primary production (C/N ratio of 6·6 by number of atoms for marine plankton; Redfield 1958). The sediments from the Saudafjord had a significantly higher organic carbon content compared with the sediments from the two other fjords.

All sediments were fine-grained. Particle sizes in the mud fraction alone were less than 20 μm and in most sediments were less than 10 μm.

Mobilization of PAH from Sediments

There are limited data on naturally occurring concentrations of PAH in water (Anon. 1983; Mix 1984; Neff 1985; Eisler 1987; Müller 1987; Law and Whinnett 1993). Concentrations of benzo(a)pyrene, or B(a)P, and total PAH in areas with no point source but with general human activty would probably not exceed 1 and 50 ng L^{-1}, respectively. This is consistent with <0·1 ng L^{-1} of B(a)P and 5–10 ng L^{-1} of total PAH in the water fed to the sediment samples from the Oslofjord. A total concentration of 11 PAH of less than 1 ng L^{-1} has been found in remote coastal and offshore Baltic waters (Broman 1990).

After the incoming water had passed over the sediment surfaces, the analyses showed that mobilization of PAH (Fig. 3) was dependent on the PAH concentration in the sediment and the degree of disturbance to which the sediment was subjected, and concentrations of up to 1000 ng L^{-1} total PAH and 70 ng L^{-1} B(a)P (uncorrected for the adsorption efficiency of XAD-8) were detected from the most polluted sediments. If the sediment contained 20–30 μg PAH g^{-1} but was not disturbed, the concentrations in the water after passing over the sediment surfaces were the same as those for the Oslofjord reference sediments (5–10 ng PAH L^{-1}). There was, however, a difference by a factor of 100 between moderately polluted (1·4 μg PAH g^{-1}) and extremely polluted (540 μg PAH g^{-1}) sediments (approximately 5 and 500 ng L^{-1} total PAH, respectively, calculated as a mean of five observations). Likewise, the mobilization increased by a factor of five

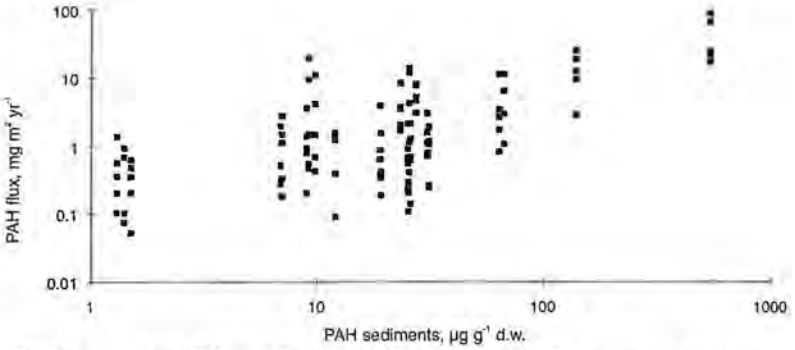

Fig. 3. Flux of total PAH as a function of PAH concentrations in the experimental sediments.

Table 4. Sediment conditions and resulting PAH concentrations in mussel tissues for the various treatments

Sediment total PAH (μg g^{-1}): mean of analysis of two replicate boxes. SPM (mg d.w. L^{-1}) as measure of bioturbation: mean and range of all measurements. Mussel total PAH (μg kg^{-1} w.w.) mean of two samples, each of 50 pooled individuals (single samples for Oslofjord)

Site	Sediment condition		Mussel PAH		
	PAH	SPM	1 month	3 months	6 months
Oslofjord	1·4	1·6 (<0·5–2·9)	6·2	6·3	10·0
	1·3	5·5 (1·4–11·6)	6·5	10·0	11·0
	1·5	6·4 (1·5–11·8)	8·1	8·2	12·7
Saudafjord	9·5	2·8 (0·9–12·8)	24·3	28·3	30·0
	28·5	2·4 (0·9–10·5)	16·1	15·5	30·3
	10·5	2·8 (0·9–12·8)	26·5	26·8	33·3
	24·5	3·4 (0·9–5·0)	48·9	54·2	56·7
	341·5	2·8 (0·9–12·8)	654·7	1086·9	479·0
Sunndalsfjord	7·0	2·0 (0·6–12·9)	9·7	22·4	18·5
	22·5	2·7 (0·9–11·7)	8·0	7·0	16·2
	25	2·0 (0·6–12·9)	16·7	30·6	37·8
	29·5	5·2 (1·9–10·7)	36·2	32·8	57·0
	65·5	2·0 (0·6–12·9)	26·1	78·2	85·1

to 10 if the sediment surface (20–30 μg PAH g^{-1}) was disturbed by shrimps, creating an increase in SPM by a factor of three.

There were pronounced variations in PAH mobilization from the same type of sediment at the various samplings. Generally, PAH are largely particle-bound (Readman *et al.* 1984), but there was no significant correlation with SPM concentration. However, SPM analyses were limited to particles greater than approximately 1 μm (filtered onto a GF/C filter). Analysis of the effluent water with the Coulter counter showed that the particle size in general was less than 4 μm. However, when the sediment was disturbed, resulting in a SPM concentration of > 10 mg L^{-1}, particles of sizes up to 10 μm were resuspended. There were no significant differences among the fjords in PAH mobilization at given sediment concentrations.

The SPM concentration varied from 0·5 to 12·9 mg L^{-1}, with a mean and standard deviation of 3·2 and 3·0 mg L^{-1} (n = 114) (Table 4), respectively. There were no time trends in SPM (or in PAH) during the experiment that could indicate systematic changes in faunal activity. The SPM concentrations caused by the faunal disturbances were within the range often observed *in situ* in near-bottom fjord waters (Syvitski and Murray 1981; unpublished data from Norwegian fjords). The PAH mobilization as a function of the sediment concentration was therefore estimated on the basis of all observations from all sediment types. Since the variation in the total PAH flux changes with variations in the independent variable, it is appropriate to log-transform the dependent variable (Sokal and Rohlf 1969). This produced the following significant linear relationship (Fig. 3)

$$\log (\text{F-tot. PAH}) = -0{\cdot}074 + 0{\cdot}00343 \times (\text{tot. PAH-sed.}),$$

$$r = 0{\cdot}59,\ P < 0{\cdot}001,\ n = 115, \quad (1)$$

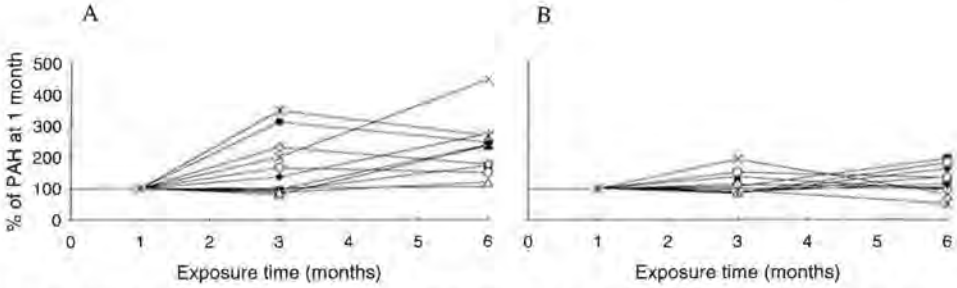

Fig. 4. Increase in total PAH in mussel tissues at three and six months as a percentage of the concentration at one month. Lines are drawn through values from the same treatment. (*A*) Sunndalsfjord experiment; (*B*) Saudafjord experiment.

where (F-tot. PAH) is the flux of total PAH in mg m^{-2} $year^{-1}$ and (tot. PAH-sed.) is the sediment concentration ($\mu g\ g^{-1}$).

There was a significant correlation at the <0·001 level between the flux of total PAH and B(a)P. The equation of B(a)P flux as a function of total PAH sediment concentration was

$$\log\ (\mathrm{F-B(a)P}) = -25{\cdot}6 + 5{\cdot}20 \times (\text{tot. PAH-sed.}),$$

$$r = 0{\cdot}55,\ P < 0.001,\ n = 115. \qquad (2)$$

Concentrations of PAH in Mussels

The mussels exposed to water from the polluted sediment boxes reached tissue concentrations of PAH significantly above those in mussels exposed to water from the reference boxes (Table 4). The tissue concentrations increased gradually up to three months but changed rather unsystematically after that, suggesting that the populations had reached a steady state between uptake and loss of PAH. The relative increase in tissue concentration after one month was less in the Saudafjord test population than in the Sunndalsfjord test population (Fig. 4), indicating that the former reached an apparent accumulation equilibrium faster than did the latter.

Net accumulation of PAH in the mussels correlated positively with the concentrations of PAH in the outlet water from the test sediments at one, three and six months and with the PAH concentrations of the test sediments themselves (Fig. 5). The PAH composition in the mussels did not change systematically with the sediment concentrations.

The net accumulation was also positively correlated with the amount of suspended solids in the outlet water, and accumulation was significantly higher in mussels exposed to the Saudafjord sediments than in those exposed to the Sunndalsfjord sediments at comparable concentrations of suspended solids (Fig. 6). The PAH composition in the mussels was also affected by the degree of disturbance of the sediments. Mussels exposed to water from disturbed sediments accumulated a PAH composition clearly different from that of the water within one month, whereas those downstream of undisturbed sediments first showed such a deviation at six months.

The proportion of CPAH in the mussels was in the range of 31–43% of total PAH. The percentage increased gradually with time, this being most pronounced in the Sunndalsfjord experiment (Table 5). The CPAH percentage in mussels was significantly higher than that in the outlet waters after one month but lower than that in the sediments. At three and six months the CPAH percentages in mussels were not significantly different from those in the sediments. This shows that the mussels selectively accumulated CPAH. The results in Table 5 further suggest that the Saudafjord mussels reached a stable CPAH percentage in one to three months. In the Sunndalsfjord mussels the CPAH percentage increased gradually throughout the experiment.

Discussion

PAH in the sediments occur in the following forms: incorporated in the biota, associated with particulate organic and inorganic matter, associated with dissolved organic matter, and truly dissolved in the interstitial water. Generally, the remobilization from sediments is mainly dependent on desorption, physical resuspension, bioturbation and gas convection (Larsson 1985; Larsson and Södergren 1987). Since the flow rate did not induce resuspension, the bioturbation by infauna and added shrimps, and also desorption, were the main mechanisms for mobilization of the sediment PAH. This biologically mediated mobilization includes both particle resuspension and pore-water advection.

The pronounced variations in PAH mobilization from the same type of sediment at the various samplings were probably a function of the mechanical disturbances of the sediment by the shrimps and other benthic animals in the boxes and illustrate the importance of fauna to physical/chemical processes in the sediment, as also pointed out by others (Rhoads and Young 1970; Aller

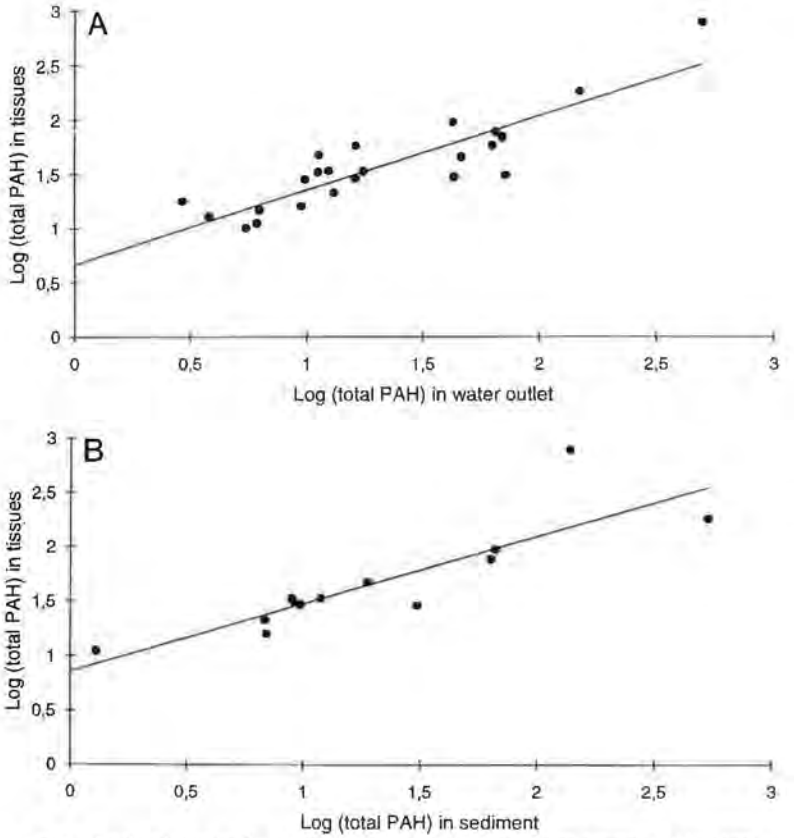

Fig. 5. Total PAH (μg kg^{-1} w.w.) in mussel tissues at six months as a function of (*A*) total PAH in water outlets (ng L^{-1}, means of all measurements for each treatment, all boxes from the test fjords included), and (*B*) total PAH in test sediments (μg kg^{-1} d.w.) at the end of the experiment (only boxes with medium bioturbation included).

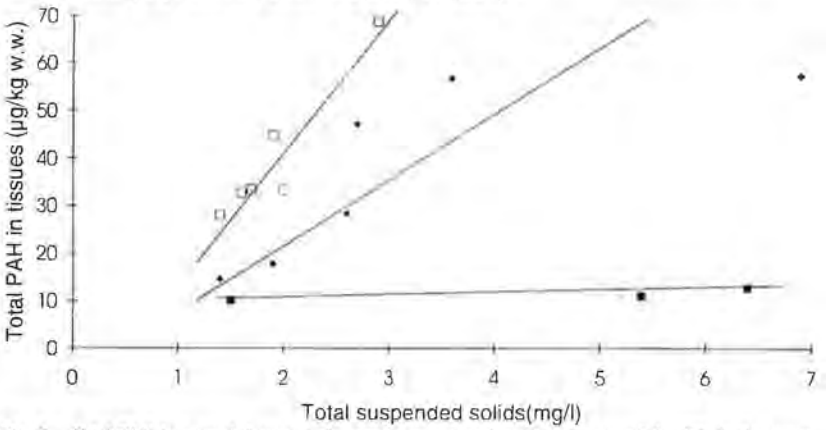

Fig. 6. Total PAH in mussel tissues at six months as a function of total suspended solids in the water outlets. □, Saudafjord; ◆, Sunndalsfjord; ■, Oslofjord.

Table 5. Mean (±s.d.) CPAH percentages in sediments, outlet waters and mussel tissues

Sample type	Saudafjord	Sunndalsfjord	Both fjords
Sediments at 6 months	46·5±3·6	39·4±6·9	43·0±6·5
Water, mean of Month 1	28·6±9·9	24·7±11·2	26·7±10·5
Water, mean of Months 1–3	27·7±9·0	25·1±10·3	26·4±9·5
Water, mean of Months 1–6	27·1±10·9	23·5±11·2	25·3±10·9
Mussels at 1 month	37·5±14·5	30·8±9·1	34·1±12·3
Mussels at 3 months	40·7±13·1	38·7±14·7	39·7±13·6
Mussels at 6 months	40·2±7·4	42·9±6·9	41·6±7·1

1977, 1978; Rhoads and Boyer 1982; Karichoff and Morris 1985). In light of the potentially different forms of occurrence of PAH, one cannot expect that the SPM analyses alone should adequately explain the mechanisms of mobilization. Lack of correlation between SPM and mobilized PAH suggests that mobilized PAH was largely present in the size fraction smaller than 1 μm.

There are obvious limitations on the general application of the results from the present experimental situation to a natural fjord. The estimates of PAH mobilization are derived from experiments with 0·25-m^2 fjord bed sections and focus on the unidirectional transport from the sediment to the overlying water. The fate of mobilized PAH in a natural fjord will depend on transport, redeposition, uptake, etc. as governed by the local conditions at the site in question and cannot be assessed on the basis of laboratory experiments alone. Further, the pronounced variations in concentrations from equivalent treatments will reduce the precision of the flux estimates. However, it must be emphasized that the main objective of this experiment was to estimate orders of magnitude rather than to attain exact quantitative measurements of the importance of 'hot spot' sediments as a secondary source of pollution compared with the direct discharges from the production plant. If it is assumed that the disturbance (giving SPM <13 mg L^{-1}) caused by naturally occurring infauna and the added shrimps is representative for a situation *in situ*, Eqn 1 would imply that from a 1-km^2 fjord bed with 100 μg PAH g^{-1} in the sediments, annual mobilization would be of the order of 20 kg total PAH and 1 kg B(a)P (corrected for an approximate 50% adsorption efficiency of the XAD-8). The estimate is biased because it does not account for the subsequent fate of the mobilized PAH on an actual fjord site, as pointed out above, and does not apply to accidental disturbances such as dredging, propeller turbulence or rapid water displacements.

Still, within the inherent limitations, the experiments indicate that mobilization from 'hot spot' sediments would be insignificant if the direct discharge to the receiving waters is in tonnes per year but would gradually increase in significance as the direct annual discharge diminishes and may counteract further improvement. The strong initiatives taken to reduce environmental effects have for some smelters resulted in outputs that are comparable to the calculated fluxes (Table 1; Leinum 1992).

The primary purpose of the mussel experiment was to compare the relative bioavailabilities of the mobilized PAH under the various experimental conditions. The results showed that tissue concentrations increased during the first three months but not systematically after that for any treatment. The fact that water concentrations of PAH and SPM did not show any clear increasing or decreasing trend with time suggests that an apparently steady state in accumulation was reached within three months. One reason could be that the lipid content in the mussels most likely decreased during the experiment owing to a small supply of particles, although we do not have any analytical evidence for this. However, if starvation was the only cause, the trend should be opposite because the Saudafjord sediments (and consequently the SPM from these sediments) had higher TOC than did the Sunndalsfjord sediments.

Although the accumulation responses to PAH exposure were significant, the maximum concentrations of PAH in the tissues, even after three and six months, were relatively modest and about one order of magnitude lower than those found in mussels sampled from the Saudafjord proper in the autumn of 1990 (Knutzen 1991). According to Norwegian quality criteria (Rygg and Thélin 1993), most of the mussel samples would be classified as good to fair on the basis of both total PAH and B(a)P, whereas the test sediments were classified as heavily to extremely polluted. Still, on the basis of the slope of the regression line in Fig. 5*A*, the bioconcentration factor (log BCF) relative to the water was 3·4 after six months (range 3·1–3·4 for the whole experiment), which is comparable to log BCF values in other studies. Pruell *et al.* (1986) found a log BCF of 2 to 3·5 within 20 days in a similar experiment with *M. edulis* conducted at 15°C. Murray *et al.* (1991) recorded BCF values of 0·007–0·034×10^6 for selected PAH in *M. edulis* from an urban environment, which corresponds to a log BCF on a wet-weight basis of approximately 3·2–3·9. The present log BCF is also reasonably below the theoretical maximum log BCF of 4·5–5 outlined by Knutzen *et al.* (1992) for organic micropollutants.

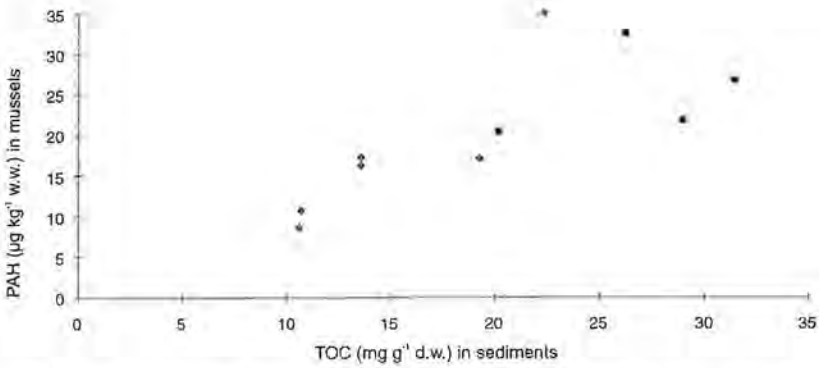

Fig. 7. Total PAH in mussel tissues at six months as a function of total organic carbon (TOC) in the sediments. Only treatments with medium bioturbation are included. ■, Saudafjord; ◇, Sunndalsfjord.

The relative importance of dissolved and particulate PAH as source for PAH accumulation in mussels is uncertain. Several authors have claimed that even in suspension-feeders the dissolved phase is the main source for tissue accumulation of organic xenobiotics (Roesijadi *et al.* 1978*a*, 1978*b*; Pruell *et al.* 1986). The present experiment was not designed to address this problem directly, but the similarity in PAH composition between the mussel tissues and the sediments, reflected in the PAH profiles and the proportion of CPAH, and the correlation between tissue concentrations and SPM suggest particulate PAH as the main source. As stated earlier, the PAH were probably mainly present in the size fraction smaller than 1 μm, which is below the size limit of 2 μm for effective retention by the mussel gill (Vahl 1972) and also below the size range of preferred food particles (2–10 μm, Bayne *et al.* 1977). Still, the particle counts showed resuspension of particles up to about 10 μm, particularly from the disturbed sediments, and direct PAH uptake from contaminated particles passing through the intestine is therefore likely. Another possible mechanism is desorption of PAH from the smaller particles and colloids passing across the gill membrane followed by adsorption through the membrane (Broman *et al.* 1990). We believe that a combination of these mechanisms has caused the accumulation, with ingestion of particles trapped by the gill as the main route of entry. The fact that most of the PAHs released from the sediments seem to have been associated with particles that would pass through the mussel gill may be one explanation why the tissue accumulation in general was low.

The results indicated a difference in bioavailability of the PAH from the two fjord sediments, with PAHs from the Saudafjord sediments being most bioavailable to the mussels. The rates of PAH accumulation (Fig. 4) and changes in CPAH percentage (Table 5) as well as PAH profiles in the tissues over time suggested that a 'steady state' was reached earlier in the Saudafjord experiment. Furthermore, the tissue concentrations downstream of boxes with comparable sediment concentrations of PAH and comparable concentrations of SPM (i.e. comparable fluxes of particulate PAH) were clearly higher in the Saudafjord experiment than in the Sunndalsfjord experiment (Fig. 6).

There is no obvious reason for such a difference, but several factors may have played a role. PAH composition and occurrence must be expected to differ somewhat between the two fjords. The Saudafjord test sediments had a significantly higher CPAH percentage ($P < 0{\cdot}05$) than did the Sunndalsfjord sediments, but this was not reflected in the final tissue CPAH (Table 5). Also, the PAH in the sediments from the Saudafjord are associated with sludge from scrubbing of pot fume that is discharged to the sea (at present after cleaning), whereas the Sunndalsfjord PAH-containing waste from scrubbing of hall gas, electrofilter dust and the anode factory is deposited on a landfill separated from the sea by a gravel and sand dike. This will probably allow only very fine particles to escape to the receiving waters. It is therefore possible that the PAH-contaminated particles in the Saudafjord sediments were coarser and of a more appropriate size for ingestion by *M. edulis* if resuspended.

Another factor may have been the difference in sediment organic carbon between the fjords. The Saudafjord sediments had on an average twice as much total organic carbon (TOC) as did those from the Sunndalsfjord (28·2 and 14·6 mg g^{-1}, respectively), and there was a significant positive correlation ($P < 0{\cdot}01$ for log-transformed values) between TOC in the sediments and PAH in the mussels at six months across both fjords

pooled (Fig. 7). The organic content may have influenced bioavailability in at least two ways: as a carrier for sorbed PAH into the mussel gut and as a stimulator for increased filtration and/or food ingestion during a period of low food availability between the short periods of microalgal addition twice a week. Although most experimental evidence shows that the filtration rate in mussels is relatively unresponsive to changes in particle density and quality within wide ranges (Navarro and Winter 1982; Bayne *et al.* 1987), some studies with very low amounts of particle loading have shown that an increase in particle densities or a change in food quality can stimulate filtration rate and growth (Riisgård and Randløv 1981; Strümgren and Cary 1984; Bayne *et al.* 1984, 1987).

It is therefore possible that elevated TOC in the water coming from the Saudafjord boxes may have stimulated higher filtration, food uptake and/or gut absorption rates (rates were not measured) and thus resulted in the higher uptake of PAH. If that is the case, the bioavailability of PAH to mussels may be a function not only of the PAH composition itself but also of the organic enrichment status of the resuspended sediments and the physiological or behavioural response to this by the filter-feeders at various nutritional seasons. A crucial question that should be pursued is whether organic enrichment in general, as found in many eutrophic fjords, is making micropollutants such as PAH more readily available for uptake by filter-feeders, especially during winter with low availability of seston as food, and the more so since the opposite relationship (reduced bioavailability with increased sediment organic carbon) has been shown for deposit-feeders (Knezovich *et al.* 1987; Weston 1990).

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Paper III

Multivariate Approach to Distribution Patterns and Fate of Polycyclic Aromatic Hydrocarbons in Sediments from Smelter-Affected Norwegian Fjords and Coastal Waters

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The environmental significance of polycyclic aromatic hydrocarbons (PAHs) generated by the production of aluminum and manganese alloys is an important issue in numerous Norwegian fjords. The fate of these compounds have been elucidated by analyzing surface and subsurface sediment samples collected over a 15-year period from seven smelter-affected fjords applying principal component analysis (PCA). Consistent patterns within and between fjords and between production types suggested causal relationships and formed the basis for formulating hypotheses regarding the fate of the compounds. The PCA was able to differentiate between various combustion origins at receptor locations, so far hidden in the traditional treatment of the data restricted to individual fjords. This differentiation reflected industrial processes and most probably cleaning arrangements at the different plants. The source-specific signals were discernible over considerable distances and down to concentrations 3–4 times background levels. Compound-specific transformation reactions occurring during transport and incorporation into the sediments contributed little to the total variance and did not suppress the source-specific signals. This may imply a rather low availability to the bottom fauna and raise questions as to the potential harm inflicted by smelter-generated PAHs.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous contaminants of coastal marine sediments. PAHs arise from incomplete combustion of organic matter in flames, engines, and industrial processes and from emissions of noncombustion-derived petrogenic oils. PAHs also occur naturally from events such as forest fires and volcanic eruptions (*1, 2*). The relative composition of the compounds, the PAH profile, is dependent on the origin, but the compounds may undergo specific biotic and abiotic transformation reactions that may lead to changes in the profiles. The environmental concern about PAHs is due to their potential to form carcinogenic and mutagenic diols and epoxides (*3, 4*).

The metallurgical industry may constitute important sources of combustion-derived PAHs. These PAHs appear to be strongly particle associated. Compared to petroleum-derived PAHs, they are less available to desorption processes and presumably also less bioavailable (*5, 6*). The microbial degradation of PAHs with three or less condensed rings is well documented. Less is known about microorganisms capable of utilizing higher molecular weight PAHs as a sole source of carbon and energy, although recent research has reported that this may apply for pyrene (*7, 8*). In general, PAHs of higher molecular weight may be subject to co-metabolic transformations.

In Norway, aluminum and manganese alloy smelters discharge PAHs in effluent wastewater to marine recipients. In the Al plants, the PAH originates mainly from seawater scrubbing of ventilation air and pot fumes from production processes based on the Søderberg technology and, in some cases, also from the production of anodes. The PAH problem is mainly related to the use of coal tar pitch as a binder in the production of ramming paste and anode paste and the fabrication of anode and cathode blocks. In the Mn alloy smelters, the scrubbing of pot fumes is the main source of PAHs, and in these plants, coke used as a reduction agent is probably more important than pitch. The PAH is introduced to recipient waters through pipelines or from settling basins separated from seawater by sand and gravel dikes. Annual discharges have been estimated to be at least 1–10 t of PAH per smelter, but the figures have been reduced drastically in recent years. However, discharge data are uncertain due to the lack of standardized methods for sampling and analysis.

PAH contamination has been monitored in Norwegian recipient waters since the mid-1970s. In the sediments, parent PAH compounds, in particular fluoranthene, chrysene/triphenylene, benzofluoranthenes, benzopyrenes, and benzo[*ghi*]perylene, are quantitatively the most important (*9*). Generally, the monitoring has addressed total concentrations and occurrences of selected compounds, while little attention has been paid to patterns of distribution, although Bjørseth et al. (*10*) revealed distance-related profile changes in one fjord.

In the present study, monitoring data of surface and subsurface sediments collected over a 15-year period from a number of smelter-affected Norwegian fjords have been assembled and analyzed using principal component analysis (PCA) in a search for common patterns and trends. Particular emphasis has been placed on fjord comparisons. Consistent patterns suggest causal relationships and form the basis for formulating hypotheses regarding the fate of the compounds. Another important aspect of the analyses has been to discern and render visible minor variation patterns that are usually overlooked or viewed as noise in routine statistics. The use of PCA in evaluating environmental chemistry data, e.g., for source recognition and transport pathways, has recently been encouraged (*11, 12*).

Sampling, Chemical Analysis, and Data

Data from seven smelter-influenced fjords and coastal areas (four Al, three Mn alloy) were selected for the present study (Figure 1, Table 1). Most Norwegian smelters are located near the heads of fjords or on semi-enclosed coastal waters. In total, the data comprise 199 surface and 41 subsurface stations, covering water depths from 8 to 570 m and spanning distances from immediate proximity to 46 km from the effluent outfall points. The sediment particle composition at the

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FIGURE 1. Smelter locations and sampling sites. The Oslo Fjord and border waters between Norway and Sweden are unaffected by smelter point sources.

stations ranged from sand to almost pure silt and clay; however, the grain size variations were minimized by sampling the fine-grained sediments in the deeper parts of the fjords. Most samples were taken using a 5 cm i.d. gravity corer. The cores were sliced into 1–2 cm sections, and three–eight replicate cores were pooled from each station to ensure enough material.

PAH concentrations were determined by GC–FID or GC–MSD equipped with a split/splitless injector and a 25 m × 0.25 mm i.d. DB-5 fused silica (FID) or a 12 m × 0.2 mm i.d. HP-1 column (MSD) after Soxhlet extraction of freeze-dried sediment with cyclohexane. The GC was calibrated using the Supelco standard containing 16 individual PAHs. The relative accuracies were checked against SRM 1941 from NIST (U.S. National Institute of Standards and Technology). The detection limits depend on sample amounts, final extract volumes, and instrument conditions but were normally 0.5 ng g^{-1} dry weight for individual PAHs. Details of the methods are given by Næs et al. (*9*).

The number of quantified PAHs varied from fjord to fjord in the original monitoring programs. The present analyses were performed on 13 compounds that had been measured regularly and dominated the sedimentary profiles: phenanthrene (Phe), anthracene (Ant), fluoranthene (Flu), pyrene (Pyr), benz[*a*]anthracene (BaA), chrysene/triphenylene (Chr), benzo[*b*+*j*+*k*]fluoranthene (BFs), benzo[*e*]pyrene (BeP), benzo[*a*]pyrene (BaP), perylene (Per), indeno[1,2,3-*cd*]pyrene (Ind), dibenz[*a,c*/*a,h*]anthracene (DBA), and benzo[*ghi*]-perylene (BP). However, occasionally a few values were missing (not analyzed, below the detection limit) in which cases the compounds concerned had to be omitted from analysis. Prior to the final PCAs, preliminary analyses were performed to unveil possible chemical analysis errors and to identify outliers. The data span a wide range of concentrations from background to nearly 800 000 ng g^{-1} dw of PAH_{13} (= sum of the 13 compounds) (Table 1). A value of 300 ng g^{-1} dw is set by the Norwegian sediment quality criteria (*13*) as the upper background limit for fine-grained fjord sediments not influenced by point sources. An overview of the occurrence of PAH in sediments and organisms in Norwegian marine waters affected by Al smelters may be found in Næs et al. (*9*).

PCA [CANOCO version 3.10 software (*14*)] were used to apportion the variance in the data sets. The analyses were performed as "ordinary PCA" (variable-centered) and "double-centered PCA" (variable- and sample-centered) on log-transformed data. The latter option is comparable to standardized PCA by eliminating the effects of scale, thus focusing on relative patterns. The PCA results are presented in combined plots (biplots) [CanoDraw 3.0 software (*15*)] of variables and stations. The stations are shown as points, while the variables are represented by arrows running from the origin of the plot to the position of the variable scores (loadings). The direction of an arrow represents the direction of increasing variable concentration across the stations, while the length represents the strength of the increase. The angle between pairs of arrows indicates the approximate correlation between variables.

Results

Surface Sediments. An obvious trend in all fjord data sets is a decline in total concentrations with distance from the effluent outfall. In ordinary PCAs for the individual fjords, this appears as a very strong first axis (PC1) capturing more than 80% of the total variance (Table 2). The successive axes (PC2, PC3) consequently represent minor components of the total variation. These variance components, however, may be interpreted as profile patterns superimposed on the concentration gradient and may thus reflect source signals and processes transforming individual PAHs in the recipient waters.

In order to focus on the profile patterns, the effects of concentrations were deducted from subsequent analyses, which were performed double centered. Figure 2 shows analyses of surface sediments in fjords influenced from Al and Mn alloy smelters, respectively. The Al fjord data (Årdal, Sunndal, Karmsund, Vefsn) comprised more than a 100 stations and also some replicate samples (Figure 2A). One sample series (Vefsn 1989) was omitted because it showed large and apparently unsystematic variations that suppressed the patterns among other samples. In the plot, the samples formed groups for fjords, to some extent (e.g., Årdal) also for sample series from different years. This clustering of samples according to constituent data sets indicates that each fjord had a typical PAH profile, in some cases even each sampling. Looking at the samples in relation to the variable pattern (PAHs) allows a characterization of the profiles, showing for instance that Karmsund, Vefsn (1978), and Årdal (1983) had a relative dominance of lighter weight compounds, while the second series from Årdal (1989) had a higher representation of heavy weight compounds. These characteristic profiles must actually be discernible along the entire PAH concentration gradient in the fjords. There was a tendency, however, for stations occupying the area near the mouth of the fjords to be displaced to the right in the plot (not illustrated), indicating a relative increase of lighter PAHs as compared to stations near the source.

The individual PAH compounds were arranged in a sequence that roughly followed their molecular weights from light to heavy. This arrangement implies that compounds with similar molecular weights had similar distributions across the stations (high correlations), while compounds of different molecular weights varied proportionate to the size of the difference. The strongest trend (PC1) separated the light and

TABLE 1. Summary Data for Stations Sampled in Different Fjord Areas

fjord area	receiving water	sampling year	no. of stations (core stations)	distance from source, km[a]	water depth, m[a]	sediment section (depth), cm[a,b]	PAH_{13}[a,c] ng g^{-1} dw
Årdal	narrow fjord	1983	9 (2)	0.6–15	84–570	0–1 (20)	3 401–784 296
		1989	21	0.6–3.5	48–165	0–1	
Sunndal	open fjord	1986	21 (1)	0.1–44	16–345	0–1 (14)	341–772 506
		1990	10	0.5–3.2	82–123	0–2	
Karmsund	coastal sound	1988	16 (2)	1.1–11	13–93	0–1 (18)	8 500–88 700
Vefsn	open fjord	1978	6	2.5–20	159–485	0–2	
		1984	8 (8)	1.8–12	39–458	0–1 (3)	684–173 590
		1989	10 (2)	1.8–20	35–487	0–1 (10)	
Feda	narrow fjord	1984	13 (1)	0.1–19	23–384	0–2 (10)	357–144 858
Sauda	open fjord	1976	6 (3)	0.3–20	80–500	0–2 (8)	
		1981	11 (1)	0.3–20	40–375	0–1 (20)	158–499 401
		1986	18 (2)	0.3–20	84–322	0–1 (5)	
		1990	10	0.4–2.5	55–178	0–2	
Grenland	narrow fjord	1986	14	2.3–20	22–113	0–2	342–60 572
		1989	26 (19)	1.1–46	8–217	0–2 (18)	

[a] Distance from source, water depth of stations, and sum of 13 PAHs are given as ranges. [b] Sediment section refers to the thickness of the analyzed sediment slice, depth (in parentheses) to the subbottom depth of the deepest slice. [c] See text for definition of PAH_{13}.

TABLE 2. Data for Ordinary PCAs of PAHs in Separate Fjord Areas

fjord area	variable excluded	no. of stations included	variance explained (%)		
			PC1	PC2	PC3
Årdal		26	87	10	2
Sunndal	Per	26	95	3	1
Karmsund	Ant, DBA	16	88	5	4
Vefsn	Ant, Per, DBA	20	83	9	4
Feda	BeP	9	97	2	0.5
Sauda	Per	39	89	6	3
Grenland	Per, DBA	32	87	8	2

heavy compounds, which appeared to show a negative correlation and diverged on a relative scale. Most medium weight compounds deviated from the pattern and were fitted on PC2, which was most distinct for triphenylene/chrysene (Table 3).

The Mn alloy fjord data (Feda, Sauda, Grenland) comprised nearly 100 stations (Figure 2B). Some stations in Grenland located near petrochemical industry were omitted. These stations were markedly dominated by lighter compounds that suppressed the dissolution in the plot. The PAHs were arranged in a pattern similar to that found in the Al fjords, but the samples were less clearly grouped for fjords, although Sauda and Grenland tended to be separated. The latter showed a relative preponderance of light and medium weight PAHs.

Figure 2C shows an analysis for Al and Mn alloy fjord data put together. Most samples were grouped according to type of production. The Al sites tended to have more light and medium weight PAHs, while Mn alloy sites tended to have more heavy PAHs. The groups may be almost separated by projecting the site points on an axis running from the lower left to the upper right in the direction of the arrows for indeno-[1,2,3-*cd*]pyrene and benzo[*ghi*]perylene on the one side and pyrene and benz[*a*]anthracene on the other. The results suggest that for the present data selection these compounds were particularly typical of the type of smelter and that their relative amounts may be used to distinguish between profiles.

Considering that source-related profiles were detectable for the fjords, further comparisons were made with areas that lacked point sources. Figure 3 shows smelter fjord profiles compared to profiles from the Oslo Fjord (3 stations) and the open coast border waters between Norway and Sweden (9 stations). Both areas represent environments that are moderately influenced by general human activity (mean PAH_{13} < 2000 ng g^{-1}). The most strongly contaminated fjord stations (> 5000 ng g^{-1} PAH_{13}) were omitted to restrict the analysis to samples with comparable PAH concentrations. It appeared that the smelter-affected fjords may generally be distinguished from the diffusely affected areas. The samples from Vefsn, the Oslo Fjord, and the border waters formed clear groups, while the Mn alloy fjords (Sauda, Feda, Grenland) formed less distinct groups and partly overlapped with the border waters. The difference between the Oslo Fjord and the border waters suggests that diffusely affected areas may also have characteristic profile patterns.

Downcore Profiles. The data for downcore profiles comprised 68 samples (core sections) from 18 stations in six fjords (Årdal, Feda, Karmsund, Sauda, Sunndal, Vefsn). Most samples were grouped according to fjords (Figure 4). Further, within fjords there was a clear tendency to group sections according to stations and sampling years. As in the analyses of surface sediments, PC1 mainly fitted the lower and higher molecular weight compounds, while PC2 fitted the intermediate molecular weight compounds. Benzo[*a*]pyrene deviated somewhat and correlated most strongly with PC3. The Mn alloy fjords (Feda, Sauda) were associated with the heavier PAHs.

Discussion

The amount and distribution of PAHs in the fjord sediments depend on the effluents involved, transport, and sedimentation as well as on possible transformation reactions. The present study demonstrates that most fjords had a characteristic PAH profile or "fingerprint". The fingerprints were particularly clear for the Al smelter-influenced fjords, where also different samplings could be distinguished. Aluminum production could be distinctly separated from manganese alloy production. The patterns were also evident downcore in the sediments in the fjords. Comparison with areas without smelter point sources indicated that the profile patterns were discernible down to 3–4 times background levels.

The systematic nature of the fingerprint patterns suggests that these reflect source characteristics. There is no indication that the patterns could be artifacts from analytical performance, considering also that all chemical analyses were performed by the same laboratory. Verification of the source characteristics can hardly be made, however, because discharge data for the smelters are scarce and mainly represent total PAH levels. Yet, some information may be deduced on the basis of knowledge about the production processes and cleaning arrangements. In the Al smelters there are various installations for cleaning of emissions, e.g., scrubbing of ventilation air and pot fume, dry cleaning with alumina, and electrostatic precipitation (*16*). Generally, scrubbers are efficient for retaining large- and medium-sized particles and

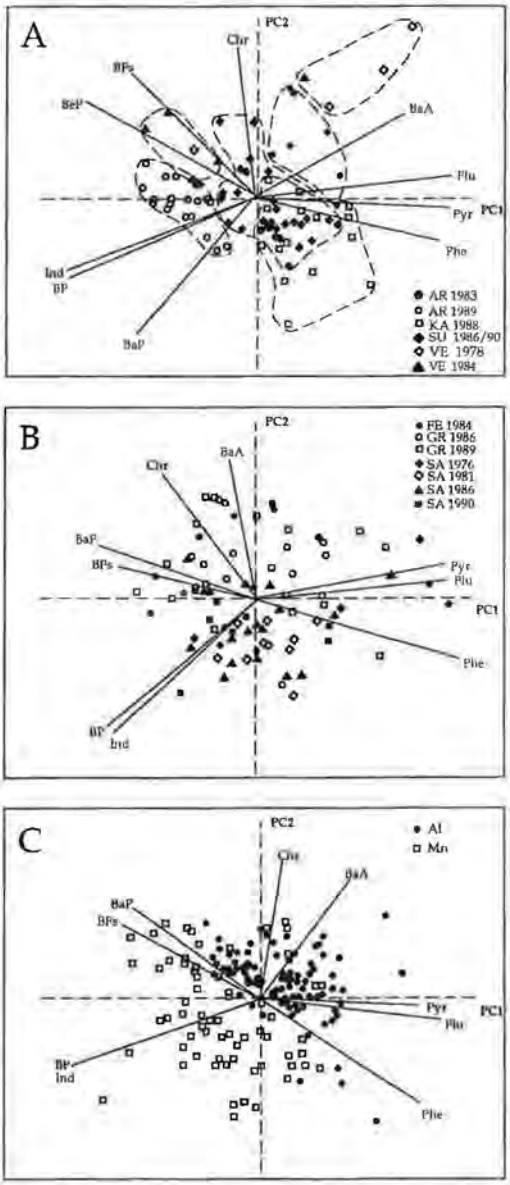

FIGURE 2. Double-centered PCA for inter-fjord comparison. (A) Al smelter-influenced stations, number of samples = 82, PC1 and PC2 account for 51 and 16% of variance, respectively. (B) Mn alloy smelter-influenced stations (N = 83, PC1 = 48%, PC2 = 21). (C) Combined Al and Mn alloy smelter-influenced stations (N = 159, PC1 = 49%, PC2 = 19). Abbreviations: AR, Årdal; KA, Karmsund; SU, Sunndal; VE, Vefsn; FE, Feda; GR, Grenland; SA, Sauda.

inorganic compounds, while electrostatic precipitation is efficient for the removal of small particles and organic compounds (*17*). Retention of individual PAHs therefore may be supposed to vary, essentially more or less in relation to molecular weight as the heavier PAHs generally have the strongest affinity for particles. The efficiency may also gradually change with time. For instance, in Årdal the efficiency of the electrostatic precipitation declined during the 1980s (information from the plant), which may explain the transition to profiles dominated by higher molecular weights from 1983 to 1989. Profile differences could also

TABLE 3. Cumulative Variance Fit for Individual PAHs in PCA for Al Smelter-Influenced Fjords

fraction fitted variable	PC1 0.51	PC2 0.16	PC3 0.09	PC4 0.08
Phe	0.75	0.81	0.98	0.98
Flu	0.77	0.78	0.83	0.88
Pyr	0.62	0.62	0.79	0.89
BaA	0.12	0.17	0.23	0.60
Chr	0.01	0.50	0.56	0.56
BFs	0.23	0.54	0.77	0.77
BeP	0.49	0.72	0.73	0.73
BaP	0.20	0.53	0.60	0.84
Ind	0.62	0.79	0.79	0.81
BP	0.67	0.82	0.83	0.93

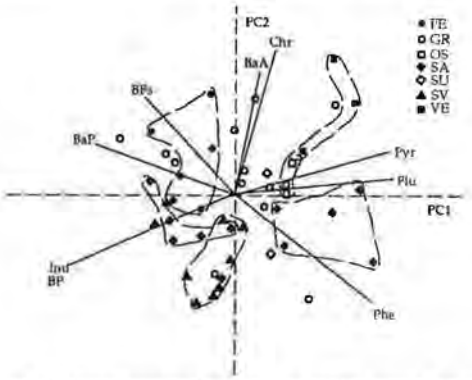

FIGURE 3. Double-centered PCAs of smelter-influenced stations with PAH_{13} concentrations of less than 5000 ng g^{-1} combined with stations unaffected by point sources (N = 47, PC1 = 54%, PC2 = 19%). OS, Oslo fjord; SV, border waters Norway/Sweden.

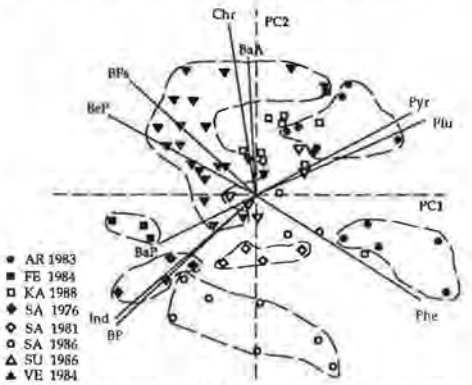

FIGURE 4. Double-centered PCA for downcore samples (N = 68, PC1 = 30%, PC2 = 30%).

stem from the PAH source material (coal tar pitch). Differences in pitch type and softening point influence the PAH profile (*18*), but there is no evidence that this caused systematic differences in the effluent PAH.

Fjords influenced by Mn alloy smelters were less clearly differentiated than the Al fjords, although most Grenland samples were distinguishable from Sauda samples. Mn alloy smelters have similar cleaning systems, which translates into similar routes of PAH entry into the recipient waters. The smelters also have the same central supplier of anodes. Accordingly, there are no particular source characteristics among the smelters that would be expected to cause different effluent profiles. The differences in the Grenland samples

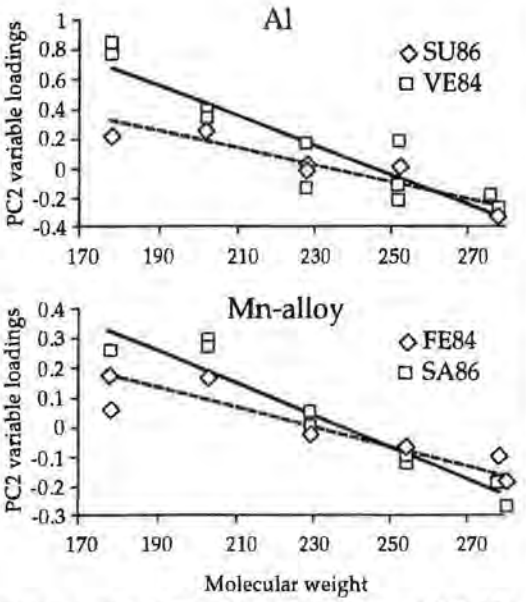

FIGURE 5. Plot of molecular weights of the individual PAH compounds against PC2 coordinates based on variable-centered PCA on individual fjord data sets. Fjords affected by aluminum production (Al) include Vefsn sampled in 1984 and Sunndal in 1986. Fjords affected by manganese alloy production (Mn) include Feda sampled in 1984 and Sauda in 1986. All regression lines are significant at $p < 0.001$.

probably reflect influences from other sources in the area, in particular PAHs of petrogenic origin dominated by low molecular weight PAHs which is quite typical of fossil fuel sources (*19*). Grenland is the most heavily industrialized area of Norway, receiving numerous anthropogenic inputs.

Comparisons of the fjords with different production types indicated that the Mn alloy industry generates a PAH profile dominated by higher molecular weights. Presumably, this is due to temperature differences in the production processes. In Mn alloy smelters, PAHs originate in high temperature pot rooms (1200−1500 °C), while in Al smelters, the PAH generating processes (evaporation from anode, anode production) operate at far lower temperatures. Higher temperatures will facilitate the evaporation of PAHs of higher molecular weights, thereby shifting the profile toward heavier compounds (*20*). It is also possible that the pH of the effluent waters (approximately 7 in Al production as compared to 10 in Mn alloy) affects the profiles as PAH degradation and sorption to particles can be pH dependent (*21*). However, nothing is known about the significance of such processes in effluent waters.

The arrangement of the variables (PAHs) in the analyses reflects the variation of the individual PAHs in the recipient waters. All analyses depicted the PAHs in a consistent pattern that can be related to molecular weight. Generally, molecular weight may be taken as a descriptor of structure−property and structure−activity relationships (*22*). Most probably, the variable patterns therefore represent processes operating on the PAHs in the sea. In the present analyses combining data set from different fjords and samplings, however, source differences may confound the individual variation, but the variable pattern also emerges in analyses of single sample series. This is demonstrated in Figure 5 showing variable scores (loadings) related to the molecular weight of the compounds. The illustrated results are from ordinary PCAs (see Table 2) in which PC2 captures the pattern appearing on PC1/PC2 in the double-centered PCAs. The compound-specific processes may operate during transport, during sedimentation, and after incorporation in the sediments. Various studies have shown that PAHs in seawater may be transported as dissolved (truly and/or apparent), sorbed to natural organic matter such as humics, or associated with different-sized particles (*23, 24*). Thrane (*17*) and Bjørseth (*20*) showed that in the Al smelters the PAHs were present in both the gaseous and particulate phases of ventilation air. Hence, in the smelter effluents, the PAHs may occur both dissolved and associated with particles, which suggest subsequent selective transport in the fjord. Correspondingly, this could account for the general seaward shift toward a lighter profile in the fjords. Similar findings are supported by studies elsewhere (*10, 25, 26*).

In the sediments, microorganisms may degrade PAHs and alter the profile (*7*). The analyses of downcore profiles, however, demonstrated also that subsurface samples retained a profile related to source characteristics. These results contrast with experiments, mostly in the laboratory, showing that PAH may be biodegraded after incorporation into the sediments (*8, 21, 27, 28*). However, disagreement in laboratory-predicted half-lives and field observation data may be a consequence of differences in environmental factors (*29, 30*). Also, the binding type of the PAH molecule and its associated matrix is of fundamental importance. The PAHs in the sediments are probably to a great extend bound to oxides and black carbon-containing particles (soot). The latter association is known to render the PAHs resistant to degradation and also to imply that desorption and re-partitioning into the porewater is strongly restricted (*5, 6, 31*).

The PCAs were able to differentiate between various combustion origins at receptor locations, so far hidden in the traditional treatment of the monitoring data restricted to individual fjords. In the litterature, various approaches have been proposed for PAH source recognition, including alkyl homologue series, binary PAH ratios, and parent PAH profiles (*19, 20, 32*). The results from the present data show that parent PAH profiles analyzed with multivariate data processing tools such as PCA are powerful means of source resolution and could also facilitate the early detection of sediment responses to changes in input. The consistency of the source-specific signals shows that the PAHs were resistant to or have a severely restricted potential for natural post-depositional transformation reactions. This may imply a rather low availability to the bottom fauna and raise questions as to the potential harm inflicted by smelter-generated PAHs. Studies of benthic communities in the fjords have indicated that the ecological effects are small (*33*), but further studies regarding PAH uptake and their metabolization by benthic organisms are needed.

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THE DISTRIBUTION AND ENVIRONMENTAL RELATIONSHIPS OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) IN SEDIMENTS FROM NORWEGIAN SMELTER-AFFECTED FJORDS

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ABSTRACT

PAH distribution was examined in 119 surface sediment samples collected in connection with monitoring surveys in four Norwegian fjords affected by aluminium and manganese alloy smelters. Regression, principal component analysis (PCA) and redundancy analysis (RDA) were used to identify common patterns and possible correlations with explanatory variables (distance, depth, grain size, TOC, C/N-ratio, metals). The best-fitting variables corresponded to the deposition of PAH, expressed either as a function of distance from source or as the amount of PAH-bearing material. This supports the assumption that particulate, combustion-derived PAHs are resistant to transformation during transport and incorporation into surface sediments. Compound-specific processes leading to minor, but systematic and strikingly similar profile variations in the fjords could not be adequately explained by the environmental variables. © 1997 Elsevier Science Ltd

INTRODUCTION

Aluminium (Al) and manganese-alloy (Mn-alloy) production represents an important anthropogenic source of combustion-derived PAHs in Norwegian fjord and coastal waters. Concentrations in excess of 1000 times background levels have been observed in sediments and monitoring organisms in the vicinity of some plants [1]. PAHs are potentially hazardous substances which may have carcinogenic and mutagenic effects [2,3]. As a consequence, Norwegian nutritional authorities have issued dietary warnings for, and prohibited the sale of, some edible organisms from some of the fjords. This has had some financial repercussions for the harvesting of natural fjord resources.

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In Al plants, PAHs originate mainly from the sea water scrubbing of ventilation air and pot fumes resulting from production processes based on Søderberg technology, and, in some cases, also from the production of anodes. The PAH problem is mainly related to the use of coal tar pitch as a binder in connection with the production of ramming paste and anode paste, and the fabrication of anode and cathode blocks. In the Mn-alloy smelters, the scrubbing of pot fumes is the main source of PAHs, and coke used as a reduction agent is probably a more significant source than pitch. PAHs are introduced into the receiving waters through pipelines or from settling basins separated from sea water by sand and gravel dikes.

Typically, parent compounds dominate the PAH composition of sediments. In terms of quantity, fluoranthene, chrysene/triphenylene, benzofluoranthenes, benzopyrenes and benzoperylene are important [1]. The relative composition, ie, the PAH profile, appears to be unique for each fjord. The profiles reflect the sources and seem to depend on the type of industrial processes and cleaning systems used by the various smelters [4]. The source specific profiles are readily recognizable and discernible over a broad range of concentrations from the most contaminated areas in the vicinity of effluent discharge points to distant areas featuring low contaminant concentrations. The presence of widespread, source-dependent patterns indicates that the transformation reactions that act on PAHs during transport and incorporation into the sediments are of minor importance in the fjords [4].

Sedimentary combustion-derived PAHs appear to be strongly particle-associated. However, studies by Bjørseth [5] and Thrane [6] suggest that the PAHs in the smelter effluents occur both in a dissolved state and in association with particles. Dispersion and sedimentation in the receiving waters may therefore be a function of the particle affinities of the PAHs as well as the dynamics of particle transport and deposition. Generally, organic matter adsorbs PAHs in aquatic environments, but this association will vary between individual PAHs, since the carbon-normalized partition coefficient is linearly related to the hydrophobicity of the contaminants [7,8]. The effluent particle-associated PAHs are, to a certain extent, bound to black carbon (soot) and seem strongly resistant to degradation and desorption [9,10]. Little is known of the quantitative relationship between the various processes and the potentially different fates of effluent PAH components.

The present paper examines PAH distribution patterns in surface sediments from four Norwegian smelter-affected fjords. The patterns have been linked to topographical and sediment characteristics in order to describe the underlying processes. The data have been compiled from monitoring programmes conducted in the fjords since the mid-1970s. Various numerical techniques were employed, including regression, principal component analysis (PCA) and redundancy analysis (RDA). PCA was used to summarize and partition the variation in the data, thus elucidating the main patterns in PAH distribution. Regression and RDA were used

to examine environmental relationships which were explored by entering variables for the various environmental conditions. In particular, sediment-borne organic matter and other effluent contaminants were used to represent the particulate materials carrying PAHs.

MATERIAL AND METHODS

Sampling and chemical analysis

Data were collected from two fjords affected by aluminium production (Årdal, Sunndal) and two fjords affected by manganese-alloy production (Feda, Sauda) (Figure 1, Table 1). PAHs were measured at a total of 119 samples, covering water depths from 16 to 570 m and spanning distances from immediate proximity to 44 km from the effluent outfall points. The values range from nearly 800 000 ng g^{-1} d.w. of total PAH to background levels (Table 1). A value of 300 ng g^{-1} d.w. has been set as the upper background limit for finely grained fjord sediments under Norwegian sediment quality criteria [11]. Most samples were taken using a 5 cm i.d. gravity corer. The cores were sliced into 1-2 cm sections.

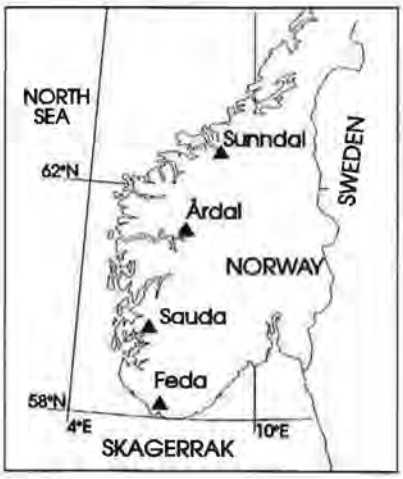

Figure 1: Map of Southern Norway with smelter locations.

PAH concentrations were determined by GC-FID or GC-MSD equipped with a split/splitless injector and a 25 m x 0.25 mm i.d. DB-5 fused silica (FID) or a 12 m x 0.2 mm i.d. HP-1 column (MSD) after Soxlet extraction of freeze-dried sediment with cyclohexane. The GC was calibrated using the Supelco standard which is based on 16 individual PAHs. The relative accuracies were checked against SRM 1941 from NIST (the US National Institute of Standards and Technology). Although detection limits depend on sample amounts, final extract volumes and instrument conditions, they were normally 0.5 ng g^{-1} dry weight for individual PAHs. Details of the methods are given by Næs et al. [1].

Table 1. Summary data for the stations sampled in the different fjord areas. Distance from source, water depth of stations, silt-clay percentages, TOC contents and C/N ratios are given as ranges. Sediment section refers to the part of the surface sediment analyzed.

Fjord area	Sampling year	No. of stations/ samples	Distance from source, km	Water depth, m (sediment section, cm)	Silt-clay %	PAH_{13} ng g^{-1}	TOC %	C/N
Årdal (AR)	1983	9	0.6-15	84-570 (0-1)	n.a.	43 204-712 916	5-18	21-90
	1989	4/21	0.6-3.5	48-165 (0-1)	25-64	45 565-784 296	3.2-11	23-108
Sunndal (SU)	1986	13/21	0.1-44	16-345 (0-1)	21-90	1 118-772 506	0.4-5	10.5-25.1
	1990	3/10	0.5-3.2	82-123 (0-2)	n.a	6 481-66 209	1.1-1.9	9.1-12.7
Feda (FE)	1984	13 2	0.1-19	23-384 (0-2)	n.a.	337-144 858	1.9-8.2*	-
Sauda (SA)	1976	6	0.3-20	80-500 (0-2)	n.a.	262-89 711	0.4-8.3*	-
	1981	11	0.3-20	40-375 (0-1)	n.a.	686-143 203	n.a.	-
	1986	18	0.3-20	84-322 (0-1)	n.a.	866-87 117	2.2-5.1*	-
	1990	3/10	0.4-2.5	55-178 (0-2)	n.a.	8 362-499 401	1.7-4.9	14.3-26.5

* = Loss on ignition divided by 2.5
n.a. = Not analyzed

Grain size, organic matter and metals were measured in some of the fjord surveys (Table 1). Grain size was expressed as the silt-clay percentage by wet sieving on a 0.063 mm sieve. Organic matter was determined as total organic carbon (TOC) and total nitrogen (TN) with a Carlo Erba CHN analyzer following the removal of carbonates by acidification. In some surveys, organic matter was determined as loss on ignition (550°C). Generally, the textural variation between stations was minimized by sampling the fine-grained sediments in the deeper parts of the fjords.

Numerical analyses

Thirteen PAHs which had been measured regularly and dominated the sedimentary profiles were selected for the analyses. These were: phenanthrene (Phe), anthracene (Ant), fluoranthene (Flu), pyrene (Pyr), benz(a)anthracene (BaA), chrysene/triphenylene (Chr), benzo(b+j+k)fluoranthene (BFs), benzo(e)pyrene (BeP), benzo(a)pyrene (BaP), perylene (Per), indeno(1,2,3-cd)pyrene (Ind), dibenz(a,c/a,h)anthracene (DBA) and benzo(ghi)perylene (BP). Total concentrations are represented by the sum of the compounds (= PAH_{13}).

The environmental (explanatory) variables consisted of topographical factors (water depth, distance from source), sediment characteristics (TOC, C/N ratio, silt-clay %) and other contaminants (Cd, Pb, Cu, Mn, F). The contaminants were included to evaluate possible correlations with PAH distributions which might indicate common origins or similar distribution mechanisms. Sediment characteristics had not been measured regularly in Sauda, so they were replaced by a four-class categorical variable for sediment type (anoxic mud, perturbed sediment, sand-mixed mud, mud). Loss on ignition was recalculated to TOC by division by 2.5 (TOC was set at 40 per cent organic matter). In analyses combining data from different surveys, a categorical variable with one class for each survey (year) was entered to account for temporal variation.

Simple and multiple regression were used to relate the changes in total concentrations (PAH_{13}) to the explanatory variables. Distance from source and sediment characteristics were considered to be of greatest interest. In multiple regressions, combinations of various explanatory variables (distance, TOC, grain size) were tested to determine their contribution to the relationship.

Principal component analysis (PCA) was used to examine profile patterns and apportion the variance in the PAH data for each fjord. PCA represents the patterns and trends by arranging the PAHs (variables) and stations along axes (principal components) which are assumed to represent basic factors or relationships. The first axis (PC1) illustrates the most prominent trend, while the successive axes (PC2, PC3, etc.) represent secondary trends in decreasing order of importance. All axes are uncorrelated. The present analyses were performed as 'ordinary PCAs' (variable-centred), meaning that the concentration differences were taken into account. Occasionally, single PAH values were missing, in which case the compounds or stations in question had to be omitted from the analysis. Preliminary PCAs were performed to unveil possible chemical analysis errors and identify outliers.

Redundancy analysis (RDA) was used on data from two fjords (Sunndal, Sauda) to examine the relationship between PAH profiles and explanatory variables. RDA is a technique related to PCA, but it includes an additional data set of explanatory (environmental) variables for the stations. The axes represent linear combinations of the explanatory variables. Thus the analysis will detect specific variation patterns among PAHs, which correlate with the explanatory variables. Both 'ordinary' and 'double-centred' RDAs (variable- and sample-centred) were performed. The double-centred option eliminates the effects of scale, focusing particularly on PAH profile patterns. During analysis, the procedure of 'forward selection' was used to rank the variables by importance and to identify a subset of variables which were maximally related to PAH distribution. The significance of the variables was tested using Monte Carlo permutation tests.

The analyses were performed using Statgraphics v. 2 and CANOCO v. 3.10 software [12,13] for regressions and PCA/RDA, respectively. All PAH values and strongly skewed explanatory variables (depth, distance) were log-transformed prior to analysis. Plots from PCA and RDA were made using CanoDraw 3.0 software [14]. Combined plots (bi-plots/tri-plots) of variables (individual PAHs and explanatory variables) and stations are given. The variables are represented by arrows pointing in the direction of increasing values. The length represents the strength of the increase, while the angle between pairs of arrows indicates the approximate correlation between variables.

RESULTS

Variation in total concentrations

The total PAH concentrations (PAH_{13}) decreased strongly with distance from the effluent outfall points in all fjords. This relationship fits well into a straight line based on regressions performed on log-log transformed data. Regressions generally explained 75-90 per cent of the variance in PAH concentrations (Table 2). In all cases, regressions were found significant at the 1 per cent level or better. Multiple regressions that combined sedimentary characteristics with distance as independent variables explained an additional 5 to 10 per cent of the variance. Generally, TOC was the best choice for a second variable, but the added contribution was not consistently statistically significant. TOC was, however, clearly related to PAH concentrations, and, in Årdal, TOC was actually a more viable explanatory variable than distance when taken alone in simple regression. Grain size added very little to the explained variance.

Table 2: Results of fitting a linear model to describe the relationship between log (PAH_{13}) and log (explanatory variables). All relationships are significant at $p<0.01$.

Fjord - sampling year	No. of stations	Distance from source	R^2: Explanatory variable: Distance	R^2: Explanatory variables: Distance, TOC*
Årdal, 1983	8	0.35-15	84	93[A]
Feda, 1984	11	0.5-20	89	98
Sunndal, 1986	21	0.1-44	82	95
Sauda, 1986	18	0.3-20	75	80[B]

* : For Feda and Sauda, TOC was calculated as loss on ignition divided by 2.5

A: When TOC was added, the contribution of distance was not significant ($p_{Distance} = 0.12$)

B: The contribution of TOC as an additonal explanatory variable was not signifiant ($p_{TOC} = 0.07$)

Variation of individual compounds

The individual PAHs decreased with distance from source roughly in proportion to total concentrations. In the PCAs, this appeared as a very strong first axis that explained most of the variation (Table 3). PAH scores (variable loadings) on the axis were similar and generally high, while the stations were arranged in order - from those closest to the effluent sources to those furthest away. This trend is illustrated in the diagrams (Fig. 2) by the similar orientation and lengths of the PAH arrows on the first axis, all pointing in the direction (left) of increasing PAH concentrations. The second and higher axes separated the compounds into generally regular sequences from positive to negative scores. These axes depict patterns which may be interpreted as minor profile variations superimposed on the concentration gradient.

Table 3: Data for ordinary PCAs of PAH compounds in separate fjord areas.

Fjord area	Variable excluded	No. of samples included	Variance explained (%) PC1	PC2	PC3
Årdal		26	87	10	2
Sunndal	Ant, Per	26	95	3	1
Feda	BeP, BFs	9	97	2	0.5
Sauda	Per	39	89	6	3

The PAH sequence on the second axis (Fig. 2) roughly followed the molecular weights of the compounds from light to heavy. This arrangement implies that compounds with similar molecular weights had similar distributions across the stations (high correlations), while compounds of various molecular weights differed proportionately to the size of the difference. This particular pattern may be associated with the processes that act upon individual PAHs in recipient waters. To a certain extent, the pattern also represents source variability, ie, in the cases where data from different years have been combined. However, the small variance components explained on PC2 and higher axes show that such variations are minor compared to the changes along the concentration gradients.

Data for different years were combined in the analyses for Årdal, Sunndal and Sauda. The years were clearly separated in Årdal and Sunndal, with the samples from 1989 and 1986 containing relatively more of the heavier PAHs (Fig. 2 A, B). In Sunndal, some compounds deviated from the main pattern, eg, benz(a)anthracene which was high in 1986 and benzo(ghi)perylene which was high in 1990. The 1981, 1986 and 1990 Sauda samples had similar profiles, while the 1976 samples were characterized by relatively more of the lower molecular weight compounds.

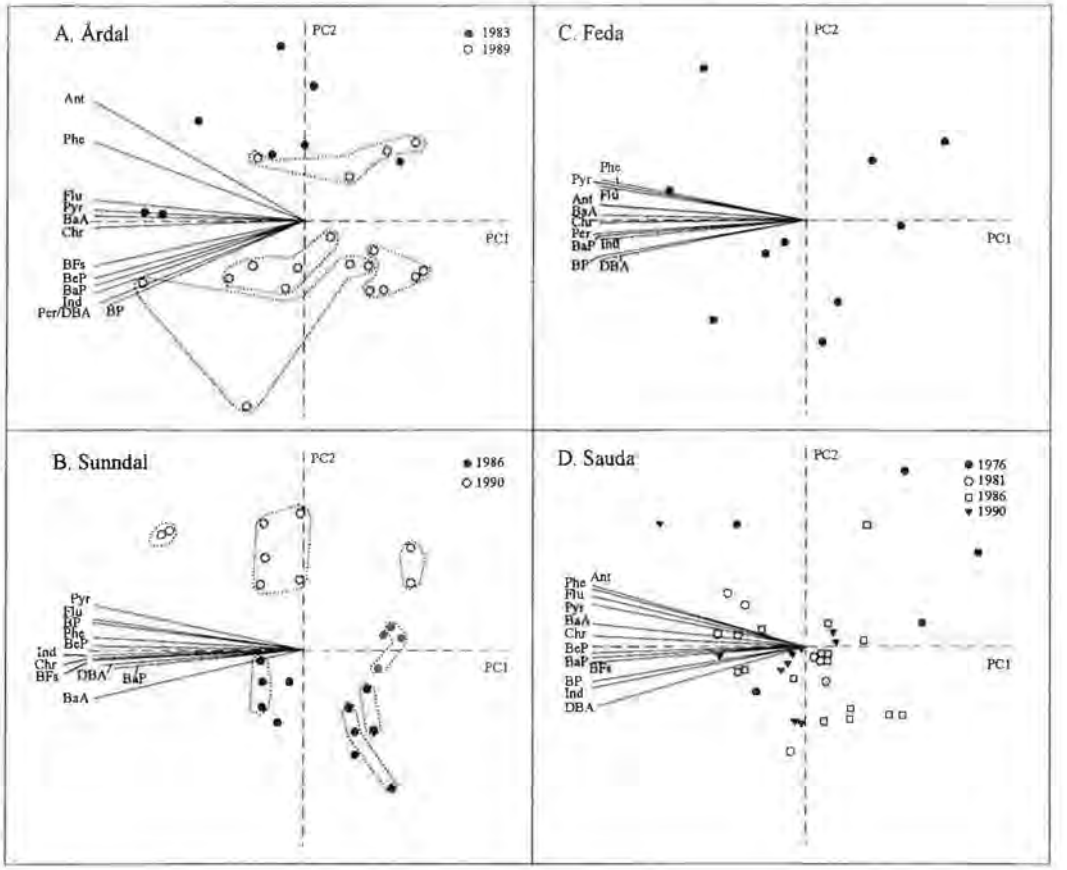

Figure 2: PCAs for separate fjords (variable-centred PCA). A: Årdal (replicate samples from same station are circled), B: Sunndal (replicate samples from same station are circled), C: Feda, D: Sauda.

Replicate samples were taken in Årdal and Sunndal. In Årdal, the replicates were somewhat scattered across the plot (Fig. 2A), but most of the scatter was along PC1. This scatter represents variations in total concentrations with up to a 20-fold difference between replicates. The low scatter along PC2 shows that the relative proportions of the compounds among the replicates were similar. This indicates that the stations were actually better characterized by profile patterns than by their total concentrations. In Sunndal, the replicates were closely grouped, showing that both concentrations and profiles were similar for parallel samples. The general similarity of the replicate profiles is implicit evidence of the quality of the chemical analyses, which is reassuring considering the large variation in total concentration at some stations.

Relation to environmental factors

Sunndal

The ordinary (variable-centred) RDA confirmed that the distance from source could explain a large part of the variation in PAH distribution (Table 4). However, the variables TOC, C/N and depth could explain as much or even more of the variation. These four variables were mutually correlated, ie, by selecting one of them, the other added very little to the explained variance. There is a high correlation between the variables because depth increases with distance from the head of the fjord, where the highest sediment carbon content is found. The variables for the sediment fine fraction (silt-clay) and metal contaminants could account for lower amounts of variation, but the contribution made by lead (Pb) was found to be statistically significant. The six best fitting variables explained 92 per cent of the variation. The plot from the analysis (Fig. 3A) illustrates that TOC, C/N and distance may all account for concentration changes. The second axis correlated with the years and, to a certain extent, with lead (Pb). The plot was generally similar to the corresponding PCA plot.

Table 4: Ranking of explanatory variables in redundancy analyses (RDA) of PAH distributions in Sunndal. A. Ordinary RDA, focusing on PAH concentrations. B. Double-centred RDA, focusing on PAH profile patterns (1986 data only). Exp: explained variance (%) attributable to each individual variable; Added: variance accounted for by variables chosen in 'forward selection'. Variables found significant ($p < 0.05$) in Monte Carlo permutation tests were selected. Asterisks indicate classes of the nominal variable year. Fluoride (F) was not measured in 1990 so it was omitted from the ordinary RDA.

A. Ordinary RDA			B. Double-centred RDA		
	Exp	**Added**		**Exp**	**Added**
TOC	81	81	C/N	28	28
C/N	79	4	Depth	27	-
Distance	73	3	TOC	25	-
Depth	69	-	Distance	22	-
Pb	31	3	F	19	12
Silt-clay	27	-	Cd	14	-
Cd	22	-	Pb	14	-
Cu	10	-	Silt-clay	8	12
1986 *	9	1	Cu	6	-
1990 *	9	-			
Total (all/selected)	95	92		76	52

The analysis of profile patterns (double-centred) was restricted to the 1986 data, when fluoride was recorded along with metal contaminants. The best fitting variables were found to be the carbon/nitrogen ratio, fluoride and the fine fraction, which, collectively, explained 52 per cent of the variance (Table 4). TOC and metals generally correlated with the C/N ratio and did not contribute any particularly unique information. The relationships are depicted in the plot where the latter variables have been entered passively in accordance with their correlations (Fig. 3B). The first axis contrasted the lower molecular weight PAHs (phenanthrene, fluoranthene, pyrene) and the medium-weight PAHs (chrysene, benzofluoranthenes, benzo(e)pyrene) along a distance-carbon/contaminant gradient. Chrysene and benzo(e)pyrene appear to have correlated well with carbon and metals, while benzofluoranthenes correlated with fluoride. The second axis illustrated a weak relationship between benzo(a)pyrene and the silt-clay fraction. The heaviest compounds (indenopyrene, benzoperylene) showed no particular correlations (most of the variance was extracted on the third axis). Further, none of the parameters explained the deviant distribution of benz(a)anthracene

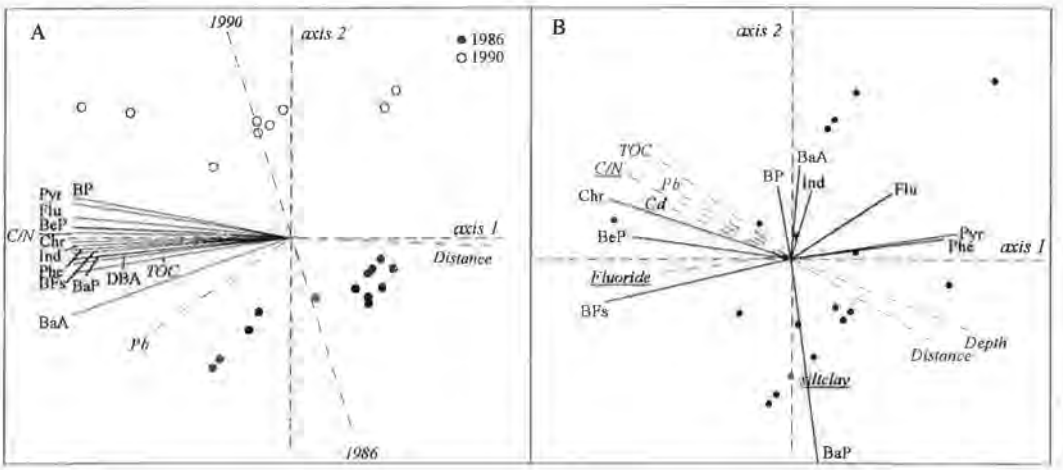

Figure 3: RDAs for Sunndal. (A) variable-centred plot with most concordant environmental variables from forward selection. (B) double-centred plot for 1986 data featuring the most important variables. The explanatory variables consisted of water depth, distance from source, silt-clay content, TOC, C/N ratio, metals (Cd, Pb, Cu) and fluoride. Underlined variables indicate active variables selected by forward selection, other variables were entered in the diagram as passives variables.

Sauda

In ordinary RDA, lead (Pb) and distance were the top-ranked factors, explaining about 70 per cent of the variance (Table 5). The year classes 1976 and 1981 accounted for a minor but significant amount of the variance, and, collectively, the four most important variables accounted for 84 per cent of the variance. The sediment classes, which were entered to compensate for poor data regarding grain size and organic matter, were presumably rather unspecific, accounting only for small amounts of the variation. The plot from the analysis (Fig. 4A) was similar to the corresponding PCA plot. Lead and distance correlated with the first axis, while the year classes correlated with the second axis.

Table 5: Ranking of explanatory variables in redundancy analyses (RDA) of PAH distributions in Sauda. A. Ordinary RDA, focusing on PAH concentrations. B. Double-centred RDA, focusing on PAH profile patterns (1976, 1986, 1990). Asterisks indicate classes of the nominal variables' sediment type and year. Year was entered as a covariable in the double-centred analysis to remove variation due to differences between the years. #: explained variance after fitting covariables. TOC was not measured in 1981 so it was omitted from the ordinary RDA.

A. Ordinary RDA	**Exp**	**Added**	**B. Double-centred RDA**	**Exp**	**Added**
Pb	70	70	Distance	18	18
Distance	69	8	Cd	16	5
Depth	58	-	Depth	14	-
Mn	56	-	Mn	14	-
Cd	44	-	Pb	13	6
Mud *	33	-	Anoxic sed *	12	-
Anoxic sediment *	14	-	Mud *	10	-
Perturbed sediment *	13	-	TOC	10	9
1976 *	10	4	Perturbed sed *	3	-
1981 *	8	2			
1990 *	6	-			
1986 *	5	-			
Sandmixed mud *	1	-			
Total (all/selected)	87	84		44 (59)#	38 (51)#

The analysis of profile patterns (double-centred) comprised data from 1976, 1986 and 1990, so it included total organic carbon (TOC) among the environmental variables. The categorical variable year was entered as a covariable to cancel out systematic differences between the surveys. The best fitting variables were found to be distance, organic matter, lead (Pb) and cadmium (Cd), which (together) explained 38 per cent of the variance (Table 5). This accounted for more than 50 per cent of the residual variance after accommodating

the covariables. The plot shows that the first axis contrasted the lower molecular weight PAHs (phenanthrene, fluoranthene, pyrene) with the heavier PAHs on a distance-metal (Pb, Cd) gradient (Fig 4B). The medium-weight PAHs (chrysene, benzo(e)pyrene, benzo(a)pyrene) were most closely related to the metals and organic matter. The heaviest compounds (indenopyrene, benzoperylene) correlated weakly with the explanatory variables, although the third axis (not shown) indicated a slight correlation with cadmium and lead.

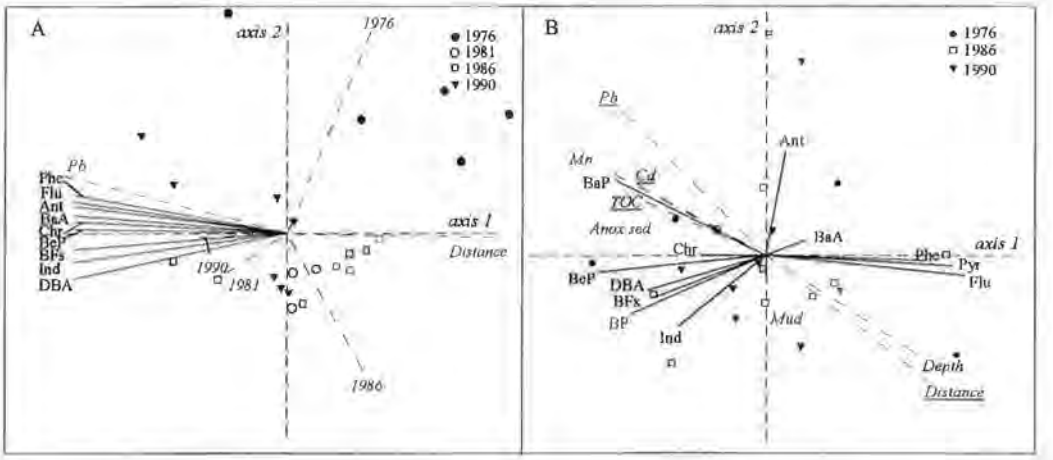

Figure 4: RDAs for Sauda. (A) variable-centred plot with the most concordant environmental variables from forward selection (all year classes shown). (B) double-centred plot for 1976, 1986 and 1990 data with the most important variables. Year entered as co-variable. Underlined variables indicate active variables selected by forward selection, other variables were entered in the diagram as passives variables.

DISCUSSION

PAH distribution patterns were generally similar in the four fjords investigated. Most of the variation was accounted for by the well-known fact that concentrations decrease with distance from effluent outfall points. This variation could also be described by examining variables linked to materials known to bind PAH, eg, carbon, or other contaminants originating from the same sources. The PAH profiles varied in the same manner in the fjords, as demonstrated by the recurrent molecular-weight correlated variable arrangement in the analyses. Næs & Oug [4] demonstrated that such patterns were a general attribute of PAH distribution in smelter-affected fjords.

The relationship between total PAH concentrations and the distance from the effluent outfalls can be expressed by the equation for the linear regression:

$$\log(PAH_{13}) = a + b*\log(\text{Distance})$$

which is equivalent to fitting a power function on the original data (natural logarithms):

$$PAH_{13} = e^{a} * \text{Distance}^{b}$$

The parameter b, the slope of the regression line, can be interpreted as a percentage change in PAH_{13} per unit change in distance. The high percentage of explained variance (80 to 90 per cent) indicates that a simple dispersion model can account for most of the PAH distribution in the fjords. Although other characteristics, such as TOC and metal contaminants, were better explanatory variables in some cases, the improvements were not always significant. Distance from source may therefore be sufficient for explaining the PAH variations, as long as total PAH is considered.

It appears that most PAHs settle out of the fjord waters in direct relation to distance from effluent outfalls. This result suggests that PAH distribution should be a function of their particle affinities and the hydrophysical processes transporting the particles. The intercorrelation with TOC and metal contaminants corroborates this assumption, as these describe the input of particulate materials to the sediments. The correlation with metals, in particular, indicates that the same type of particles and the same transport mechanisms may be involved in the dispersion of different effluent contaminants.

The high correlations with the TOC and C/N-ratio variables demonstrated in Sunndal indicate that the PAHs were associated with organic carbon or carbon-containing material. The method employed for measuring the carbon (elemental analyzer) implies that this material represents a mixture of detritus from plankton production, river-borne terrestrial plant materials and soot particles from the smelter effluents. It is not possible to assess the role of each of the components for PAH distribution, but the high correlation between C/N and total PAH suggests the importance of the soot particles, which are depleted in nitrogen. The situation is complicated, however, by the influx of river-borne material, which generally has a low nitrogen content, into the fjord close to the effluent discharge point. In other words, a distance gradient for sedimented terrestrial organic carbon could parallel PAH distribution patterns.

In Sauda, the metals were good explanatory parameters. The Sauda smelter effluent discharges contain trace metals originating from the manganese ore used for production. The result therefore suggests that most PAHs were associated with effluent particles. The organic material showed far less concordance. In this case, the measured organic material may largely represent naturally occurring carbon materials since the measurements were performed by loss on ignition. This method does not include soot particles from high temperature combustion processes.

The characteristic profile variations correlated with molecular weights may be attributed to compound-specific processes that occur in the receiving waters. Generally, molecular weight may by perceived as a descriptor of structure-property relationships [15]. The processes were not able to suppress source-specific profiles in comparisons between fjords [4], so they generally seem to be of minor importance. The present study did not show any clear environmental correlations with the specific patterns. However, it appeared that the lower molecular weight PAHs increased in direct proportion to distance, ie, the concentrations of light PAHs decreased somewhat less rapidly than the concentrations of the other compounds. Further, medium-weight PAHs appeared more closely associated with TOC and metal-containing material than the other compounds. The results suggest that water solubility and particle affinities may be of importance. Generally, heavier PAHs have the strongest affinity for particles.

Profiles from different surveys in the same fjord were generally separated in the analyses. These patterns most probably reflected source variations. Variations in the RDAs were best explained by the unspecific nominal variable for year, while depth and sediment characteristics for example, which might have differed between the surveys, were unimportant. Presumably, the variations represent minor changes in production processes or cleaning installations. For instance, according to information from the Årdal plant, the efficiency of the cleaning equipment (electrostatic precipitation) declined during the 1980s. This may have led to less efficient particle retention which may in turn explain the relative increase in heavier PAHs. The source characteristics can hardly be verified, however, because smelter discharge data are scarce and generally limited to total PAH levels. There is no indication that the differences are attributable to analytical performance considering the laboratory quality assurance programme and the consistency of the profile patterns.

The study shows that the environmental variables used at present, and which have been measured more or less routinely as part of the monitoring programmes, can account for the most obvious PAH distribution patterns in the fjords. However, the best fitting variables simply represented the deposition of PAH, expressed either as a function of distance from source or as an amount of PAH-bearing material. This supports the previous conclusion that PAHs are resistant to transformation during transport and incorporation into the surface sediments [4]. The compound-specific processes leading to minor, but

systematic and strikingly similar profile variations in the fjords could not be adequately explained by the variables. Further evaluations of these relationships would require better descriptions of sediment properties and PAH speciation, for instance, by analyzing variables such as the different particle types, carbon-containing materials and the dissolved/particulate state of the PAH in question.

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Paper V

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Source and Species-dependent Accumulation of Polycyclic Aromatic Hydrocarbons (PAHs) in Littoral Indicator Organisms from Norwegian Smelter-affected Marine Waters

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ABSTRACT

The accumulation responses of littoral indicator organisms to different sources of combustion-derived PAHs have been analysed using principal component analysis (PCA). Further, biota profiles were compared with sediment profiles. The data consisted of a total of 272 samples of Mytilus edulis, Modiolus modiolus, Littorina littorea *and* Patella vulgata *collected over a 20-year period from seven smelter-affected fjords. The results show that the indicator organisms reflect contaminant levels in the ambient environment and that their metabolic regulation was not strong enough to suppress source-specific signals from the smelter-generated PAHs. The indicators generally captured the same patterns as the sediments, demonstrating their ability to integrate over time as well as space. The differences in PAH patterns between the various indicator organisms were mainly a function of their depth and habitat preferences, and thus of ambient PAH composition. A simple dispersion model would suffice for illustrating the main distribution of total PAH concentration.* © *1998 Elsevier Science Ltd. All rights reserved*

Keywords: PAH, smelters,, littoral indicator, organisms, principal component analysis.

INTRODUCTION

Sedentary benthic organisms have been used for the past 20 years to monitor contaminant levels in aquatic systems (NAS, 1981; Knutzen and Sortland, 1982; Farrington *et al.*, 1983; Claisse, 1989; Martin and Richardson, 1991; Prest *et al.*, 1995). The most commonly used organisms are those able to accumulate contaminants without adverse effects. It is generally held that the contaminant levels in the organisms represent a time-integrated picture of concentrations in the ambient environment. The monitoring of contaminant

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levels in organisms is therefore used as an alternative to water sampling, which requires labour-intensive, costly programmes to identify the most important trends.

In Norway, various species of molluscs, crustaceans and macroalgae have been used to assess the levels of polycyclic aromatic hydrocarbons (PAHs) in fjords and coastal waters affected by aluminium and manganese alloy smelter effluent (Næs *et al.*, 1995). The PAHs originate from the seawater scrubbing of ventilation air and pot fumes and, in some cases, from the production of anodes. They enter the recipient waters through pipelines or settling basins separated from the sea by sand and gravel dykes. The most commonly used monitoring organisms are the blue mussel (*Mytilus edulis*) and the horse mussel (*Modiolus modiolus*), but the common periwinkle (*Littorina littorea*) and the common limpet (*Patella vulgata*) have also been employed (Næs *et al.*, 1995). Although the choice of monitoring organisms has been standardised insofar as possible among the various areas investigated, it is inevitably dependent on local availability and occurrence.

Numerous design criteria must be met when using organisms for monitoring purposes (Phillips and Segar, 1986; Martin and Richardson, 1991). Generally, monitoring programmes are established to describe spatial gradients and variation within and between areas of interest, and to register temporal changes. It is, therefore, a basic assumption that the contaminant levels do not vary due to the activity and metabolism of the organisms to a degree that skews the patterns at issue. Attention should therefore be devoted to sampling design and biotic factors, such as metabolic regulation and the ability of the indicator organisms to integrate the contaminant in question over time. The use of different indicator species could hamper comparison between areas because the species could have different accumulation characteristics. In particular, this may be important with respect to the accumulation of potentially carcinogenic compounds, and hence to the question of whether such organisms lend themselves for human consumption.

The present study is an analysis of data on PAHs found in monitoring organisms. Collected over a 20-year period, the data have been analysed to highlight trends and common patterns. Gradients in total concentrations and variations in the relative composition of compounds, ie, the PAH profile, were considered. In particular, the study focuses on comparing profiles between different species and geographical regions. Næs and Oug (1997*a*) demonstrated that there were consistent profile patterns in the sediments of the smelter-affected fjords, and that it was possible to relate those patterns to certain characteristics of the smelters as well as to type of production. Using principal component analysis (PCA), they were able to differentiate between various sources of combustion and detect early changes in input, which had not been observed during traditional processing of the data. The present study evaluates the possible occurrence of corresponding source-dependent patterns in monitoring organisms. Further, the organisms have been compared with the sediment profiles. Principal component analysis was the procedure employed. The use of PCA in evaluating environmental chemistry data, eg, for source recognition and transport pathways, has been encouraged by Zitko (1994) and Yunker *et al.* (1996), among others.

MATERIALS AND METHODS

Sampling

Data were collected and examined from seven fjords and coastal areas influenced by aluminium (Årdal, Sunndal, Lista, Vefsn) and manganese alloy smelters (Feda, Sauda,

Brevik) (Fig. 1). Most Norwegian smelters are located near the heads of fjords or on semi-enclosed coastal waters. A total of 272 PAH samples were included (Table 1), comprising the four species: the blue mussel (*Mytilus edulis*), horse mussel (*Modiolus modiolus*), common periwinkle (*Littorina littorea*) and limpet (*Patella vulgata*). Each sample consisted of a homogenate of a number of individuals, generally 50 for blue mussels, 100 for periwinkles, and 5–10 for horse mussels and limpets. Blue mussels, periwinkles and limpets were sampled intertidally, while the horse mussel samples were collected by scuba diving (10–15 m). If possible, specimens of about the same size were chosen. All the soft parts were used, except the adductor muscle of the mussels, which was removed.

Data on PAH levels in the sediments (0–1 cm or 0–2 cm layer) were used to make comparisons among some of the fjords. The sediment samples were obtained with a gravity corer or van Veen grab and were taken at depths of between 8 and 570 m. Reports on the distribution of PAHs in the fjord sediments have been published previously by Næs *et al.* (1995) and Næs and Oug (1997*a*).

Fig. 1. Smelter locations and sampling areas.

TABLE 1
Summary of Samplings of Indicator Organisms in the Different Fjord Areas. The Station and Sample Numbers Refer to Those Used in the Principal Component Analysis

Fjord area	*Organism*	*Sampling year*	*No. of stations*	*No. of samples*
Årdal	Modiolus	1983/1990/1992/1994	7	25
Sunndal	Mytilus	1987/1991	8	13
	Modiolus	1987/1991/1992	6	13
	Littorina	1987/1991	4	7
Lista	Littorina	1985/1986/1987/1988/1989/1990	3	20
	Patella	1980/1981/1982/1983/1984/1985/1986	4	17
Vefsn	Mytilus	1978/1984/1985/1989/1990/1991	11	38
	Modiolus	1978/1989/1990	7	16
Feda	Mytilus	1984	6	10
Sauda	Mytilus	1976/1980/1981/1986/1990/1991/1992/1993/1994	7	36
	Modiolus	1976/1980/1981/1986/1990/1991/1992	6	29
Brevik	Mytilus	1980/1983/1984/1985/1986/1990/1991/1992	5	48

Chemical analysis

PAHs were analysed using GC–FID or GC–MSD. A modified version of the method described by Grimmer and Bøhnke (1975) was used to extract and clean PAH from the biological samples. Internal standards (3,6-dimethyl phenanthrene and B,B-binaphthyl for FID, seven deuterated PAHs for MSD) were added to 10 to 20 g samples which were saponified by refluxing for 8 h with KOH/methanol. PAHs were extracted from the solution with cyclohexane. Details of sampling, work-up and analytical procedures have been reported previously by Næs *et al.* (1995).

Since this study emphasises trends and common patterns in data collected over a long period of time, attention also had to be devoted to analytical performance and the comparability of the different sets of data. The same extraction and clean-up method was used throughout the study and the GC was calibrated against certified standards. Further, analytical accuracy was tested against standard reference material. Finally, all analyses were performed by the same laboratory, which has also participated in international intercalibration exercises during the period in question.

PAH data

The number of quantified PAHs varied among the surveys and to some extent between samples. A standardised assortment consisting of the twelve most important compounds was chosen for the present numerical analyses. The compounds were (abbreviation in parentheses): phenanthrene (Phe), anthracene (Ant), fluoranthene (Flu), pyrene (Pyr), benz(a)anthracene (BaA), chrysene/triphenylene (Chr), benzo(b+j+k)fluoranthene (BFs), benzo(e)pyrene (BeP), benzo(a)pyrene (BaP), indeno(1,2,3-cd)pyrene (Ind), dibenz(a,c/a,h)anthracene (DBA) and benzo(ghi)perylene (BP). Table 2 gives a summary of the total concentrations represented by the sum of the selected compounds ($=PAH_{12}$). In most areas, there was a considerable difference between minimum and maximum values because the samples were collected along a distance gradient that ranged from the

TABLE 2
Sum PAH Concentrations of PAH_{12} ($\mu g\ kg^{-1}$ dry wt) in the Different Organisms

Fjord area	*Organism*	*Min.*	*Max.*	*Median*	*Average*	*No. of samples*
Årdal	Modiolus	381	487 150	7721	36 314	25
Sunndal	Mytilus	154	26 356	1203	3883	13
	Modoilus	212	124 467	1492	18 415	13
	Littorina	901	56 024	1129	12 450	7
Lista	Littorina[a]	197	271 510	2669	56 107	20
	Patella[b]	26	142 925	4142	19 197	17
Vefsn	Mytilus	2294	267 585	22 012	37 756	38
	Modiolus	674	102 941	2845	16 424	16
Feda	Mytilus	1401	17 650	7812	8328	10
Sauda	Mytilus	4	1 619 520	859	90 644	36
	Modiolus	6	480 516	1131	38 434	29
Brevik	Mytilus	51	14 581	1543	2833	48

[a]DBA not analysed.
[b]DBA and Ind not analysed.

immediate vicinity of the effluent outfall to areas that were virtually unaffected. There were very high concentrations near the effluent discharge points, which resulted in rather pronounced differences between median and average concentrations.

Numerical analyses

Gradients in total concentrations (PAH_{12}) were related to distance from effluent outfalls using linear regression. PAH values and distances were log-transformed prior to making calculations. A series of separate analyses was performed for species and surveys.

PAH profile patterns were evaluated by principal component analyses (PCA) for separate species and combined data for species, sediments and fjords. The analyses were performed by the 'double-centred' (variable- and sample-centred) form on log-transformed data, an option comparable to using standardised PCA and eliminating the effects of scale. Preliminary PCAs were performed prior to the final analyses to unveil possible chemical analysis errors and identify outliers. In the case of missing individual PAH values (e.g. below the detection limit), the compounds or samples in question had to be omitted from the analysis.

The PCAs were performed using CANOCO version 3.10 software (ter Braak, 1988). Plots were made using CanoDraw 3.0 (Smilauer, 1992). PCA depicts the patterns and trends by arranging the PAHs (variables) and samples along axes (principal components) which are assumed to represent basic factors or relationships. The first axis (PC1) illustrates the most prominent trend, while the successive axes (PC2, PC3, etc.) represent secondary, tertiary, etc., trends in decreasing order of importance. The amount of variance explained by each axis, stated as a percentage of the total variance, may be taken as a measure of the importance of the axis. The results are depicted in combined plots (biplots) of variables and samples with the variables represented by arrows running from the origin of the plot to the position of the variable scores (loadings). The arrows point in the direction of increasing variable concentrations, while the lengths of the arrows represent the strength of the increases.

RESULTS

Gradients in total PAH concentrations

In all fjords, the PAH concentrations in the indicator organisms decreased proportional to the distance from the effluent outfalls. Generally, it was possible to represent the relationship by a straight line on log–log transformed data. Table 3 shows statistics of the regression lines for a selection of data sets. Generally, the regressions explained 80 to 95% of the variability in the PAH concentrations, illustrated by the values of the r^2 statistic. In most cases, the regressions were found to be significant at the 1% level. The estimated slopes of the lines were fairly equal, except that Lista (periwinkles) showed a steeper slope (more rapidly decreasing concentrations) and Feda (blue mussels) showed a less severe slope. There were no systematic differences between the species.

PAH profiles in the various species

Blue mussels

Blue mussels had been collected in most of the fjords and represented the largest and most comprehensive data set. A series of PCAs examined temporal profile changes within fjords, profile patterns between fjords and profile patterns related to the type of production processes used by the various smelters. The main patterns of the analyses were generally similar (Fig. 2). The samples were largely configured according to constituent data sets, and the variables (PAHs) were displayed in basically congruent arrangements. The clustering of the samples indicates that each sample set represents a specific PAH profile. The arrangement of the variables may be related to the molecular weights of the PAH

TABLE 3

Statistics for Fitting a Linear Log–Log Regression Model of Concentrations of PAH_{12} (wet wt, dry wt was not available for all data sets) against Distance from Source for Different Indicator Organisms from Selected Surveys and Fjord Areas

Fjord area	*Organism–sampling year*	*Distance from source (km)*	*Negative slope*	r^2	*n*	*p (model)*
Årdal	Modiolus–1983	0.3–14	1.29	0.97	7	<0.0001
Årdal	Modiolus–1992	0.3–14	0.80	0.99	5	0.0003
Årdal	Modiolus–1994	0.3–14	0.84	0.96	6	0.0006
Sunndal	Mytilus–1987	6.5–50	1.49	0.81	6	0.01
Sunndal	Mytilus–1991	1.5–50	0.83	0.94	7	0.0003
Sunndal	Littorina–1987	0.3–50	0.78	0.99	4	0.005
Feda	Mytilus–1984	0.2–16	0.39	0.60	10	0.008
Sauda	Mytilus–1981	2.5–28	1.51	0.996	4	0.002
Sauda	Mytilus–1986	0.5–38	1.16	0.90	6	0.004
Sauda	Mytilus–1990	0.5–38	1.00	0.88	6	0.005
Sauda	Modiolus–1976	0.5–15	1.00	0.96	4	0.02
Sauda	Modiolus–1986	0.5–28	1.18	0.94	5	0.006
Lista	Littorina–1995	0.5–10	2.21	0.94	12	<0.0001
Vefsn	Modiolus–1978	1.5–24	1.13	0.84	7	0.004

Distance from source shows the range among sample locations.
r^2 is the coefficient of determination. *n* denotes number of samples and *p* is the probability of no relationship.

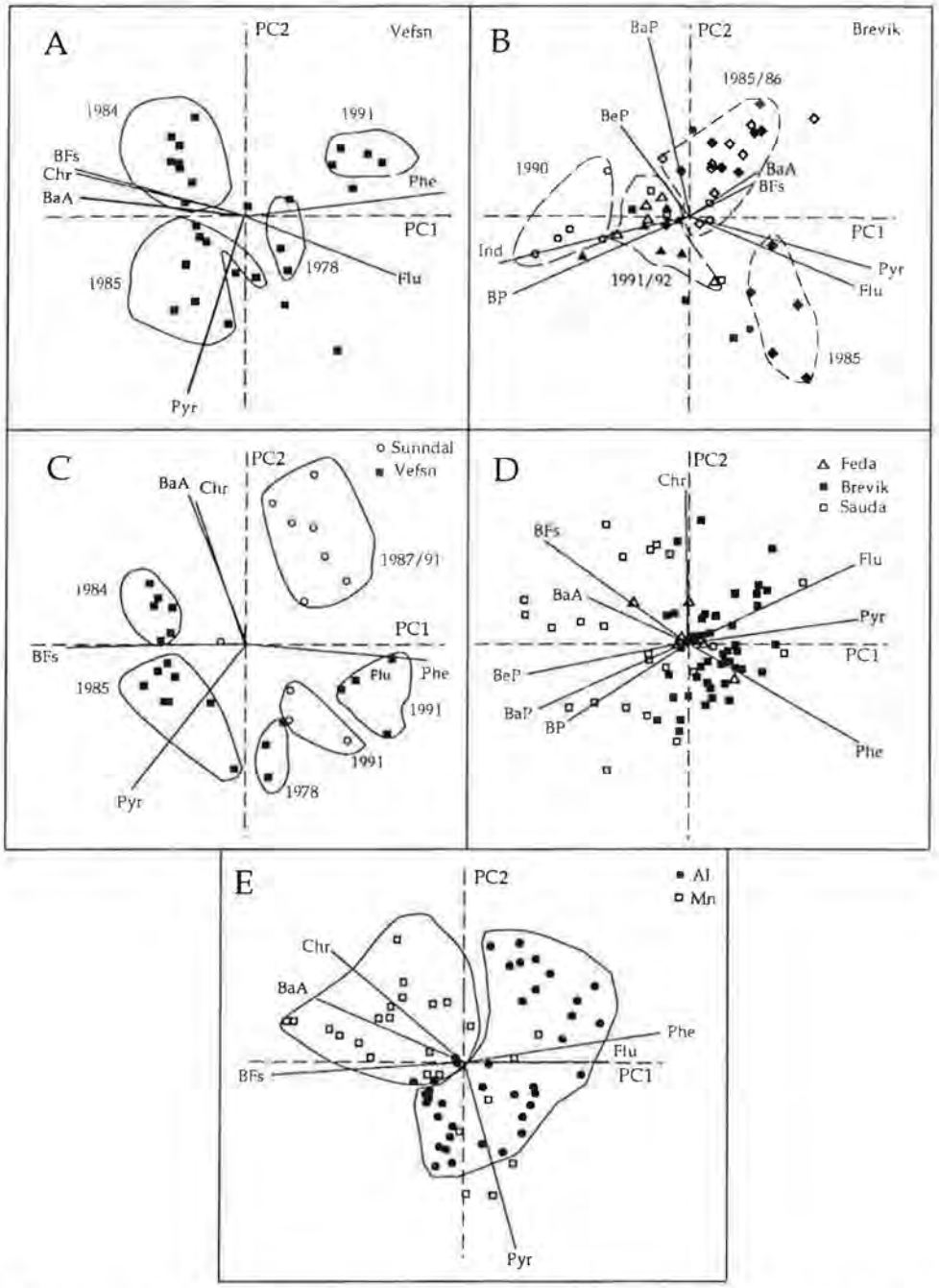

Fig. 2. PCA ordination diagrams of PAHs in the blue mussel. (A): Vefsn, variance explained PC1;PC2 = 83;9%. (B): Brevik, PC1;PC2 = 44;20%. Different symbols are used for separate surveys. (C): Sunndal and Vefsn combined (Al production), PC1;PC2 = 70;15%. (D): Feda, Brevik and Sauda combined (Mn alloy production), PC1;PC2 = 53;18%. (E): Al- vs Mn-alloy production, PC1;PC2 = 70;14%.

compounds. The first axis (PC1) generally contrasted the PAHs with the greatest differences in molecular weights, while the second axis (PC2) correlated with compounds having intermediate molecular weights. This arrangement implies that compounds with similar molecular weights were correlated and that variation among PAHs with lower molecular weights were inversely proportional to the compounds with higher molecular weights.

Temporal profile changes were examined in Vefsn and Brevik where a number of surveys had been conducted between 1978 and 1992. In Vefsn (Al production), most of the variance was explained by PC1 (Fig. 2(A)). The different surveys were clearly distinguishable, particularly 1984, 1985 and 1991. It appears as though the 1991 samples had a relative dominance of lighter molecular weight compounds (phenanthrene, fluoranthene), while the 1985 samples had a relative dominance of pyrene. From 1978 to 1985, there seemed to be a progression towards heavier compounds, although that trend was reversed in 1991. One of the surveys (1989) was, however, omitted altogether. The data appeared to deviate radically, actually suppressing the patterns produced by the other samples when included in the analysis. Corresponding deviations were observed in the sedimentary PAH profiles from the same year in Vefsn (Næs and Oug, 1997).

In Brevik (Mn alloy), the variance was more equally distributed among the axes (Fig. 2(B)). The samples were less distinctly allocated according to surveys, although the 1985/1986, 1990 and 1991/1992 surveys were fairly readily distinguishable. A subgroup of the 1985 samples stood out somewhat as it was relatively rich in fluoranthene and pyrene. The analysis indicates that the temporal trends were rather weak, and that there might be other important sources of variation in the area. Brevik is one of the most heavily industrialised areas in Norway, and there are additional PAH sources from petrochemical industry in the area.

Profile patterns between fjords were evaluated for two fjords affected by aluminium production (Vefsn and Sunndal), and three fjords affected by Mn alloy production (Brevik, Feda and Sauda). In the Al-fjords, the samples were principally grouped according to the surveys (Fig. 2(C)). Except for the 1991 samples from Vefsn, the fjords differed on PC1, indicating a relative preponderance of light-weight compounds (phenanthrene, fluoranthene) in Sunndal. In the Mn alloy fjords, Brevik and Sauda differed, while the Feda samples fell in-between. Brevik showed a relative preponderance of lighter molecular weight PAHs (Fig. 2(D)). The separate surveys were not differentiated and have not been indicated on the diagram.

Data from all fjords were combined to examine the profiles in relation to smelter production type. Most samples were allocated according to production type (Fig. 2(E)). It appeared (for the present selection of variables) that the Al fjord samples were relatively rich in lighter compounds, particularly phenanthrene and fluoranthene, while the Mn alloy fjord samples were rich in heavier compounds such as chrysene and benzofluoranthenes.

Horse mussels

The analyses of horse mussel data revealed very much the same patterns as those found in blue mussel data. The separate surveys were distinguishable both within fjords (Fig. 3(A)) and in comparisons between fjords (Fig. 3(B)). It appears that the differences between the surveys were stronger than the general differences between the fjords. When comparing Al and Mn alloy affected fjords, the two types of production were easily distinguishable (Fig. 3(C)).

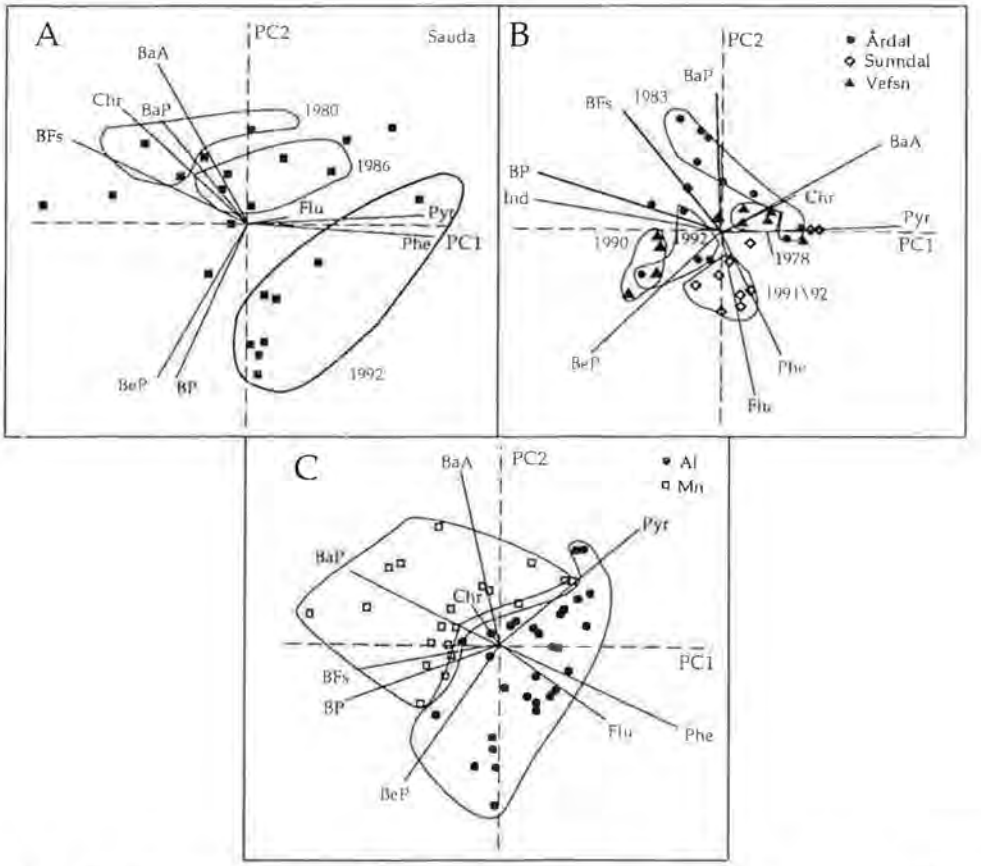

Fig. 3. PCA ordination diagrams of PAHs in the horse mussel. (A): Sauda, variance explained PC1;PC2 = 44;24%. (B): Årdal, Sunndal and Vefsn combined (Al production), PC1;PC2 = 46;22%. (C): Al- vs Mn-alloy production, PC1;PC2 = 44;25%.

Profile variations among species

Two or three indicator organisms were sampled simultaneously in some fjords (Table 1). These data were analysed for the various fjords in order to evaluate possible species-dependent profile variations (Fig. 4). Blue mussels and horse mussels were compared in Vefsn (Fig. 4(A)), while mussels and periwinkles were compared in Sunndal (Fig. 4(B)). Horse mussels differed clearly from the other species, showing a relative affinity for the heavier PAHs. The blue mussel and the periwinkle overlapped and appeared to have rather similar profiles (Fig. 4(B)). Notwithstanding the difference between the mussel species, it was possible to distinguish between the sample surveys (Fig. 4(A)).

The common periwinkle and the common limpet were compared at Lista (Fig. 4(C)). The monitoring programme was initially based on the limpet (1980–1986), but later changed to the periwinkle (1985–1990) as the limpet population declined. No particularly clear patterns appeared in the analysis, although the periwinkle tended to have a heavier PAH profile (Fig. 4(C)).

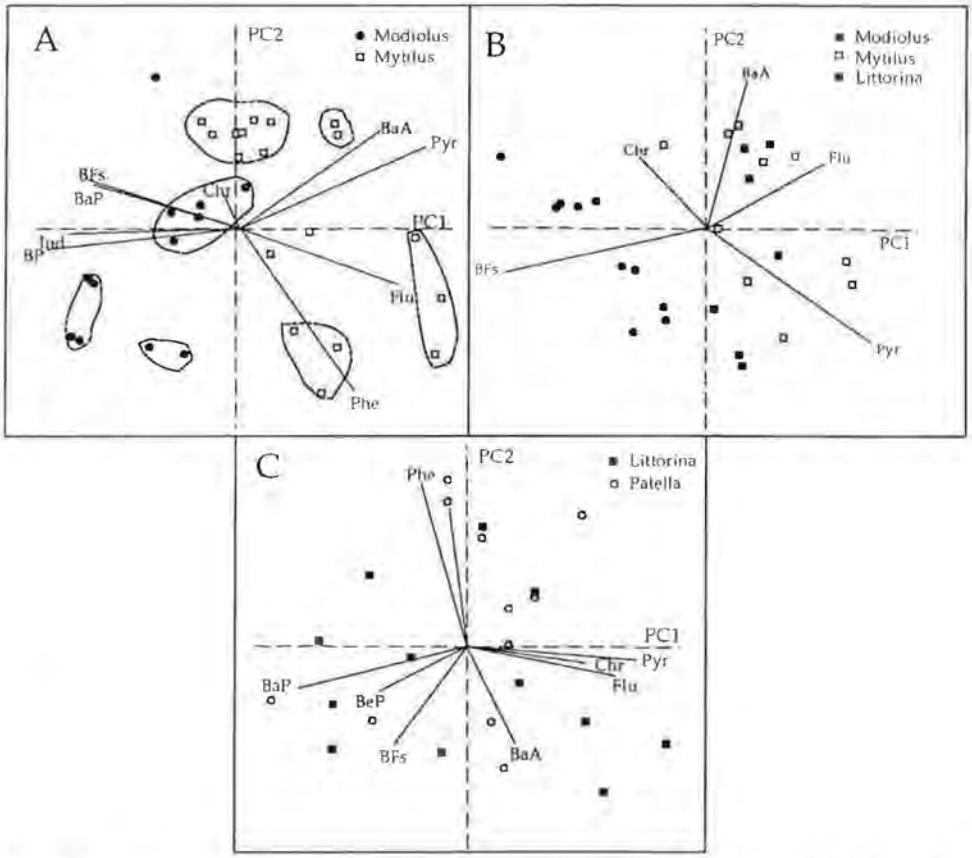

Fig. 4. PCA ordination diagrams of PAHs, species comparisons. (A): Blue mussels and horse mussels (Vefsn). Different sampling years are encircled. Variance explained PC1;PC2 = 63;21%. (B): Blue mussels, horse mussels and periwinkles (Sunndal), PC1;PC2 = 62;21%. (C): Periwinkles and limpets (Lista), PC1;PC2 = 36;22%.

Comparison between indicator organisms and sediments

PAHs were also monitored in the bottom sediments of most of the fjords. Compared with the indicator organisms, the sediments were distinguished by a relative dominance of heavier PAHs (Fig. 5). The distinction appears quite clearly in Årdal where horse mussels and sediments were compared (Fig. 5(A)). However, it was somewhat less clear in Vefsn where blue mussels, horse mussels and sediments were compared (Fig. 5(B)). This analysis indicated that blue mussels and horse mussels were more dissimilar than the horse mussels and sediments being separated on PC1 and PC2, respectively. In Sunndal, the mussel species and periwinkles were compared with the sediments (Fig. 5(C)). The greater distinction was between the sediments and the organisms (PC1), while the horse mussel was separated from the other species as the second most significant pattern (PC2).

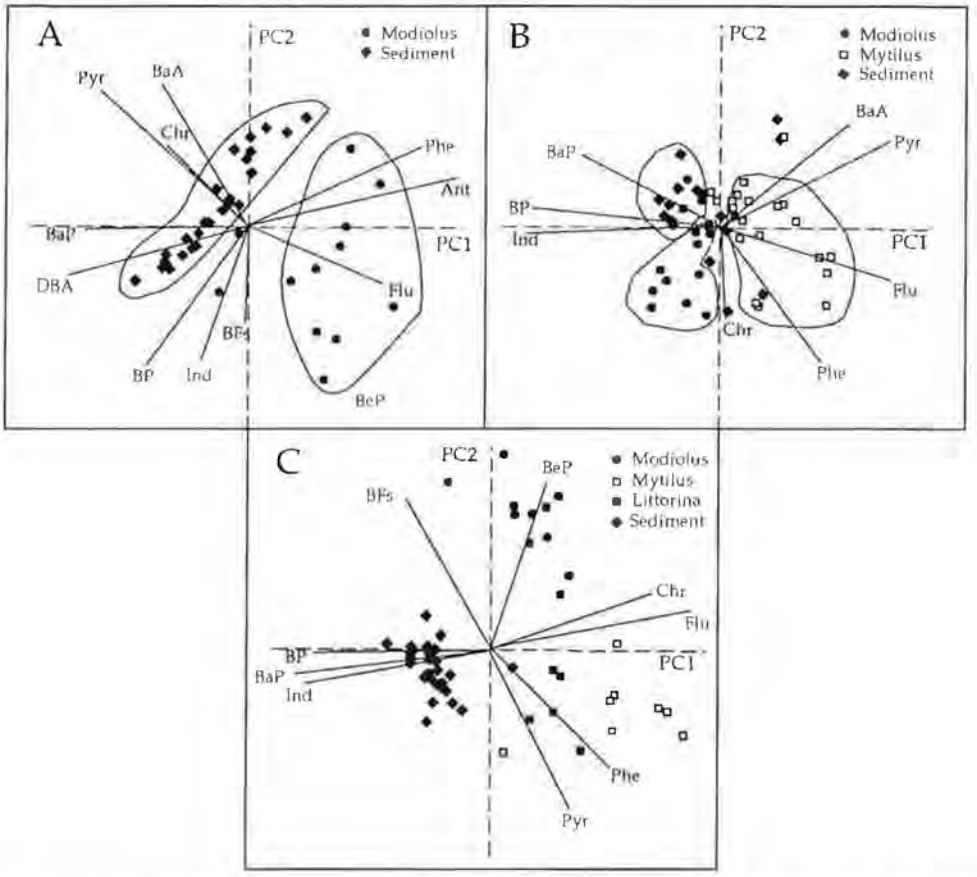

Fig. 5. PCA ordination diagrams comparing PAH in monitoring organisms and fjord sediments. (A): Horse mussels and sediments from Årdal. Variance explained PC1;PC2 = 52;24%. (B): Horse mussels, blue mussels and sediments from Vefsn, PC1;PC2 = 54;17%. (C): Horse mussels, blue mussels, periwinkles and sediments from Sunndal, PC1;PC2 = 64;16%.

DISCUSSION

This study demonstrated the presence of consistent PAH profile patterns in indicator organisms, and showed that the patterns were related to individual surveys, the type of smelter production and the species involved. To a certain extent, the fjords could also be distinguished by the different profiles. The marked tendency towards consistent clustering of samples according to surveys, which was particularly evident in the mussels from aluminium-influenced fjords, suggests that the characteristics of the profiles are discernible all along the PAH concentration gradients in the fjords. This, together with the clear separation between Al and Mn alloy production, suggests that the profiles represent source characteristics and that very little modification of the source signals takes place along the concentration gradient. There is no indication that the profile patterns could be artefacts from analytical performance. This conclusion is based both on the analytical

QA/QC and on the interpretability of the patterns in view of production processes and knowledge about the effluent PAH composition (see below).

The profile patterns generally correspond to the patterns observed in the fjord sediments. Næs and Oug (1997*a*) demonstrated that most fjords have a characteristic PAH profile, or 'fingerprint', which was discernible even a good distance from the sources and down to concentrations at three to four times background levels. The fingerprints were interpreted to reflect the industrial processes and the cleaning arrangements in the different plants. Al smelters have various devices for cleaning emissions, while Mn alloy smelters have similar cleaning systems, leading to similar routes of PAH entry into recipient waters. It also seemed that temporal profile changes in the fjord sediments could be attributed to variations in the efficiency of the cleaning devices. The consistency of the source-specific signals indicated that the PAHs were not subject to specific transformation reactions in the receiving waters. Presumably, most PAHs are bound to oxides or black carbon-containing (soot) particles from the smelter processes (Næs and Oug, 1997*a*,*b*; Næs *et al.*, unpublished). This particle association is known to render the PAHs resistant to degradation and repartitioning (Butler and Crossley, 1981; McGroddy and Farrington, 1995; Gustafsson *et al.*, 1997). Direct verification of the source characteristics can hardly be made, however, because discharge data for the smelters are scarce and mainly represent total PAH levels (Næs and Oug, 1997).

The general profile differences between the species may be associated with their depth and habitat preferences. The blue mussel, periwinkle and limpet all live on the rocky shore, while the horse mussel is subtidal and generally lives partly buried in sandy or soft sediment substrates. The three intertidal species showed rather similar profiles while the horse mussel accumulated relatively more of the higher molecular weight PAHs. Comparing the species with the fjord sediments, it was found that the sediments contained even more of the heavier PAHs. The organisms and sediments therefore seemed to represent a gradient with the amount of heavier PAHs increasing in direct proportion to depth. Presumably, this pattern reflected a trend in ambient PAH composition. Recent investigations have shown that lighter molecular weight PAHs have a strong propensity to be in a dissolved state (Næs *et al.*, unpublished) and may be transported in the brackish surface waters in the fjords. High molecular weight compounds are almost always associated with particles which tend to be deposited in the deeper and hydrodynamically quieter area of the fjords.

All the indicator species are microphagous feeders which can ingest PAHs with food particles. Periwinkles and limpets scrape particles and microalgae from the rock with their horny rasping tongues, while mussels collect food particles from the water by filter feeding. The PAH profiles in periwinkles, limpets and the blue mussels were similar. Although they show different profiles, the blue mussel and the horse mussel have similar particle preferences (Bayne *et al.*, 1977; Møhlenberg and Riisgård, 1978; Vahl, 1972). Consequently, for the present species, the feeding mode does not seem significant for tissue PAH composition.

Although organisms may metabolise PAHs, their metabolic capacity depends on specific enzymatic systems which differ among groups of aquatic organisms (cf. Cerniglia and Heitkamp, 1989). The main component in the breakdown of PAHs is the cytochrome P450 system. Although molluscs in general appear to have low cytochrome P450 mixed-function oxydase enzymes (Stegeman and Lech, 1991), there is support for mussels having an active capability to metabolise PAHs (Gilewicz *et al.*, 1984; Livingstone and Farrar,

1985; Stegeman, 1985; Broman *et al.*, 1990; Michel *et al.*, 1994). The consistent clustering of samples according to surveys indicates that significant PAH transformation does not take place in the mussels. It may be assumed that most of the PAHs are unavailable for metabolism because of strong particle affinities.

It was possible to relate total PAH concentrations in the organisms to distance using linear regression on log–log transformed data. This is equivalent to fitting a power function on the original data:

$$\mathrm{PAH} = e^{a} \times D^{b}$$

where D = distance from source, e = basis of the natural logarithm, a = intercept and b = slope of the regression line. In this model, the slope of the regression line can be interpreted as a percentage change in PAH concentrations per unit change in distance. The results indicate that the model is generally applicable for PAH concentrations in the indicator organisms. It may therefore be assumed that the organisms are exposed to PAH in direct relation to their distance from the effluent outfalls. The same model can be applied for PAH concentrations in the sediments of the smelter-affected fjords (Næs and Oug, 1997*b*). This simple and apparently general relationship supports the assumption that explanations of PAH concentrations in the smelter-affected fjords should take account of particle affinities and hydrophysical particle transport processes (Næs and Oug, 1997*b*). The steepest slope of the regression was found at Lista which is an open coastal environment, while the smallest slope was found in Feda which is a narrow fjord. The other fjords are comparable large (15–50 km long) with roughly the similar topography and freshwater input at the head.

This study has shown that the monitoring organisms reflect ambient PAH levels and detect specific profile patterns related to the source in the smelter-affected fjords. The metabolic transformations in the organisms appeared to be of relatively little importance, and were not strong enough to suppress source-specific signals. The organisms generally displayed the same trends as were found in the sediments, indicating their ability to integrate time and space dependent variability. These properties are prerequisites for the use of organisms in contaminant monitoring (Phillips and Segar, 1986). Further, the fact that simple dispersion models may be used to explain the main distribution of the PAHs allows for simple designs and statistical testing in monitoring programmes.

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Paper VI

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The relationship between soft bottom macrofauna and polycyclic aromatic hydrocarbons (PAH) from smelter discharge in Norwegian fjords and coastal waters

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ABSTRACT: Data from monitoring surveys were used to test the responses of soft bottom fauna in Norwegian fjords and coastal waters affected by effluents from aluminium and manganese alloy smelters to effluent discharges. Generally, PAHs have been considered the main threat to the biota in the receiving waters. Data from a total of 57 quantitative stations, of which 30 had been simultaneously sampled for sedimentary PAHs, were analysed. Canonical Correspondence Analysis (CCA) was used to evaluate species patterns in relation to sediment PAH concentrations and natural environmental variables such as depth, sediment characteristics and freshwater inflow. The PAHs varied from very high concentrations (760,000 ng g^{-1} total PAH) at the most impacted sites to background levels

in reference areas. Despite previous reports of little to no effects of the effluent discharges, the analyses identified significant species changes along PAH gradients, which could be associated with feeding modes. In particular, a number of carnivorous polychaetes had their distribution optima in the most affected areas. Based on species statistics calculated by CCA, response functions were fitted for the species on PAH and used to establish a generalised expression of community composition in relation to PAH concentrations. At low PAH levels, the carnivores represented 15 to 20 per cent of the specimens in the communities, but the proportion began to increase from about 10,000 ng g^{-1} and exceeded 50 per cent at 100,000 ng g^{-1} total PAH. The faunal changes were most evident in shallow waters, possibly reflecting the effects of dissolved PAHs to which the species may react more directly than they do to sedimentary particle-associated PAHs.

KEY WORDS: Soft bottom fauna · Canonical Correspondence Analysis · PAH · environmental effects · species response functions · trophic structure

INTRODUCTION

The production of primary aluminium and manganese alloys is an important industrial activity in Norway, and Norwegian production accounts for a significant share of overall international capacity. A number of smelters are located along the west coast, mainly in narrow fjords or semi-enclosed coastal waters. Since the 1970s, environmental studies have been conducted in waters which receive waste discharge from the smelters. Waste effluents contain PAHs, fluoride, soot particles from combustion processes and, in some cases, metals. PAHs have received the most attention as they have generally been considered the main threat to the receiving waters. In monitoring studies, very high PAH concentrations have been documented in

sediments and indicator organisms near effluent discharge areas (Næs et al. 1995). The concentrations decrease rapidly with distance from source, although elevated levels have been registered in the fjords several tens of kilometres away. However, the ecological effects appear to be small, except within restricted zones in the immediate vicinity of the effluent outfalls. Generally, it has been difficult to assess the role played by PAH in terms of the functioning of organisms in the receiving waters (Knutzen 1995).

In two of the fjords (Årdal and Sunndal), sediment PAH concentrations in excess of 700,000 ng g^{-1} dry weight, that is, more than 1000 times background levels, have been observed (Næs et al. 1995). In Årdal, the seabed was lifeless in the most impacted area, but a species-rich fauna was present in sediments containing 100,000 to 400,000 ng g^{-1} PAH at rather short distances (< 600 m) from the lifeless zones (Knutzen 1995). In most fjords, no correlation has been found between faunal diversity and PAH levels. Knutzen (1995) actually suggested that the physical damage done to the habitat, rather than the PAH level, was the determining factor for benthic fauna in the most impacted areas.

The apparently limited ecological effects contrast with the toxicity of PAH reported from laboratory experiments and field studies conducted elsewhere (see e.g. compilations by Long 1992; Long et al. 1995). Knutzen (1995) suggested that the discrepancy might be related to the particle affinities of the PAHs. The majority of smelter effluent PAHs is associated with soot particles, which are presumably less available to organisms than PAHs in a dissolved state. Similar conclusions were reached by Paine et al. (1996), as they were unable to document toxicity from heavily PAH contaminated sediments near a Canadian aluminium smelter. On the other hand, it is also possible that certain specific biological responses have been overlooked. The ecological effects in the Norwegian fjords were mainly assessed on the basis of faunal diversity at selected localities. Various recent studies (e.g. Olsgard & Gray 1995) have shown that measures of

diversity are not sufficiently sensitive to describe the full range of the effects of contaminants on soft bottom fauna.

The present study represents an integrated evaluation of soft bottom community data from various Norwegian fjords and surveys with particular emphasis on species patterns as related to PAHs and natural environmental factors. Different species' feeding modes were incorporated into the analyses to assess the relationships in view of biological features of the organisms. The analyses were performed using Canonical Correspondence Analysis (CCA), a multivariate direct gradient technique which relates species patterns to environmental variables (ter Braak 1986, Jongman et al. 1987, ter Braak & Verdonschot 1995). PAH was treated as an environmental variable on a par with depth, sediment characteristics and freshwater influence. The main aims of the study were: (a) to describe the species assemblages along gradients based on PAHs and natural environmental factors, (b) to examine species distribution related to PAH gradients, and (c) to evaluate trophic community structure along PAH gradients. Data from fjords and coastal waters which do not receive industrial effluents were included in the analyses as a reference to provide environmental gradients in unimpacted systems.

MATERIAL AND METHODS

Fjords and sampling sites. Data were collected (Fig. 1) from four fjords which receive effluents from Al and Mn alloy smelters (Årdal, Feda, Sauda, Sunndal). A total of 30 stations were sampled simultaneously for macrofauna and sedimentary PAH, covering water depths from 28 to 375 m and spanning distances from immediate proximity to more than 10 km away from the effluent outfall points (Table 1). At the most contaminated sites, the PAH levels exceeded 100,000 ng g^{-1}, far above the defined limit for very

strong contamination (sum PAH >20,000 ng g^{-1}) set by Norwegian sediment quality criteria (Rygg & Thélin 1993). Values decreased with distance from the outfall points and approached background levels in some of the fjords. All smelters are located near the head of the fjords and are close to major river outlets. Details of the occurrence and distribution patterns of PAHs in the fjords are given by Næs et al. (1995) and Næs & Oug (1997a, b).

Data gathered from five fjords and coastal areas (Topdal, Kvinnherad, Kårstø, Surnadal, Tresfjord) were selected to depict gradients in fjords unaffected by smelter effluents (Fig. 1, Table 1). Altogether, data were entered for 27 stations, representing, insofar as possible, the same depths and sediment types as in the smelter affected fjords. In particular, fjords were selected on the basis of the influence exerted on them by freshwater input in order to describe estuarine gradients. In the smelter-affected fjords, estuarine gradients may confound species patterns related to PAH contamination due to the smelter locations.

Fig. 1. Sampling sites in smelter-affected fjords (filled) and reference areas (hatched).

Table 1. Summary data for macrofaunal stations in Norwegian fjords affected by smelter effluents and in reference fjords not influenced by industrial effluents. The type of smelter (aluminium, manganese alloy) is indicated. Distances from smelter effluent points, distances from major river outlets and PAH levels at the stations are shown. PAH_{13} = sum of PAHs from phenanthrene through benzo(ghi)perylene (see text). * = given background value.

Fjord area	Sampling year	No. of stations	Depth	Distance from smelter	Distance from river	PAH_{13}
			m	km	km	$\mu g\ g^{-1}$
Årdal (Al)	1983	3	90-152	0.8-3.5	0.4-3.4	261-760
	1989	4	110-165	1.0-3.5	0.7-3.4	96-298
Feda (Mn alloy)	1984	5	28-88	0.3-2.8	0.4-2.8	7-144
Sauda (Mn alloy)	1976	1	180	2.2	2.2	1.9
	1981	5	40-375	0.4-9.5	0.5->5	1.9-143
Sunndal (Al)	1986	8	60-328	0.8->10	1.6->5	1.1-123
	1995	4	111-113	1.1->10	1.7->5	0.5-17
Topdal	1983	8	33-198	3.0->10	0.4->5	0.5*
Kvinnherad	1990	5	31-186	>10	0.5->5	0.5*
Kårstø	1983	5	35-134	>10	0.5->5	0.5*
Surnadal	1991	5	36-210	>10	0.7->5	0.5*
Tresfjord	1990	4	45-93	>10	1.2->5	0.5*

Sampling and analysis. The faunal samples were taken using 0.1 m^2 Petersen, Day or van Veen grabs. At each station, 2 to 8, usually 4 to 5, replicate samples were taken, sieved on 1 mm mesh sieves and preserved in 4% buffered formaldehyde solution. Collected specimens were identified to the species level in the majority of animal groups examined. The species were classified into four broad categories according to their general feeding modes (carnivores, surface deposit feeders, subsurface deposit feeders,

suspension feeders). Most carnivores are vagile forms which can actively move about, whereas the other groups comprise sessile forms having very low mobility.

Surface sediment samples (0-1 cm) for PAH-analysis were usually obtained with a 5 cm internal diameter gravity corer, but subsamples were occasionally taken from the grabs. PAHs were analysed using GC-FID or GC-MSD on samples Soxhlet extracted in cyclohexane. The internal standards were 3,6-dimethylphenanthrene and B,B-binaphthyl for analysis with FID and seven deuterated PAHs for analysis with MSD. Details of the sampling and analytical methods, along with an overview of the distribution of the PAHs in the receiving waters, are presented by Næs et al. (1995).

Measurements were taken of the fine fraction and sedimentary organic matter in some of the fjords. The fine fraction (silt-clay) of surface sediments (0-1 cm) was determined by wet sieving. Carbon and nitrogen were determined according to the measurement routine for total organic carbon (TOC), using a CHN analyser after the removal of inorganic carbonates. Visual characterisation of the sediment has been given for all stations.

Environmental variables. Environmental variables for the numerical analyses were designated for topography (water depth, distance from source, distance from river inflow points) and seabed conditions, as well as PAH and sediment characteristics. The distances to effluent outfalls and freshwater inflow points were used to describe spatial faunal patterns in the fjords. The distance from river inflow points represents the possible influence of freshwater and terrestrial river-borne materials, and was applied in order to distinguish natural fjord gradients from smelter effluent gradients. Seabed conditions were defined as a four-class categorical variable based on the visual characterisation of the sediments (effluent-impacted sediment, organically enriched sediment, sandmixed mud and

mud). Topographical variables and seabed conditions were determined for all stations.

The PAHs were described using the total concentration (PAH_{13}) of 13 routinely measured compounds (phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(bjk)fluoranthenes, benzo(e)pyrene, benzo(a)pyrene, perylene, indenopyrene, dibenzanthracene, benzo(ghi)perylene). Stations in the reference fjords were given an estimated background value (PAH_{13} = 500 ng g^{-1}), so that PAH values were entered for all stations.

Numerical analyses. Canonical Correspondence Analysis (CCA) provides a simultaneous ordination of species, samples and environmental variables. This technique arranges species and samples along axes which are constrained to be linear combinations of the environmental variables. Accordingly, the faunal patterns displayed can be related to the variables (ter Braak 1986, Jongman et al. 1987, ter Braak & Verdonschot 1995). The importance of the relationships is reflected by the amount of species variance (referred to as 'inertia') that can be extracted on the axes. This is measured by the eigenvalues of the axes, which can be compared with the total variance in the species data ('total inertia') for the purpose of quantifying how much of the variation for which the variables may account. The total inertia is calculated as the sum of all unconstrained eigenvalues in Correspondence Analysis (CA). Diagrams are presented as combined plots (biplots) of species and environmental variables. Species are marked by points, representing their most preferred position (distribution optimum) in relation to the variables. The variables are indicated by arrows (quantitative variables) pointing in the direction of maximum value of the variable. Long arrows indicate strong trends, and the angle between pairs of arrows approximates the correlation between the respective variables. Categorical variables are designated by points for the variable classes.

The analyses were carried out with the program CANOCO, version 3.10 (ter Braak 1988, 1990), while diagrams were constructed with the drawing program CanoDraw 3.0 (Smilauer 1992). Three analyses were carried out. In the first analysis, general faunal gradients and responses to PAH contamination in the fjords were illustrated using data from all stations. Data on depth, distance from smelter, distance from river inflow points and PAH were entered as quantitative variables, while the bottom sediments were represented by the categorical variable for seabed conditions. In the second analysis, faunal gradients were assessed from two fjords (Sunndal and Surnadal), where quantitative sediment variables had also been obtained. Prior to analysis, topographical variables and PAH concentrations were log-transformed to make adjustment for skewed distributions. Species data for replicate samples were pooled for each station, recalculated as densities (ind m^{-2}), and square-root transformed to down-weight high densities. The procedure of 'forward selection' and Monte Carlo permutation tests were used to rank the variables in order of importance, and to identify a subset of significant variables maximally related to species distribution. In the plots, species' feeding modes were entered in order to illustrate functional relationships on PAH and environmental gradients.

The third analysis focused particularly on species distribution related to PAH. This analysis was carried out with PAH as the only environmental variable, implying that the first CCA axis turns into a linear gradient for PAH on which species are arranged in order. The variables of depth and distance from river outlet points were entered as covariables in order to eliminate variation related to these factors. For each species, statistics were calculated to determine the distribution centre on the gradient (optimum) and the spread along the gradient (tolerance). These statistics were utilised in a subsequent analytical step to fit response curves for the species on the

PAH gradient. The curves were fitted according to the Gaussian function, assuming unimodal species distributions

$$Y_i = c_i \exp(-0.5(x-u_i)^2/t_i^2)$$

where Y_i is the estimated density of species i for the value (x) on the gradient (PAH concentrations), c_i the maximum density, u_i the optimum and t_i the tolerance ('standard deviation'). The maximum density c_i was estimated by taking the total species weight (the sum of observed densities) divided by the parameter N_2, measuring the number of samples in which the species is well represented ('the effective number of occurrences': ter Braak 1990, ter Braak & Verdonschot 1995). Values for u, t, total weight and N_2 are supplied in the output from the CANOCO processing. The analysis was performed on untransformed species values due to the fitting of the species curves.

The fitted species curves were used to develop a generalised expression of community changes along a PAH gradient. To manage this, all species were classified according to their feeding modes and their response functions were added up across species within the different feeding groups. The resultant diagram illustrates a model of the community's trophic composition in relation to PAH.

RESULTS

Fauna and sediments

In most fjords, the number of species sampled per station varied from 40 to 70 (Table 2). In Årdal, however, very few species were found at the stations on the periphery of the lifeless zone close to the outfall. Moreover, a

reduced number of species was detected at the most contaminated stations in Feda and Sauda. For the most part, the Shannon-Wiener diversity index ranged from 2.5 to 5.0. Apart from at the most highly impacted stations, species numbers and diversity did not differ between the smelter-affected fjords and the fjords not receiving industrial effluents. Table 3 lists the species included in the analyses (a total of 97 species) and includes summary information about their occurrence in the fjords.

Some stations close to effluent outfalls had dark grey or black sediments with a high TOC value and C/N ratio (Årdal, Sunndal) (Table 2). This is presumably due to effluent soot particles having become embedded in the sediments (Næs & Oug 1997a, b). Soot carbon is not distinguished from naturally occurring organic carbon by the ordinary analysis for TOC. In Sunndal, the TOC value and C/N ratio correlate with PAH concentrations (Næs & Oug 1997b).

Table 2. Summary data for macrofauna and sediment variables in Norwegian fjords affected by smelter effluents and in reference fjords not influenced by industrial effluents. H' = Shannon-Wiener diversity index (log 2 base). * = values estimated from loss of ignition (TOC = 0.25 LOI). n.a. = not analysed.

Fjord area	Year	No. of stations	No. of species	Ind m^{-2}	H'	silt+clay %	TOC %	C/N ratio
Årdal	1983	3	22-45	246-612	1.7-4.3	n.a.	5.4-15	49-80
	1989	4	45-56	1024-1479	3.1-3.9	40-57	2.5-6.6	25-65
Feda	1984	5	19-56	683-1760	2.2-5.0	n.a.	*2.6-5.1	n.a.
Sauda	1976	1	16	1670	2.5	n.a.	*1.3	n.a.
	1981	5	2-20	25-360	1.6-4.0	n.a.	n.a	n.a.
Sunndal	1986	8	30-48	1625-3835	2.5-3.9	30-90	0.4-3.9	11-25
	1995	4	98-127	3500-5703	4.3-5.6	36-74	0.6-1.4	8-12
Topdal	1983	8	40-60	450-8748	2.0-4.6	n.a.	0.9-4.4	11-15
Kvinnherad	1990	5	40-81	350-1292	3.5-5.2	n.a.	0.3-2.0	10-11
Kårstø	1983	5	23-90	516-3674	2.5-4.9	n.a.	n.a.	n.a.
Surnadal	1991	5	38-70	1090-5020	3.2-4.6	41-97	0.7-1.8	9-14
Tresfjord	1990	4	51-72	1150-1770	3.7-4.7	n.a.	3.6-5.2	10-13

Table 3. List of species included in the ordination analyses. The trophic group (TR) and number of recordings (Rec) are also indicated (total number of stations = 57). Trophic groups: c = carnivore (also including omnivore), sus = suspension feeder, sd = surface deposit feeder, ssd = subsurface deposit feeder. The trophic group classification is in accordance with Fauchald & Jumars (1979), Josefson (1986) and Gaston (1987). All species with a total number of specimens >15, equivalent to 0.5 per thousand of total abundances are included.

Species	TR	Rec	Species	TR	Rec
ANTHOZOA			*Pista cristata*	sd	22
Edwardsiidae ind	c	3	*Streblosoma intestinalis*	sd	12
			Terebellides stroemi	sd	37
NEMERTINEA ind	c	47	*Chone* sp	sus	21
			Euchone sp	sus	10
POLYCHAETA			*Jasmineira* sp	sus	13
Paramphinome jeffreysii	c	36			
Harmothoe sp	c	30	OLIGOCHAETA ind	ssd	5
Pholoe minuta	c	36			
Sthenelais sp	c	7	OPISTOBRANCHIA		
Eteone sp	c	21	*Philine scabra*	c	17
Phyllodoce groenlandica	c	14	*Philine* sp	c	12
Nereimyra punctata	c	20			
Ophiodromus flexuosus	c	31	CAUDOFOVEATA ind	c	32
Exogone verugera	sd	25			
Typosyllis cornuta	c	22	BIVALVIA		
Ceratocephale loveni	sd	23	*Nucula sulcata*	ssd	8
Nephtys incisa	c	16	*Nuculoma tenuis*	ssd	18
Glycera alba	c	26	*Yoldiella lucida*	ssd	14
Glycera capitata	c	22	*Yoldiella tomlini*	ssd	9
Goniada maculata	c	35	*Thyasira croulinensis*	ssd	11
Lumbrineris gracilis	c	10	*Thyasira equalis*	ssd	31
Lumbrineris scopa	c	18	*Thyasira ferruginea*	ssd	17
Lumbrineris sp	c	35	*Thyasira gouldi*	ssd	4
Protodorvillea kefersteini	c	5	*Thyasira obsoleta*	ssd	13
Phylo norvegica	ssd	17	*Thyasira pygmaea*	ssd	9
Scoloplos armiger	ssd	9	*Thyasira flexuosa/sarsi*	ssd	27
Apistobranchus tullbergi	sd	11	*Thyasira* sp	ssd	17
Aricidea sp	sd	16	*Mysella bidentata*	sus	8
Paraonis gracilis	sd	18	*Parvicardium minimum*	sus	16
Paradoneis lyra	sd	20	*Abra nitida*	sd	34
Laonice cirrata	sd	12	*Arctica islandica*	sus	5
Polydora sp	sd	7	*Kelliella miliaris*	sus	14
Prionospio cirrifera	sd	38			
Prionospio malmgreni	sd	21	CRUSTACEA		
Prionospio sp	sd	7	*Eudorella emarginata*	sd	25
Pseudopolydora antennata	sd	24	*Eudorella truncatula*	sd	8
Spiophanes kroeyeri	sd	34	*Leucon nasica*	sd	19
Caulleriella sp	sd	24	*Diastylopsis resima*	sd	4
Chaetozone setosa	sd	43	Tanaidacea indet	sd	8
Tharyx sp	sd	30	*Eriopisa elongata*	sus	18
Diplocirrus glaucus	sd	39	*Westwoodilla caecula*	c	21
Asclerocheilus intermedius	sd	3	*Harpinia* sp	c	18
Polyphysia crassa	ssd	17			
Scalibregma inflatum	ssd	23	SIPUNCULIDA		
Notomastus latericeus	ssd	13	*Golfingia* sp	ssd	11
Heteromastus filiformis	ssd	47	*Onchnesoma steenstrupi*	ssd	7
Asychis biceps	ssd	8	Sipunculida indet	ssd	14
Euclymene sp	ssd	22			
Myriochele oculata	sd	25	ECHINODERMATA		
Owenia fusiformis	sd	6	*Amphiura chiajei*	sd	20
Pectinaria auricoma	ssd	16	*Amphiura filiformis*	sus	12
Amythasides macroglossus	sd	20	*Ophiura* sp	sd	23
Eclysippe vanelli	sd	15	*Echinocardium cordatum*	ssd	8
Melinna cristata	sd	18	*Labidoplax buski*	ssd	21
Melythasides laubieri	sd	4	*Leptosynapta* sp	ssd	8
Sosane sulcata	sd	7			

Main faunal patterns in the fjords

The basic CCA revealed significant faunal patterns related to depth, distance from freshwater outfall points, sediment types and smelter effluents (Table 4).

Table 4. Canonical Correspondence Analysis (CCA) for soft bottom macrofauna at stations contaminated by PAHs and in reference areas in Norwegian fjords, showing eigenvalues, percentages of extracted variance and correlation to environmental variables for the first four axes. The 'forward selection' procedure was used to rank environmental variables by importance. Inertia: extracted variance attributable to each individual variable. Added inertia: added variance accounted for by variables selected one by one in sequence. The variables found significant ($p<0.05$) in Monte Carlo permutation tests were selected. Asterisks denote classes of categorical seabed variables. The data comprise 57 stations and 97 species/species groups.

Axis	1	2	3	4	Total [a]
Eigenvalue	0.26	0.18	0.13	0.10	0.79
Extracted variance (%)	7.7	5.2	3.8	3.1	23.2
Environmental correlation	0.85	0.79	0.79	0.72	-

Forward selection	Inertia	%	Added inertia
Depth	0.22	6.5	0.22
Distance from river	0.17	5.0	0.16
Enriched sediment *	0.16	4.7	-
PAH_{13}	0.15	4.4	0.15
Distance from smelter	0.13	3.8	0.11
Effluent impacted sediment *	0.12	3.5	0.09
Muddy sediment *	0.09	2.7	-
Sandmixed sediment *	0.09	2.7	0.06
Sum			0.79

[a] The total inertia of the species data (calculated by Correspondence Analysis) was 3.39.

In particular, the PAH level (PAH_{13}) figured among the variables explaining the relatively higher fractions of species variance. Using the forward selection procedure, the variables of depth, distance from river, PAH level and distance from smelter were selected and ranked as the most important

variables. However, the fraction of explained species variance (23%) was rather low. To some degree, this reflects the structure of the analysis, given that data sets from different fjords with different species assemblages were put together in combination. In a separate analysis (not shown here), inter-fjord differences were estimated to account for about 40% of the total species variance.

The variables of depth and distance from river correlated most strongly with the first and second CCA axes. The plot of these axes (Fig. 2A) displays the species according to gradients in depth and freshwater inflow, which may be representative of natural faunal gradients in the fjords. Species positioned at the lower left had their distribution optima in shallow waters (e.g. *Amphiura filiformis* and *Edwardsia*), while species at the upper right had their optima at the deepest stations (e.g. *Thyasira gouldi* and *Kelliella miliaris*). Species marked near the centre of the plot were either found in intermediate depths, or were present at shallow and deep stations alike. Species at the upper left (e.g. *Thyasira flexuosa/sarsi* and *Phyllodoce groenlandica*) were found near river outfall points, suggesting that they are stimulated by riverborne materials or reduced water salinity. The variables of PAH level and distance from smelter correlated with the third CCA axis (Fig. 2B). The species in the upper part of the diagram were found near effluent outfall points (e.g. *Protodorvillea kefersteini* and *Nereimyra punctata*) while the species in the lower part were found far from the outfalls (e.g. *Onchnesoma steenstrupi* and *Echinocardium cordatum*). The different orientation of the arrows for distance from river and PAH level makes it possible to discriminate between estuarine faunal gradients and species patterns attributable to smelter effluents.

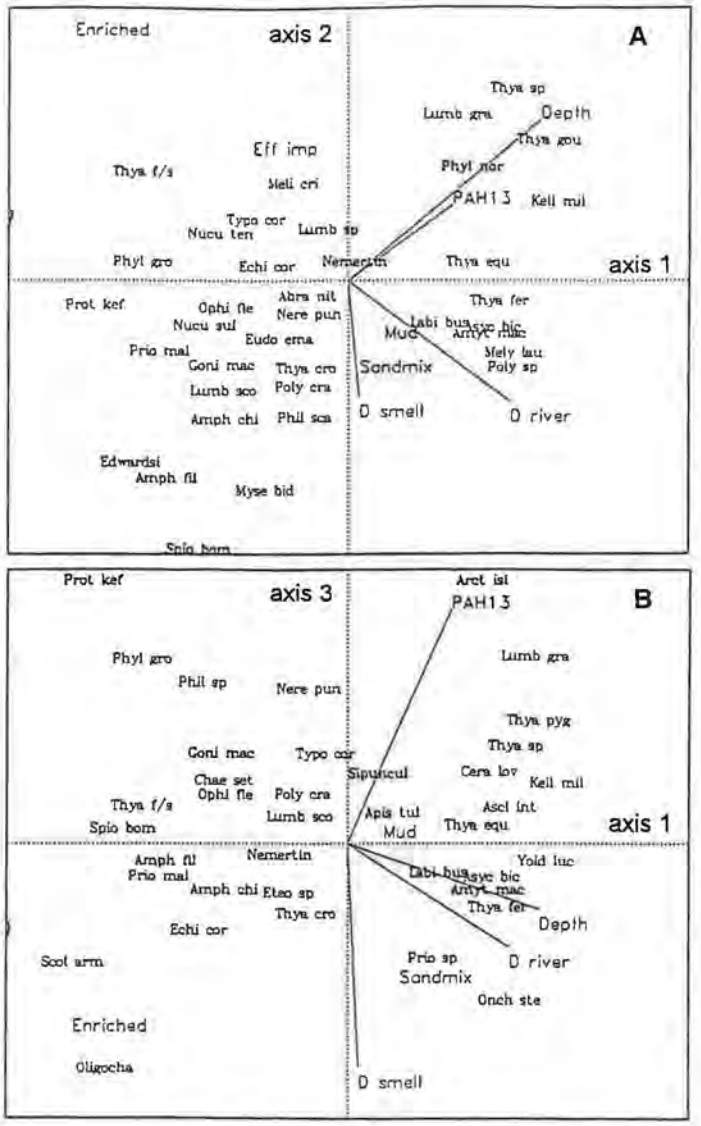

Fig. 2. Canonical Correspondence Analysis (CCA) of soft bottom macrofauna in Norwegian fjords affected by aluminium smelter effluents and in reference areas: Biplot of species and environmental variables for (A) axes 1 and 2 and (B) axes 1 and 3. Quantitative variables are indicated by arrows pointing in the direction of maximum value of the variables, D smelt = distance from smelter, D river = distance from river inflow point. A selection of characteristic species is shown, other species have been omitted for clarity. Species names are given in full in Table 3. The plots account for 13 and 11 per cent of the total species variation (total inertia) and 56 and 49 per cent of the explainable variation, respectively. Data for the analysis are given in Table 4.

The species plots which indicate feeding groups are illustrated in Fig. 3. The feeding groups formed a mosaic pattern dependent on depth and freshwater inflow, but carnivores tended to be most abundant in shallow waters, and there was a preponderance of deposit feeders in deeper waters distant from river outfall points (Fig. 3A). However, clearer patterns began to emerge in relation to PAH levels and distance from smelter effluents. In the plots for axes 1 and 3 (Fig. 3B), carnivores were in a majority in the upper left quadrant, representing shallow waters near the smelters. This contrasts with the lower left quadrant, representing shallow waters distant from the smelters, where there was an aggregation of deposit and suspension feeders. It may be noted that most deposit and suspension feeders were located in a band parallel to the first axis, reflecting their occurrence in both shallow and deep water, and at varying distances from river outlet points. The result suggests that the smelter effluents favour carnivorous species, or conversely, are negative to deposit feeders. These effects tend to be most prominent in shallow waters and moderate depths.

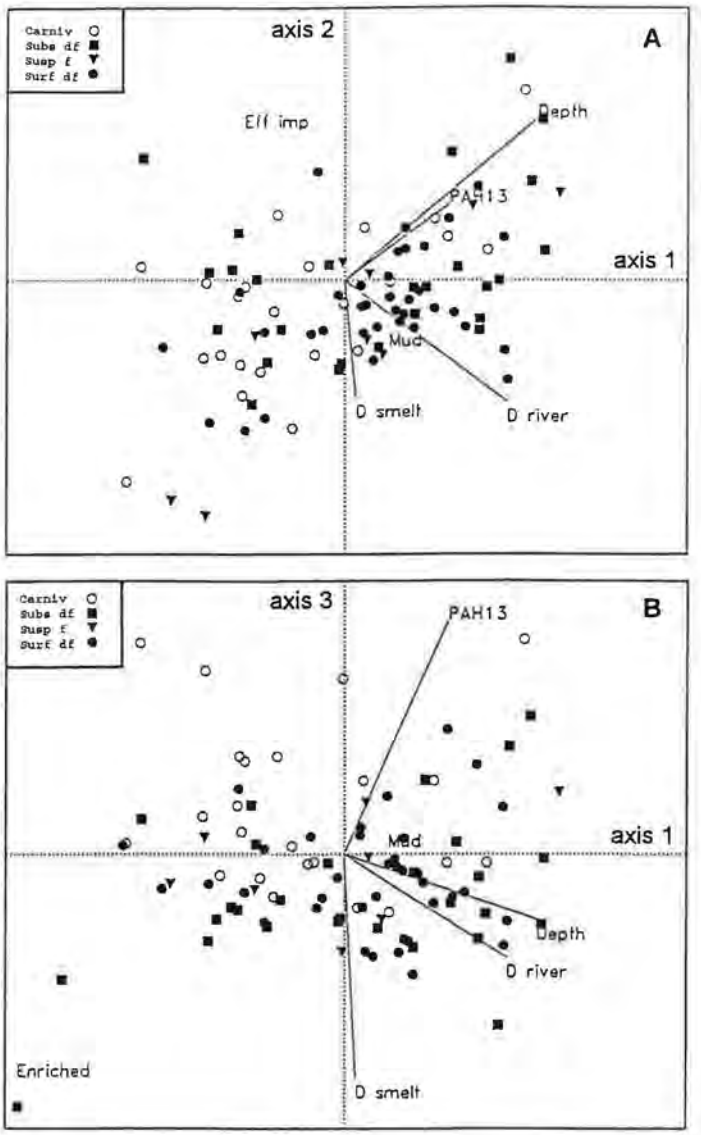

Fig. 3. CCA biplot of species and environmental variables from analyses of soft bottom macrofauna in Norwegian fjords with symbols representing feeding categories superimposed on species positions (carnivores, surface deposit feeders, subsurface deposit feeders, suspension feeders). A: axes 1 and 2, B: axes 1 and 3. The names of most important species are indicated in Fig. 2.

Sunndal and Surnadal fjords

The CCA for the Sunndal and Surnadal fjords, where quantitative sediments variables were obtained, revealed largely the same faunal patterns as in the main analysis. TOC and the C/N ratio were among the most important variables, but they were clearly related to the distance from the smelter and correlated with PAH levels (Fig. 4, Table 5).

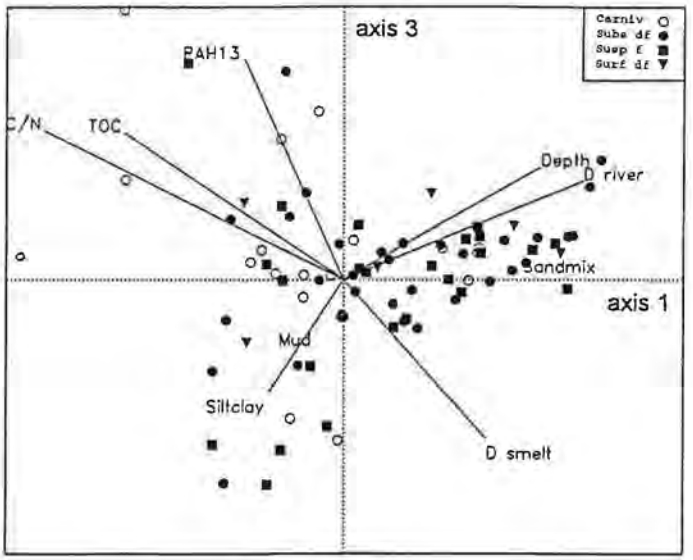

Fig. 4. CCA biplot of species and environmental variables (axes 1 and 3) of soft bottom macrofauna in Sunndal and Surnadal fjords with species shown by symbols representing feeding categories. The variables of C/N, depth, effluent-impacted sediment and PAH_{13} were active in the analysis, while other variables entered on the diagram were as passive variables. The plot accounts for 32 per cent of the total species variation (total inertia) and 62 per cent of the explainable variation. Data for the analysis are given in Table 5.

Table 5. The CCA for soft bottom macrofauna in the Sunndal and Surnadal fjords, showing eigenvalues, percentages of extracted variance and correlation to environmental variables for the first four axes. The 'forward selection' procedure was used to rank environmental variables by importance. Inertia: extracted variance attributable to each individual variable. Added inertia: added variance accounted for by variables selected one by one in sequence. The variables found significant ($p<0.05$) in Monte Carlo permutation tests were selected. Asterisks denote classes of categorical seabed variables. The data comprise 17 stations and 89 species/species groups.

Axis	1	2	3	4	Total [a]
Eigenvalue	0.28	0.20	0.17	0.08	0.73
Extracted variance (%)	20.1	14.8	12.2	5.7	52.9
Environmental correlation	0.96	0.96	0.98	0.95	-

Forward selection	Inertia	%	Added inertia
C/N ratio	0.25	18.1	0.25
Depth	0.21	15.2	0.19
Effluent impacted sediment *	0.21	15.2	0.18
Distance from river	0.18	13.0	-
TOC	0.17	12.3	-
Distance from smelter	0.16	11.6	-
PAH_{13}	0.15	10.9	0.11
Sandmixed sediment *	0.14	10.1	-
Muddy sediment *	0.13	9.4	-
Silt-clay	0.08	5.8	-
Sum			0.73

[a] The total inertia of the species data (calculated in Correspondence Analysis) was 1.38.

In the plot, most carnivores had their optima in the direction of increasing PAH levels, while most deposit feeders were located at a distance from freshwater inflow and smelter effluents with moderate and low TOC and C/N values. In forward selection, the C/N ratio was ranked at the top and selected together with depth, PAH_{13} and the categorical variable of effluent-impacted sediment. The distance variables, TOC and other sediment categories tended not to contribute any significant additional information, obviously because of intercorrelations. The silt-clay fraction showed little correlation with species distribution. The results for the C/N ratio and TOC appear to reflect the effects of effluent soot particles in the

sedimentary carbon pool (Næs & Oug 1997b). The results suggest that depth and effluents are the most salient factors for species distribution.

Faunal changes in relation to PAH contamination

The primary yield of the CCA with PAH_{13} entered as the sole variable, was an ordering of the species along a PAH concentration gradient. The species were ordered by their calculated optima with respect to PAH. The gradient (the first axis) explained a rather small fraction of the total species variance (5%), but it was found statistically significant ($p<0.05$) using Monte Carlo permutation tests. This rather small variance may, as in the basic CCA discussed above, reflect the combination of different data sets in the analysis. The ordered list of species exhibited a proportional dominance of carnivores in connection with high PAH values. This trophic pattern proved significant when tested for the distribution of carnivores versus non-carnivores along the gradient (Table 6). It was, however, among the polychaetes that a clear difference was apparent between carnivores and non-carnivores. Tests were also carried out for each of the major faunal groups to assess their overall distribution along the PAH gradient (Table 6). These indicated a significant correlation between echinoderms and low PAH values, suggesting that these species may generally avoid PAH-contaminated areas, whereas other groups were more evenly dispersed. With the notable exception of carnivores, rather few species had their optima at high PAH concentrations. The decrease was most pronounced among deposit feeders (Fig. 5).

Table 6. Results of Mann-Whitney U tests to compare the ranking of species with respect to various faunal categories along a gradient determined by PAH concentrations. A: Carnivorous species compared with non-carnivorous species. B: Systematic groups (indicated group compared with rest of species). Number of species in faunal categories (total number of species = 97) and average species ranks are shown, high ranks indicate a positive correlation with PAH. The probabilities (p) refer to two-tailed tests.

Faunal category	No. of species	Average rank	p	Sign
A: Carnivores vs non-carnivores				
All species	24 : 73	60.1 : 45.3	0.03	*
Polychaetes	17 : 40	38.7 : 24.9	<0.01	**
Non-polychaetes	7 : 33	19.0 : 20.8	0.72	ns
B. Systematic groups				
Polychaeta	57 : 40	52.7 : 43.7	0.12	ns
Mollusca	20 : 77	51.5 : 48.4	0.67	ns
Crustacea	8 : 89	47.6 : 49.1	0.89	ns
Echinodermata	6 : 91	25.1 : 50.6	0.03	*

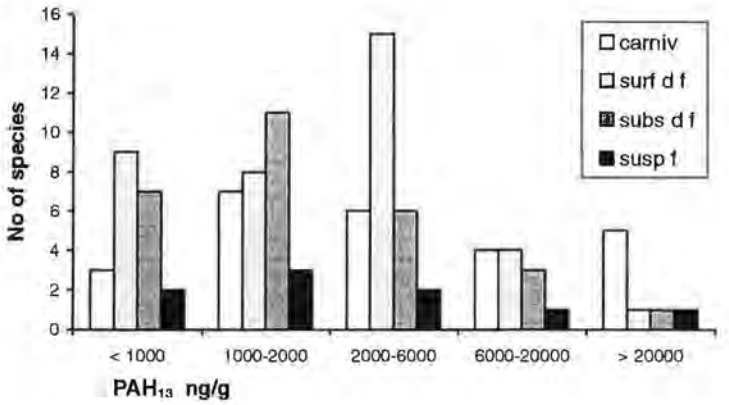

Fig. 5. Number of species in feeding categories (carnivores, surface deposit feeders, subsurface deposit feeders, suspension feeders) in relation to different PAH concentration levels. Species are represented by their optima (peak distribution centre) on the PAH gradient. The classification of species is shown in Table 3.

The generalised model of the community's trophic composition along the PAH gradient, generated from fitted response curves for the species, is shown in Fig. 6. There is a clear relative dominance of carnivores at high PAH levels. At low PAH levels, surface and subsurface deposit feeders dominate, while carnivores account for 15-20% of the specimens. The carnivores begin to increase at PAH levels of roughly 10,000 ng g^{-1} and exceed 50% at 100,000 ng g^{-1}. The carnivore curve flattens out at levels in excess of 300,000 ng g^{-1}, but the reliability of the estimates in this part of the diagram is likely to be poor since there are very few data available for these high PAH levels. The extreme left part of the figure may also be less reliable, as the estimated PAH values are somewhat arbitrary as regards background levels. A few examples of fitted species curves are shown in Fig. 7. The species shown are one echinoderm (*Amphiura filiformis*) and three polychaetes (*Eclysippe vanelli*, *Paramphinome jeffreysi* and *Nereimyra punctata*), representing different feeding modes. Although the examples illustrate quite typically the very scattered nature of the species data, it appears that the fitted functions describe the main distribution of the species adequately.

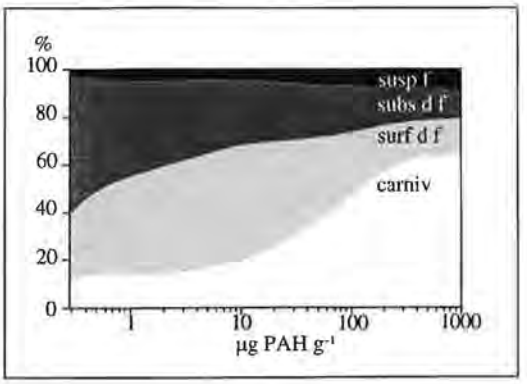

Fig. 6. The estimated trophic composition of species communities along the PAH concentration gradient. The classification of species into feeding categories (carnivores, surface deposit feeders, subsurface deposit feeders, suspension feeders) is shown in Table 3.

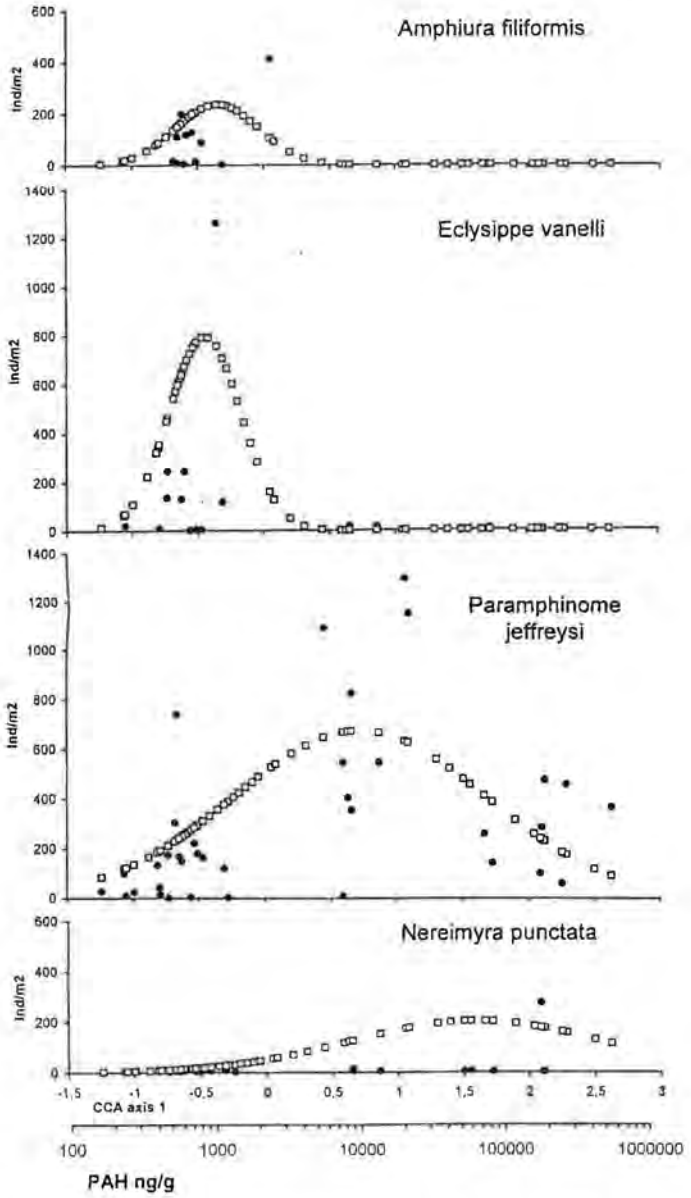

Fig. 7. Fitted response curves for species along the PAH concentration gradient showing examples of species belonging in different feeding categories. *Amphiura filiformis*: suspension feeding brittle star; *Eclysippe vanelli*: surface deposit feeding polychaete; *Paramphinome jeffreysi* and *Nereimyra punctata*: carnivorous polychaetes.

DISCUSSION

The assumption underlying the environmental studies in smelter-affected fjords is that PAH may have adverse effects on organisms in the receiving waters. It is common knowledge that PAHs may have severe pathological effects in fish, especially by inducing cancer and various skin and liver lesions (see e.g. Knutzen 1995). Fairly little is known about the effects of PAHs on other aquatic organisms. Many invertebrates, such as molluscs and crustaceans, tend to accumulate PAH without obvious detrimental effects (Law & Biscaya 1994, Næs et al. 1995). However, various studies have reported toxic effects, even at moderate PAH concentrations (see Long 1992, Long et al. 1995). Apparently, the ability to metabolise PAH differs among species and classes of organisms, which may account for some of the reasons the potential hazards tend to differ among species. In polychaetes, the metabolic ability varies considerably among species and may not be predicted on the basis of standard taxonomic classifications (Driscoll & McElroy 1996); in molluscs, the metabolic rates appear to be low. In addition, some echinoderms are known to metabolise PAHs (denBesten et al. 1993).

The actual toxicity of PAH, however, may be fundamentally contingent on speciation of the PAHs. PAHs generated by the smelting industry are, to a large extent, bound to oxides and black carbon-containing particles (soot). This association is known to render the PAHs resistant to degradation and desorption processes (McGroddy & Farrington 1995, McGroddy et al. 1996). Compared with petroleum-derived PAHs, the former are presumably less available to organisms. Knutzen (1995) suggested that the generally modest ecological effects observed in the Norwegian smelter-affected fjords might be explained by particle affinities. Indeed, studies of PAH profile patterns in the fjords have indicated that the PAHs are resistant to degradation and transformation reactions (Næs & Oug

1997a, b). Correspondingly, Paine et al. (1996) concluded that sediment PAHs present in the form of pitch or coal tar particles in smelter-affected Canadian fjords, are of limited bioavailability and do not exhibit toxic effects on the benthic fauna.

It is generally recognised that the overall ecological effects of contaminants will be exposed through the natural communities of species, since a community represents a system of species interactions and, accordingly, integrates and reflects the responses of all affected species. The study of natural species communities is therefore a key element in many monitoring programmes addressing the spatial and temporal effects of contaminants. However, the effects may be small compared with the influences of natural abiotic factors and may also be overshadowed by large biological variation. The inherent difficulty may lie in correctly linking biotic responses and environmental influences, in particular when it comes to detecting and interpreting subtle changes (Rees & Eleftheriou 1989; Elliott 1994). Despite the complexity of the systems, various authors (e.g. Joern & Hoagland 1996) stress the importance of community or ecosystem studies for risk assessments because single-species studies such as bioassays may fall short in predicting the responses of natural systems. As a consequence, capturing key response signals in natural systems will represent a major challenge in the field of ecotoxicology (Joern & Hoagland 1996).

In the present examination of data from Norwegian smelter-affected fjords, significant faunal changes along sedimentary PAH gradients have been identified. The changes may be associated with biological features of the species, and to some extent also systematic groups, inasmuch as detritus feeders were generally reduced while carnivores seemed to be unaffected or possibly even stimulated at high PAH levels. The changes appeared to commence at rather low PAH levels, before the total number of species and diversities was affected, and also before detrimental effects became

obvious. Clearly, the general effects of the PAHs on the communities were limited, but the functional changes may represent a type of early response signal preceding more fundamental changes evinced by species reductions or reduced community diversity. The changes may reflect contaminant responses which influence particular species or groups of species. The most clear and diverse responses were observed among the polychaetes, which, as a group, represent a diverse range of feeding modes and living strategies. In a general context, these results emphasise that incorporating the biological features of the organisms into analyses of the communities may enhance the capability of impact determination. Elliott (1994) notably encouraged such approaches in environmental quality assessments.

The observed changes could possibly be explained in terms of exposure to PAHs. Compared with sessile particle feeders, carnivorous polychaetes may simply be less exposed as they are able to move in and out of the strongly contaminated areas. They are also less dependent on the sediments for food and shelter. The fact that the community changes tended to be most distinct in shallow waters may also point to exposure. Recent data suggest that the smelter effluents contain substantial amounts of low-molecular weight PAHs in a non-particulate state, which are dispersed in the surface layers of the receiving waters (Næs et al., unpubl.). Further, various more toxic and soluble derived compounds may result from photo-oxidation (Ankley et al. 1997). These factors could indicate that smelter effluents influence the surface and shallow waters more extensively than the deep bottom areas in receiving waters. Nevertheless, the specific responses to the PAHs will depend on the metabolic systems and tolerance to environmental stress of the individual species. These aspects are poorly known for most of the species. Recent studies have demonstrated that metabolic abilities and tolerances vary in species-specific terms, even among systematically related forms (cf. Driscoll & McElroy 1996), and thus cannot easily be generalised to species groups.

The echinoderms represented the only systematic group which showed a negative correlation with PAH. This result ties in with recent studies in the Sunndal fjord in which biomarker responses have been detected in soft bottom sea stars and sea cucumbers (Næs et al., unpubl.). Echinoderms have also been found to be sensitive to oil pollution (Daan et al. 1992, Newton & McKenzie 1995). For instance, in the North Sea, the population of the brittle star *Amphiura filiformis* decreases in response to discharges of oil-contaminated drill cuttings (Daan et al. 1992). Possibly, particular attention should be paid to the echinoderms in PAH monitoring, as this group may seem to link biomarker and ecological responses.

In the generalised diagram of changes in the community, the main rise in the proportion of carnivores occurred at PAH levels between 10,000 and 100,000 ng g^{-1}, with the initial changes at about 5000 ng g^{-1}. Interestingly, these values are not all that distant from the effect limits for PAH established by Long et al. (1995) from a number of toxicity tests and field studies. They indicated that adverse effects first appear at about 4000 ng g^{-1} total PAH ('effect range low'), and frequently or always occur at above 50,000 ng g^{-1} ('effect range median'). Long et al. (1995) assumed that the limits would be of broad applicability, because data for a variety of species, which may differ considerably in their sensitivity to contaminants, were included. It should be remembered, however, that the pattern illustrated in the present study is based on correlations between fauna and PAH and does not necessarily express a direct cause and effect relationship.

It may also be considered that the species could possibly react to stressing factors other than PAH in the discharged effluents. These may include fluoride, particulate matter, and in some cases, metals (Knutzen 1995). In the present data sets, there were rather few quantitative data for these factors, so they could not be entered as separate variables in the analyses. However, they may be assumed to contribute to the non-specific variables of distance from effluent outfall and sediment categories. The

analyses showed that the non-specific variables were less clearly related to species patterns than PAH, and therefore did not indicate that there were any other strong effluent-related stressing factors in addition to PAH. Knutzen (1995) believed that fluoride was of little importance in the receiving waters, but suggested that the deposition of particles may constitute an important factor. The present results do not confirm the latter suggestion, however, considering the proportional decrease of deposit feeders in the contaminated areas. Elsewhere, studies of the sedimentation of inert mineral particles have found that small deposit feeding organisms are favoured (Gray 1982, Olsgard & Hasle 1993).

The fractions of the species variance which could be accounted for by the environmental variables in the two CCAs were roughly 20 and 50%, respectively. These figures may be compared with studies made by Quintino et al. (1995), Mackie et al. (1997) and Oug (1997), who were able to account for 25-40% of the variation in benthic communities, using variables related to topography, sediments, and, in some cases, contaminants. The extent of unexplained variance may imply that some important ecological relationships have yet to be characterised; these include factors pertaining to the physico-chemical properties of water and sediments, and biotic relationships which control the communities (cf. Oug 1997). However, it should also be borne in mind that much of the variance will represent generally inexplicable 'noise' emanating from patchiness and random fluctuations in the biological systems. The CCA technique provides a framework for estimating and testing species-environment relationships, even if effects are masked by large sources of variation (ter Braak & Verdonschot 1995). In the present case, the species patterns extracted on the PAH gradient represented no more than 5-10% of the total variance. These patterns were not observed in the traditional treatment of the species data. Essentially, this result corroborates Elliott (1994), who pointed out that the routine analyses most commonly applied to benthic community data do not

fully extract the biological information contained in the data. In order to correctly interprete such patterns and evaluate the ecological consequences, however, it is important to describe and quantify the influences of natural environmental factors as completely as possible. This points to a need to characterise a broader set of environmental variables and ecological relationships in monitoring of the effects of contaminants.

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Paper VII

Submitted *Environmental Toxicology and Chemistry*

Accumulation and effects of aluminium-smelter generated PAHs on soft-bottom invertebrates and fish

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Abstract - An integrated study involving measurements of PAH levels in bottom sediments, assessments of resident soft-bottom communities, the accumulation of PAHs in soft-bottom invertebrates, and biomarker responses in invertebrates and fish, was conducted to assess the impact of an aluminium reduction plant in a Norwegian fjord. The fjord sediments were heavily contaminated by PAHs in the inner reaches near the aluminium smelter, where concentrations were well above levels elsewhere reported to induce biological effects. Nevertheless, the PAH contamination in the fjord did not seem to have serious effects on the benthic biota. This conclusion can be drawn from the soft-bottom communities as well as from

biomarker analyses. Presumably, contaminant speciation is important for explaining the restricted biological effects. The results support the assumption that PAHs associated with soot-like structures have limited bioavailability. They also point to the need to link various single-species approaches to measurements of effects on higher levels of organization and with an understanding of the speciation of the chemical contaminant.

Keywords: Polycyclic aromatic hydrocarbons Sediments Benthos Community structure Toxicity

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) result from the incomplete combustion of organic matter and are ubiquitous contaminants of coastal marine ecosystems. Apart from a natural background level, elevated concentrations are attributed to anthropogenic sources such as releases of combusted and uncombusted petroleum products and emissions from the metallurgy industry. Some PAHs have well-documented toxic, mutagenic and carcinogenic potentials that make them priority pollutants [1, 2]. They may have effects on the biochemical, physiological, morphological and ecological levels. However, the bioavailability of PAHs is dependent on their origin and speciation [3, 4].

The effects of PAHs on organisms are initiated through responses at the cellular level, mainly through the biotransformation of the compounds to toxic metabolites by cytochrome P4501A-system enzymes [5]. Some intermediate metabolites of carcinogenic PAHs are highly reactive, but they are generally metabolised to water-soluble conjugates that may be excreted. However, if the reactive intermediate metabolites are not inactivated, they

may bind to membrane lipids or nucleic acids, causing molecular and cellular damage such as DNA adducts. In addition, PAH metabolites may act as free radicals or generate oxyradicals through redox cycling [6]. Such free radicals may have deleterious effects if not inactivated through cellular defence mechanisms [7].

Cellular responses associated with contaminant impact on the environment are commonly referred to as biomarkers. In recent years, biomarkers have been used increasingly as early warning signals of environmental deterioration, qualifying them as important tools for risk assessment. However, various authors have pointed out that such methods should not be used in isolation, but in addition to measures of chemical or additional biological effects to get a better description of pollution-induced effects [8, 9].

In Norway, emissions generated by the production of primary aluminium are important anthropogenic sources of PAHs in fjords and coastal waters [10]. The PAHs originate from the seawater scrubbing of ventilation air and furnace off-gases in the smelters, although some originate from the production of anodes. High PAH concentrations have been observed in bottom sediments and indicator organisms near effluent discharge areas in the affected waters [10, 11]. The concentrations decrease rapidly with distance from source, but elevated levels have been detected tens of kilometres away in some of the fjords. Elevated sediment concentrations of PAHs may cause adverse effects on sediment-dwelling organisms [12-14]. In Norway, the assessment of effects has largely been based on the monitoring of soft-bottom communities. These studies have failed to relate ecological effects to PAH toxicity [15], but recent re-analyses of the data suggest that functional species changes may take place along PAH concentration gradients (Oug et al. in prep.). Furthermore, Knutzen [15]

concluded that tumours have not been observed in fish caught in PAH-affected recipient waters in Norway.

This study evaluates the environmental consequences of PAHs from aluminium smelter effluents through an integrated study of contamination and biological effects along a PAH concentration gradient in a Norwegian fjord. The study comprises 1) measurements of PAH levels in bottom sediments, 2) assessments of resident soft-bottom communities, 3) the accumulation of PAHs in soft-bottom invertebrates, and 4) biomarker responses in invertebrates and fish. The selected biomarkers represent a suite of metabolic responses, in addition to PAH breakdown products in fish. The study was performed in the Sunndalsfjord, which receives effluents from an aluminium reduction plant situated at the head of the fjord. Previous studies have shown sediments and indicator organisms in the fjord to be strongly contaminated [10, 16]. According to Norwegian criteria for the assessment of environmental quality [17], the most strongly affected areas are characterised as "markedly" to "extremely contaminated". Owing to the high levels, Norwegian authorities have issued dietary warnings against eating mussels or fish liver from the inner reaches of the fjord.

MATERIALS AND METHODS

Sampling sites and sample collection

The Sunndalsfjord (Figure 1) is approximately 60 km long with bottom depths mainly ranging from 100 to 150 m. Samples were collected in four areas located along a distance gradient from the smelter. Most samples were taken at a standardised depth of 110 m (Stns SD1-4), while additional fish samples were collected at 5 to 20 m (Stns SS1-2,4). Stations SD4 and SS4

were located outside the fjord proper and were taken for reference. Station positions were obtained using GPS/differential GPS that were accurate to 100/20 m, respectively. At the deep stations, samples were drawn from 27 February to 3 March 1995, while at the shallow stations, fish were collected from 6 to 8 September 1996. An overview of the sampling scheme and analyses is given in Table 1.

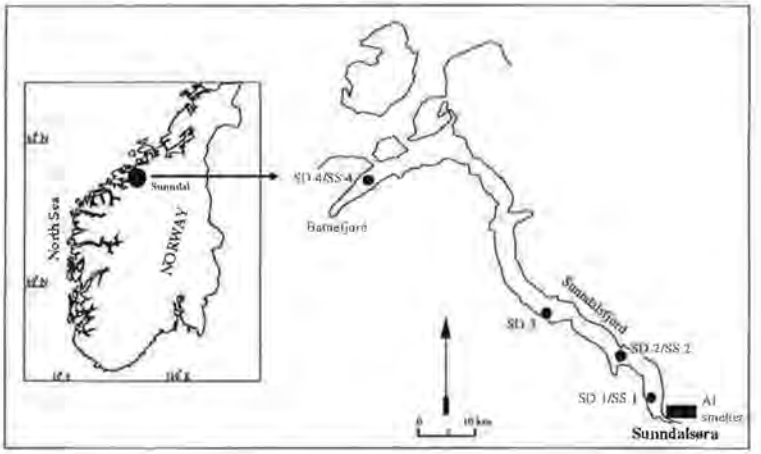

Fig. 1. Fjord area and sampling sites

Samples to test sediment chemistry and soft-bottom fauna were taken using a 0.1 m^2 van Veen grab. Four replicate samples were drawn per station. Subsamples of surface sediments (0 to 2 cm) were taken for chemical analysis using a stainless steel spoon inserted through the inspection hatches of the grab. The grab samples that were accepted were taken from undisturbed sediment surfaces covered by water. Sediment samples were deep-frozen prior to analysis. Faunal samples were sieved on 1 mm round-holed screens, and preserved in a 4 per cent buffered formaldehyde solution. All organisms were counted and identified to the lowest practical taxonomic level, generally the species level. The sediment samples were

taken as subsamples of the faunal samples to obtain maximum correspondence between sediment chemistry and fauna.

Epibenthic invertebrates for studies of PAH accumulation and biomarker responses were collected using a wide-mouth Agassiz sledge. The sledge was hauled at 1 knot for half an hour at each station. The species chosen for analyses were the scallop *Chlamys septemradiata*, the sea cucumber *Stichopus tremulus*, the sea star *Psilaster andromeda*, and the tunicate *Ascidia* sp. The species were selected to represent different types of feeders, viz. carnivores, suspension feeders and deposit feeders, in order to assess possible differences related to behaviour and ecology. Following collection, specimens were transferred to small aquaria and left for two to six hours before being processed. Scallop shell sizes were determined and all soft parts removed. The approximate length of individual *S. tremulus* specimens was measured, then the intestines with associated tissue were removed and cleaned of sediment. Individual *P. andromeda* were measured, the sex determined and the digestive glands removed. The soft parts of the tunicates were removed, rinsed in seawater to remove sediment, and blotted dry. All tissue samples were transferred to cryotubes, frozen in liquid nitrogen and stored at -80°C prior to analysis.

Fish were collected to study biomarker responses using an Agassiz sledge (deep stations) and gill nets (shallow stations). The flatfish megrim *Lepidorhombus whiffiagonis Walb.* and witch *Glyptocephalus cynoglossus L.*, were selected at the deep stations, while the flounder *Platichthys flesus L.* and the Atlantic cod *Gadus morhua L.* were selected at the shallow stations. The length of each individual fish was measured and its sex determined. Bile and liver samples were collected for analyses of cytochrome P4501A activity, DNA adducts (only *Platichthys flesus L.*) and

PAH bile metabolites were transferred to cryotubes. All samples were frozen in liquid nitrogen and stored at -80°C prior to analysis.

Table 1. Sampling and analysis. A: Stations including water depth and distance from smelter for the different sampling sites. B: Samples collected and analyses performed.

A:

Station	Water depth, m	Water depth, sledge hauls, m	Distance from smelter, km
SD 1	111	100-130	0.9
SD 2	112	100-130	8
SD 3	113	100-130	21
SD 4	112	100-130	65
SS 1	5-20		0.4
SS 2	10-20		9
SS 4	10-20		68

B:

Station	Sample type	Analyses
SD 1-4	Sediments	PAH, TOC, TN, grain size
	Soft bottom fauna	Community structure
	Epibenthic invertebrates	PAH, cellular biomarkers[1]
	Fish (witch/megrim)	Cellular biomarkers[2]
SS 1-2, 4	Fish (cod/flounder)	Cellular biomarkers[3]

1: Total protein, metallothionein (MT) -like proteins, glutathione *S*-transferase (GST) and catalase.
2: EROD activity, pyrene and benzo(a)pyrene metabolites
3: EROD activity, pyrene and benzo(a)pyrene metabolites, DNA-adducts

Chemical work-up and analyses

Sediment composition. Sediments were analysed for granulometry and organic content. Particle distribution was determined by wet sieving in the grain sizes > 500 µm, 500-63 µm and < 63µm. The organic content was

determined as total organic carbon (TOC) and total nitrogen (TN), using a Carlo Erba CHN analyser after removing carbonates by acid fumes.

PAHs in sediments. PAH concentrations were determined using GC/MSD techniques. Seven deuterated internal PAH standards were added to 0.1 to 2 g of freeze-dried sediments for quantification, while retention times and SIM (selective ion monitoring) were applied to identify the PAHs. The samples were Soxhlet-extracted with cyclohexane for 16 h and purified as described by Grimmer and Bøhnke [18] by partitioning with DMF (dimethylformamide): water. The PAHs were then eluted with cyclohexane from columns filled with 5 g of silica gel deactivated with 15 per cent water. If necessary, sulphur was removed by gel permeation chromatography. Standard reference material (SRM 1491) from NIST (US National Institute of Standards and Technology) was used for GC calibration and to control relative accuracy.

PAHs in epibenthic invertebrates. A modified version of the method described by Grimmer and Bøhnke [18] was used for extraction and clean-up of PAHs in biological material. Internal standards (as for sediments) were added to 10 to 20 g of sample, which was saponified by refluxing for eight hours with KOH/methanol. PAHs were extracted from the solutions using cyclohexane, and the extracts were subsequently washed with methanol:water. The extracts were purified following the same procedure as for sediments with DMF: water partitioning and silica gel chromatography. Subsamples of the homogenised material were taken to determine dry weight percentages.

Biomarkers in epibenthic invertebrates. The analyses of biomarker responses included total protein, metallothionein (MT)-like proteins, glutathione S-transferase (GST) and catalase. Invertebrate samples were

homogenised in ice cold 0.1 M K-phosphate buffer with pH 7.8, containing 1 mM glutathione, 0.1 mM PMSF, 0.15 M KCl and 5 per cent glycerol, using a Potter-Elvehjem teflon-glass homogeniser. An aliquot of each homogenate was transferred to an Eppendorf tube which was centrifuged at 10 000 x g at 4°C for 30 minutes. The remainder of the homogenate was stored at -20°C for subsequent PAH analyses. The supernatant (containing cytosol and microsomes) was mixed, transferred to a cryotube and frozen in liquid nitrogen. Analyses for total protein in this fraction were carried out according to the method described by Bradford [19]. In addition to being used to standardise biomarker responses, protein was used to determine protein - wet weight ratios for the different animals and tissues. Heat-stable sulphydryl-rich proteins (hereafter referred to as MT-like proteins) in the supernatant fractions were analysed by differential pulse polarography on heat-denatured samples (95°C for 3 min), according to the method described by Olafson and Olsson [20]. GST was analysed using CDNB (1-chloro-2,4-dinitrobenzene) as a substrate as described by Habig et al. [21], and catalase as described by Livingstone [22].

Biomarkers in fish. The fish samples were analysed for EROD activity, total protein, pyrene and benzo(a)pyrene metabolites in bile and DNA adducts in the liver (only *Platichthys flesus L.*). Liver samples were homogenised in ice cold 0.1 M Na-phosphate buffer, pH 7.4, with 0.15 M KCl, 1 mM EDTA and 1 mM DTT, using a Potter-Elvehjem teflon-glass homogeniser. Microsomes were prepared by a two-step centrifugation procedure as described in Goksøyr [23], and resuspended in 0.1 M Na-phosphate buffer, pH 7.4, with 20 per cent glycerol. Cytochrome P4501A activity was determined as 7-ethoxyresorufin O-deethylase (EROD) activity in the microsomal fraction, according to Stagg and Addison [24], with resorufin (megrim and witch) or rhodamine (flounder and cod) as internal standards, assay temperature 20°C, and assay pH 7.4. The analysis for microsomal

protein was as described by Bradford [19] for megrim and witch, and Lowry et al. [25] for flounder and cod. Fluorescent metabolites of benzo(a)pyrene and pyrene in bile were measured as described by Ariese et al. [26] with modifications as described by Beyer et al. 1996. The fluorescence of each bile sample was standardised based on biliverdin content. Biliverdin was determined spectrophotometrically at 650 nm on a Molecular Devices Tmax plate-reader using vertebrate biliverdin as the standard. DNA adducts were determined as described by Ericson et al. [28], basically following the method described by Reichert and French [29].

Numerical analyses

In the soft-bottom fauna, species numbers and densities were ascertained for each grab sample and for pooled samples from stations. Community diversity and constancy were calculated according to the Shannon-Wiener H' (log-base 2) and Pielou's J. To assess faunal similarities between stations and relationships to environmental factors, Canonical Correspondence Analysis (CCA) was used [30, 31]. Analysis variables were supplied for sediment characteristics (grain sizes, TOC, TN, C/N ratio), sediment PAHs and topography (distance from smelter outfall, distance from river inflow points). The procedure of 'forward selection' and Monte Carlo permutation tests were used to rank the variables by importance and to identify a subset of significant variables which best correlated to species distribution. Species data figures were transformed using square roots, while environmental variables with skewed distributions (distances, PAHs) were transformed using logarithms.

PAH profiles, i.e. the relative distribution of individual compounds accumulated in invertebrates, were compared with sediment profiles using Principal Component Analysis (PCA). The analysis was performed as

'standardised PCA', eliminating the effects of scale in order to focus on relative patterns. All data were transformed logarithmically prior to analysis.

The cellular responses in the invertebrates at the four sites were compared using ANOVA. The homogeneity of variances was checked and variables were transformed when necessary. When significant differences were detected between stations, Dunnett's test [32] was used to compare potentially affected stations with reference stations. EROD in fish livers and fluorescent bile metabolites were analysed using Kruskal-Wallis' non-parametric analysis of variance.

CCA and PCA were carried out using CANOCO, version 3.10 software [33, 34]. Plots from the analyses were made using the drawing program CanoDraw 3.0 [35].

RESULTS

Sediment composition

All stations had a grey to light grey fine-grained sediment with a mud content (i.e. material <63 μm) of 37 to 74 per cent (Table 2). The sediments were similar at SD1 and SD2 and finer than at SD3 and SD4, where the sediment contained a substantial fraction of fine sand. The organic carbon content was moderate to low (6.0 to 14.7 mg g^{-1}). The carbon to nitrogen (C/N) ratio decreased with distance from the head of the fjord. There was good concordance between the replicate samples.

PAHs in sediments

All stations in the Sunndalsfjord proper (SD1-3) were affected by smelter generated PAHs (Table 2). The combustion origins of the PAHs are indicated by the percentage of high molecular weight, potentially carcinogenic PAHs (CPAH). The PAH concentration at the reference station (SD4) was close to what is considered a natural background level (<300 μg/kg "total" PAH) in Norwegian fjords [10, 17]. The concentration at the innermost station (SD1) was about 60 times the background level.

Table 2. Granulometry (per cent), organic carbon and nitrogen (mg g^{-1} dry weight) and dicyclic and polycyclic aromatic hydrocarbons (µg kg^{-1} dry weight) content in surface sediment (0-2 cm). Figures given are mean values and mimimum-maximum values (in parentheses) of four parallels.

	Abbrevation	SD 1		SD 2		SD 3		SD 4	
Coarse sand	**>500 µm**	1	(1-2)	0	(0-1)	4	(2-5)	1	(1-2)
Fine sand	**500-63 µm**	29	(26-35)	25	(23-26)	60	(55-65)	46	(43-49)
Silt/clay	**< 63 µm**	69	(64-73)	74	(73-76)	37	(33-40)	52	(50-55)
Total organic carbon	**TOC**	14.6	(11.9-16.6)	11.4	(10.9-11.9)	6.0	(5.9-6.2)	14.7	(12.0-18.2)
Total nitrogen	**TN**	1.3	(<1.0-1.4)	1.2	(1.2-1.3)		<1.0	1.8	(1.5-2.0)
TOC/TN	**C/N**	12.3	(11.9-12.6)	9.4	(9.1-9.9)		>5.9	8.3	(7.5-9.1)
Naphtalene	**Nap**	199	(146-323)	115	(78-196)	31	(29-33)	<0.5	
1-methylnaphtalenees	**NapC1**	199	(165-277)	133	(71-212)	65	(32-99)	65	(45-77)
Biphenyl	**Bipn**	35	(28-44)	35	(7-82)	<0,5		<0.5	
2,6-Dimethyl naphtalene	**NapC2**	100	(74-169)	95	(35-175)	40	(33-46)	13	(4-28)
2,3,5-Trimethyl naphtalene	**NapC3**	76	(33-169)	117	(69-197)	40	(39-41)	11	(3-20)
Acenaftylene	**Acnle**	27	(17-45)	31	(14-79)	15	(3-29)	9	(6-14)
Acenaftene	**Acne**	279	(224-314)	174	(144-223)	31	(17-43)	12	(9-13)
Fluorene	**Flo**	130	(101-156)	73	(57-89)	25	(16-38)	<0.5	
Phenanthrene	**Phe**	1051	(758-1242)	466	(372-583)	58	(38-76)	33	(25-48)
Anthracene	**Ant**	166	(134-190)	89	(77-104)	34	(26-40)	2	(2-2)
1-methyldibenzothiophene	**DBTC1**	28	(22-38)	14	(10-20)	6	(5-7)	2	(2-2)
1-methylphenanthrene	**PheC1**	286	(263-301)	124	(102-149)	20	(16-26)	7	(4-16)
DBTC2	**DBTC2**	28	(17-47)	18	(7-37)	4	(4-4)	4	(3-5)
PheC2	**PheC2**	88	(72-103)	42	(34-53)	9	(6-12)	10	(10-10)
Fluoranthene	**Flu**	1603	(1359-1882)	703	(549-899)	117	(89-139)	45	(33-65)
Pyrene	**Pyr**	1243	(1036-1441)	534	(429-671)	71	(52-89)	14	(9-24)
DBTC3	**DBTC3**	14	(3-25)	5	(4-6)	7	(7-7)	<0.5	
Benz(a)antracen	**BaA***	1327	(1095-1577)	510	(382-687)	64	(50-83)	18	(12-22)
Chrysene	**Chr**	1947	(1470-2699)	692	(599-814)	94	(73-113)	35	(24-43)
Benzo(b)fluoranthene	**BbF***	3065	(2451-3607)	1267	(1020-1518)	170	(129-200)	74	(53-89)
Benzo(j,k)fluoranthene	**BjkF***	812	(608-949)	373	(292-440)	58	(52-68)	34	(23-42)
Benzo(e)pyrene	**BeP**	1180	(948-1375)	542	(460-631)	98	(72-117)	57	(38-72)
Benzo(a)pyrene	**BaP***	1486	(1170-1784)	602	(488-815)	78	(66-87)	26	(17-32)
Perylene	**Per**	347	(281-410)	147	(116-191)	32	(29-34)	19	(10-26)
Indeno(1,2,3cd)pyrene	**Ind***	1677	(1352-2055)	741	(612-912)	91	(69-103)	51	(26-73)
Dibenz.(a,c/a,h)anthracene	**DBA***	356	(232-495)	152	(69-232)	33	(32-34)	12	(5-24)
Benzo(ghi)perylene	**BP**	1226	(992-1470)	562	(487-673)	81	(65-89)	42	(30-50)
	Sum dicyclic 1)	608	(449-982)	466	(248-862)	120	(32-206)	86	((48-121))
	Sum NPD 2)	1008	(711-1835)	611	(384-814)	139	(60-220)	107	(81-154)
	Sum PAH22 3)	18364	(15036-21459)	7856	(6783-9659)	1167	(914-1326)	490	(321-669)
	% CPAH 4)	48	(46-49)	46	(43-48)	41	(38-44)	43	(41-46)

1) Naphthalene through C3-naphthalene
2) Naththalene, phenanthrene, dibenzothiophene and their C1-, C2- and C3-alkyl homologs
3) Acenaphtalene through benzo(ghi)perylene
4) Compounds potentially carcenogenic to humans according to IARC (1987), i.e. categories 2A+2B (possibly and probably carcinogenic). Compounds are marked with an asterix. For BDA only the (a,h,) isomer

Faunal composition and relation to environmental variables

All stations supported a rich macrofauna. The number of species varied between 98 and 127, while densities ranged from 3500 to 5700 individuals per m2 (Table 3). The highest densities were recorded at the two innermost fjord stations. The Shannon-Wiener diversity index (H') ranged from 4.3 to 5.6, showing increasing values from the innermost stations to SD3 and the reference station SD4. Polychaetes constituted the most important group, accounting for 60 to 80 per cent of the total number of individuals, although bivalves, sipunculids and echinoderms were also important. The most prominent species were the polychaetes *Paramphinome jeffreysii*, *Polydora sp.*, *Pseudopolydora sp.* and *Heteromastus filiformis*, and the bivalve *Thyasira equalis* (Table 4). Some of the relatively abundant taxa were not found at SD1. In particular, there were very few sipunculids and echinoderms lacked totally (Table 3).

Table 3. Faunal attributes of soft bottom macrofauna. H' = Shannon-Wiener index (log 2); J = Pielou's evenness; % abund. = per cent abundance of major taxonomic groups (P = Polychaeta; M = Mollusca; C = Crustacea; S = Sipunculida; E = Echinodermata). Species figures show total number of species at the stations and miniimum-maximum number of species in individual grab samples (in parentheses). Density values given are average ± SE.

Stn	Species	Ind m^{-2}	H'	J	% abund. P/M/C/S/E
SD 1	98 (52-62)	4715 ± 284	4.26	0.65	80/14/4/0/0
SD 2	124 (64-78)	5703 ± 462	4.99	0.72	60/24/6/5/2
SD 3	127 (59-79)	3500 ± 123	5.58	0.80	57/23/5/7/5
SD 4	115 (63-72)	3878 ± 269	5.55	0.81	69/13/3/7/5

Table 4. Densities (ind m^{-2}) of the most important species in macrofaunal samples. All species which are among the top-ten abundance dominants at one or more stations are shown.

	SD 1	SD 2	SD 3	SD 4
NEMERTINEA indet	75	155	80	95
POLYCHAETA				
Paramphinome jeffreysii (McIntosh)	545	355	123	175
Levinsenia gracilis (Tauber)	5	3	80	190
Polydora sp	-	550	15	10
Prionospio sp	115	-	3	338
Pseudopolydora sp	1508	885	225	103
Spiophanes kroeyeri Grube	18	35	98	128
Caulleriella sp	105	18	10	45
Scalibregma inflatum Rathke	95	18	3	3
Heteromastus filiformis (Claparede)	338	343	333	60
Notomastus latericeus Sars	123	33	3	90
Amythasides macroglossus Eliason	70	113	38	138
Eclysippe vanelli (Fauvel)	15	18	118	135
Melythasides laubieri Desbruyeres	20	60	185	28
Pista cristata (O.F.Mueller)	90	105	83	23
Streblosoma intestinalis M.Sars	15	8	20	138
Terebellides stroemi M.Sars	10	75	38	113
Chone sp	98	13	18	20
BIVALVIA				
Thyasira equalis (Verrill & Bush)	450	673	93	73
Thyasira ferruginea (Forbes)	35	138	233	198
Abra nitida (Mueller)	35	85	120	15
Kelliella miliaris (Philippi)	3	273	78	5
SIPUNCULIDA				
Onchnesoma steenstrupi Koren & Danielssen	-	238	230	248

CCA were performed for 87 species, omitting species with abundances of less than 0.1 per cent of the total. The analysis exhibited a clear station-related pattern with replicate grab samples closely grouped (Figure 2). Station SD1 was distinguished on the first axis, while the other stations were distinguished by geographical location on the second axis. In total, the environmental variables accounted for 61 per cent of the variance in the species data (Table 5). The environmental variables representing distance from river, distance from effluent outfall, the C/N ratio and PAH

concentration accounted for the highest fractions of species variation. The two distance variables each accounted for about 25 per cent of the variance. Through the forward selection procedure, distance from river, benzofluoranthenes, TOC and fine sand were found to constitute the best combination of variables. The individual PAHs, sum PAH, sum NPD and dicyclic compounds appeared to account for largely the same species patterns, but the naphtalenes and the dicyclic compounds explained somewhat less variation than the heavier compounds.

The ordination diagram of the two first axes displays the most important environmental relationships (Figure 2A). Station SD1 is clearly distinguished by its location relative to river inflow and by the C/N ratio, as well as by distance from smelter outfall and the PAH components. Stations SD2-4 are also arranged along the PAH gradient, but to some extent also along gradients based on nitrogen (TN) and grain size composition. TOC showed a different pattern, featuring high values at SD1 and SD4. The polychaetes *Scalibregma inflatum*, *Pseudopolydora* and *Heteromastus filiformis*, and the bivalve *Thyasira equalis* may be most clearly associated with elevated PAH levels and high C/N ratios (Figure 2B). The species associated with low PAH values were e.g. the polychaetes *Paraonis gracilis*, *Streblosoma intestinale* and *Eclysippe vanelli*, the sipunculid *Onchnesoma steenstrupi* and echinoderms (*Ophiura*).

The eigenvalues of the extracted axes and the total species variance in the analysis (= total inertia) were low, however, reflecting that the data were rather homogeneous. In general, eigenvalues >0.3 indicate strong gradients [31]. In a separate analysis (results not shown) which decomposed the inertias for stations and samples, respectively, it was estimated that 60 per cent of the species variation was attributable to differences between stations and 40 per cent to variation among replicate samples. No significant

relationships were found between replicate samples and the environmental variables. This indicates that the explained variance was related to differences between the stations, while very little was related to variation between the replicates.

Table 5. Canonical correspondence analysis (CCA) for soft bottom macrofauna showing eigenvalues, extracted variance and correlation to environmental variables for the first axes. The 'forward selection' procedure was used to rank environmental variables by importance. Inertia: extracted variance attributable to each individual variable; Added inertia: added variance accounted for by variables selected one by one in sequence. Variables found significant ($p<0.05$) in Monte Carlo permutation tests were selected. Abbreviated variable names (in parentheses) refer to Fig. 2.

Axis	1	2	3	4	Total [a]
Eigenvalue	0.19	0.15	0.07	0.03	0.44
Extracted variance (%)	26.2	20.5	9.5	4.6	60.8
Environmental correlation	0.99	0.98	0.96	0.94	-
Forward selection	**Inertia**	**%**			**Added inertia**
Distance from river (D river)	0.19	26.2			0.19
Distance from smelter (D smelt)	0.18	24.8			-
C/N-ratio (C/N)	0.17	23.4			-
Benzofluoranthenes (BFs)	0.17	23.4			0.14
Benzo(a)pyrene	0.17	23.4			-
Benzo(ghi)perylene	0.17	23.4			-
Phenanthrene	0.17	23.4			-
Sum PAH (Sum PAH)	0.17	23.4			-
Sum NPD (Sum NPD)	0.15	20.7			-
Sum dicyclic (Sum Dic)	0.13	17.9			-
Naphtalene C1 (NapC1)	0.13	17.9			-
Fine sand (Finesand)	0.12	16.6			0.04
Silt-clay (Siltclay)	0.12	16.6			-
TOC (TOC)	0.11	15.2			0.07
TN (C/N)	0.10	13.8			-
Coarse sand (Crs sand)	0.07	9.7			-
Sum					0.44

a: The total inertia of the species data (calculated in CA) was 0.73.

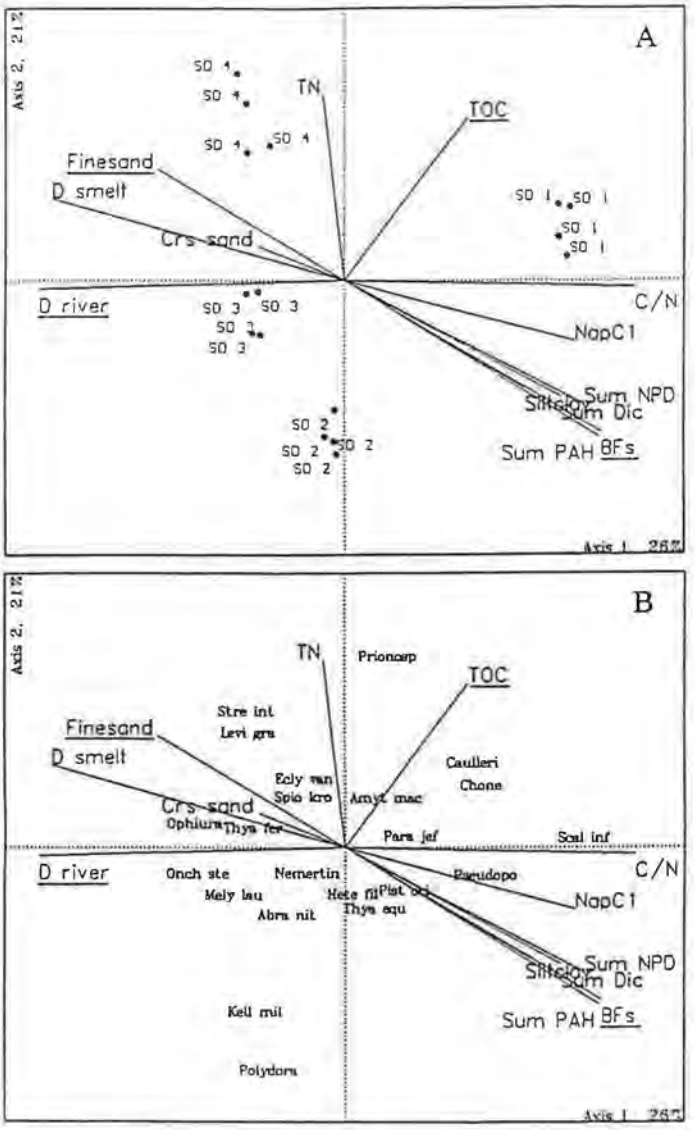

Fig. 2. Canonical Correspondence Analysis of soft-bottom macrofauna. A. Biplot of samples and environmental variables. Sample labels indicate station and replicate sample numbers. The environmental variables are represented by vectors pointing towards the maximum increase in the variable. Long vectors represent strong trends, and the angle between pairs of vectors approximates the correlation between the respective variables. Underlined variable names indicate active variables selected by 'forward selection',. Other variables in the diagram were entered as passive variables. B. Biplot of species and environmental variables. Species names are given in full in Table 4. Some species were omitted from the diagram for clarity. The plot displays 47 per cent of the total species variation (total inertia) and 77 per cent of the explainable variation.

PAH accumulation in epibenthic invertebrates

All invertebrates accumulated PAHs that coincided with sediment PAH levels, that is, showing the highest tissue concentrations at the innermost fjord stations (Table 6). Among the species, tissue concentrations decreased in the following order: *Stichopus tremulus* (deposit feeder) > *Ascidia sp.* and *Chlamys septemradiata* (filter feeders) > *Psilaster andromeda* (carnivore). The sea star *P. andromeda* was not found at the innermost station near the source, while ascidians were not found at the reference station. Low molecular weight compounds were generally below the analytical detection limit, with the exception of phenanthrene.

Table 6. Concentrations (µg kg^{-1} dry weight) of selected polycyclic aromatic hydrocarbons in epibentic invertebrates. Sum of dicyclic compounds, total sum (PAH_{22}) and % CPAH calculated as in Table 2. Concentrations represent a homogenate of 5-10 individuals.

Organism	*Psilaster andromeda*			*Ascidia* sp.			*Stichopus tremulus*				*Chlamys septemradiata*			
Station/ compound	**SD2**	**SD3**	**SD4**	**SD1**	**SD2**	**SD3**	**SD1**	**SD2**	**SD3**	**SD4**	**SD1**	**SD2**	**SD3**	**SD4**
Phe	<0.5	<0.5	<0.5	23	9	7	5	6	3	<0.5	8	11	6	<0.5
Flu	6	5	4	40	16	12	13	11	5	5	16	21	17	4
Pyr	<0.5	<0.5	<0.5	26	10	8	9	8	3	<0.5	12	15	11	<0.5
BaA	6	<0.5	<0.5	26	10	6	17	9	4	3	15	13	9	2
Chr	<0.5	<0.5	<0.5	37	15	10	29	12	6	3	25	23	14	3
BbF	5	<0.5	4	95	33	25	106	72	36	30	52	48	47	15
BjkF	3	<0.5	<0.5	57	24	16	75	47	25	21	32	27	25	11
BeP	<0.5	<0.5	<0.5	64	27	21	102	52	29	22	42	37	38	11
BaP	6	<0.5	<0.5	57	19	12	67	35	17	10	25	24	19	5
Per	<0.5	<0.5	<0.5	14	5	3	21	10	7	9	9	9	9	3
Ind	6	<0.5	<0.5	89	39	25	94	67	36	29	29	26	18	10
DBA	<0.5	<0.5	<0.5	27	10	10	32	21	11	7	9	8	6	3
BP	<0.5	<0.5	<0.5	102	35	38	216	98	55	27	25	24	17	8
Sum dicyclic	<2.5	125	<2.5	<2.5	5	<2.5	<2.5	47	28	44	<2.5	2	<2.5	<2.5
Sum PAH_{22}	37	5	9	671	253	193	797	450	237	168	304	295	239	75
% CPAH	66	0	50	52	54	49	49	56	54	59	53	50	52	62

The accummulation relative to sediment concentrations expressed as tissue-to-sediment ratios (TSRs), were in diametric contrast to the concentration gradient and increased in particular from SD1 and SD2 to SD3 (Table 7). Generally, the TSRs were elevated by a factor of 4 to 10 at SD3 compared with SD1. TSRs also differed from compound to compound, and increased roughly proportional to the molecular weight of the individual compounds. The highest TSRs were recorded for high molecular weight compounds (benzofluorathenes to benzo(ghi)perylene) in the deposit feeder *Stichopus tremulus*.

Table 7. Tissue-to-sediment ratios ($TSR*10^{-2}$, normalised to dry weight) of invertebrates.

Organism	*Ascidia* sp.			*Stichopus tremulus*				*Chlamys septemradiata*			
Station/ compound	**SD1**	**SD2**	**SD3**	**SD1**	**SD2**	**SD3**	**SD4**	**SD1**	**SD2**	**SD3**	**SD4**
Phe	2,2	1,9	11,9	0,4	1,2	4,4	n.c.	0,7	2,5	10,8	n.c.
Flu	2,5	2,3	9,9	0,8	1,6	4,7	10,6	1,0	3,0	14,1	9,9
Pyr	2,1	1,9	10,9	0,8	1,5	4,6	n.c.	1,0	2,9	14,9	n.c.
BaA	2,0	2,0	8,7	1,3	1,7	5,7	14,7	1,1	2,5	13,5	13,5
Chr	1,9	2,2	11,0	1,5	1,7	6,2	9,1	1,3	3,3	14,6	8,1
BbF	3,1	2,6	14,9	3,5	5,7	21,4	40,7	1,7	3,8	27,7	20,8
BjkF	7,0	6,3	28,2	9,2	12,5	42,7	60,6	4,0	7,2	42,7	31,0
BeP	5,4	5,0	21,1	8,7	9,5	30,1	38,9	3,5	6,7	38,9	18,5
BaP	3,8	3,2	16,0	4,5	5,8	21,9	36,6	1,7	4,0	24,2	18,7
Ind	5,3	5,3	26,9	5,6	9,1	40,0	56,9	1,7	3,5	20,3	19,9
DBA	7,7	6,6	30,0	9,1	13,6	33,1	57,2	2,5	5,3	17,9	23,7
BP	8,3	6,2	47,2	17,6	17,5	67,4	65,4	2,0	4,3	20,9	18,4

n.c.= Not calculated due to concentration in organism below detection limit.

The PCA comparing the relative distribution of PAHs (PAH profile) in organisms and sediments indicated that each species had a specific profile, and that species profiles differed from sedimentary profiles. In the plot, this was illustrated by the individual samples being arranged in groups by

species and sediment (Figure 3). The arrangement of the variables (PAHs) showed that the organisms had a relatively higher representation of intermediate and high molecular weight PAHs. Remarkably, the deposit feeder *Stichopus tremulus* had the profile which differed most from the sediment, while the suspension feeders *Chlamys septemradiata* and *Ascidia* had profiles with less deviation, but with a relative preponderance of intermediate and high molecular weight compounds, respectively. The difference between organisms and sediment was quite strong. It was depicted on the first axis (PC1), which captured as much as 85 per cent of the variance in the data. The distinction between *S. tremulus* and *C. septemradiata* (PC2) represented a far less important pattern. It may be noted that the presence of consistent profiles implies that the relative distribution of PAHs did not change much along the concentration gradient in the fjord, suggesting that the differences among species depend on species-specific processes.

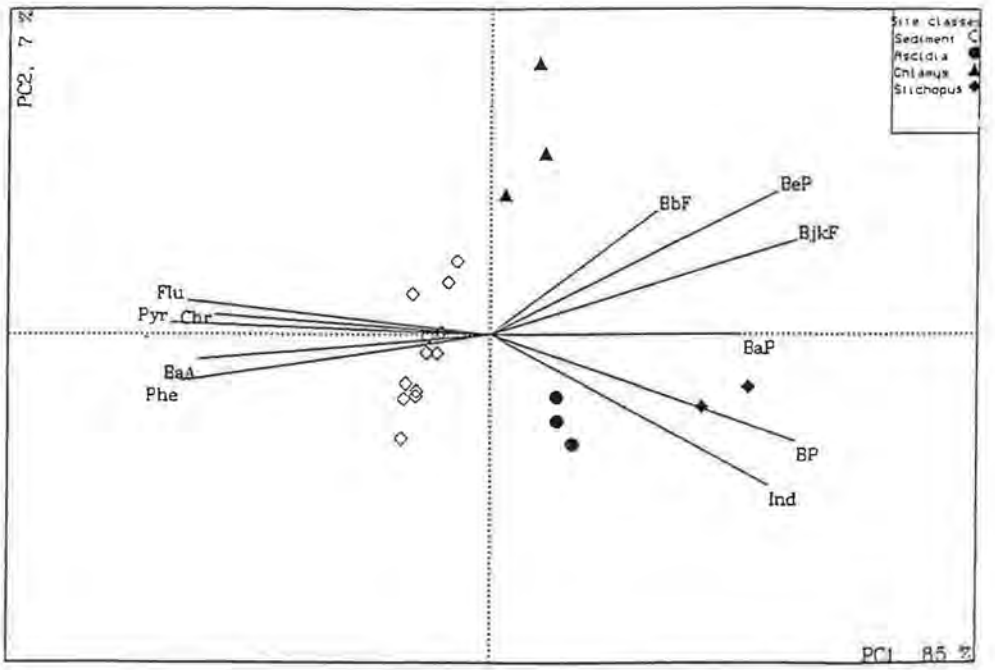

Fig. 3. Standardised PCA showing PAH profile pattern of sediments and invertebrates from the Sunndalsfjord based on 23 samples and 12 compounds. Compound names are abbreviated as given in Table 2.

Biomarkers in epibenthic invertebrates

The biomarkers did not show obvious trends which could be related to PAH sedimentary levels for the species *Ascidia sp.*, *Stichopus tremulus* and *Chlamys septemradiata*. However, there was a lowered protein-to-wet-weight ratio for *S. tremulus* at SD1 and a lowered GST for *C. septemradiata* at SD1 and SD2 compared with SD4 (Table 8). *Ascidia sp.* also tended to have reduced concentrations of protein and elevated levels of MT-like proteins at SD1 compared with the stations more distant from the smelter. The biomarker pattern was different in the sea star *P. andromeda*, as GST decreased significantly in proportion to distance from the source and MT-like proteins and catalase decreased from station SD2 to SD3. The trend for the latter biomarkers was, however, reversed at the reference station, showing values comparable to SD2. The sea star was absent at the station with the highest PAH levels (SD 1). None of the species-specific variability could be attributed to sex or size (results not shown).

Table 8. Biomarker responses in soft bottom epibenthic invertebrates. Results are presented as mean ± standard deviation (n). Asterisk (*) indicate reponses at stations SD1-3 found to be significantly different ($p \leq 0.05$) from the reference station SD4 in Dunnett's test following ANOVA. Measurement units: Protein/wwt (mg/g), MT-like proteins (MT-ekv/mg proteins), catalase (mmol/min/mg protein), glutathione *S*-transferase (nmol/min/mg protein).

Species	Variable	SD 1	SD 2	SD 3	SD 4
Ascidia sp.	protein/wwt	2.86 ± 0.42 (8)	3.94 ± 1.50 (5)	4.18 ± 2.19 (4)	-
	MT-like proteins	25.84 ± 13.64 (8)	10.10 ± 2.48 (5)	17.56 ± 14.15 (4)	-
	catalase	0.10 ± 0.18 (8)	0.21 ± 0.18 (5)	0.05 ± 0.07 (4)	-
Stichopus tremulus	protein/wwt	17.37 ± 4.53 (9)*	27.08 ± 11.52 (12)	25.74 ± 11.19 (12)	26.82 ± 5.90 (13)
	catalase	0.03 ± 0.03 (9)	0.04 ± 0.04 (12)	0.03 ± 0.04 (12)	0.05 ± 0.05 (13)
	glutathione *S*-transferase	92.72 ± 57.51 (5)	113.4 ± 49.86 (5)	50.07 ± 17.94 (5)	91.92 ± 44.71 (5)
Psilaster andromeda	protein/wwt	-	118.7 ± 7.31 (5)	102.7 ± 30.31 (12)	99.65 ± 35.11 (10)
	MT-like proteins	-	6.95 ± 1.85 (5)	5.32 ± 1.79 (12)*	7.31 ± 2.03 (10)
	catalase	-	0.76 ± 0.10 (5)	0.59 ± 0.15 (12)*	0.79 ± 0.16 (10)
	glutathione *S*-transferase	-	406.5 ± 128.5 (5)*	356.8 ± 79.37 (5)	215.3 ± 55.45 (5)
Chlamys septemradiata	protein/wwt	53.91 ± 14.95 (26)	55.49 ± 10.97 (10)	44.19 ± 12.90 (11)	45.52 ± 10.14 (11)
	catalase	0.06 ± 0.01 (26)	0.05 ± 0.02 (10)	0.10 ± 0.04 (11)	0.08 ± 0.05 (11)
	glutathione *S*-transferase	553.3 ± 115.3 (5)*	551.7 ± 181.2 (5)*	903.4 ± 318.2 (5)	921.5 ± 240.2 (5)

Biomarker responses in fish

Biomarker responses differed between deep water fish (megrim and witch) and shallow water species (flounder and cod). Megrim and witch showed a trend, although not significant, towards decreased levels of bile metabolites proportional to distance from the smelter (Figs 4, 5). In witch, the trend was also reflected in EROD activity, while there was no trend in EROD activity in megrim. In flounder and cod, PAH metabolites and hepatic EROD activity decreased from station SS1 near the smelter to SS2, but increased at the reference station, showing values similar to those near the smelter (Figs 6, 7). The ratio between pyrene and benzo(a)pyrene metabolites in bile from flounder indicated a local source of PAH for flounder collected at reference station SS4. Hepatic DNA adducts were below the detection limit (0.2 nmol adducts/mol nucleotides) in all the flounder examined (5 from each site, results not shown). Hence, no effects of PAHs on the genetic material were observed in this stydy.

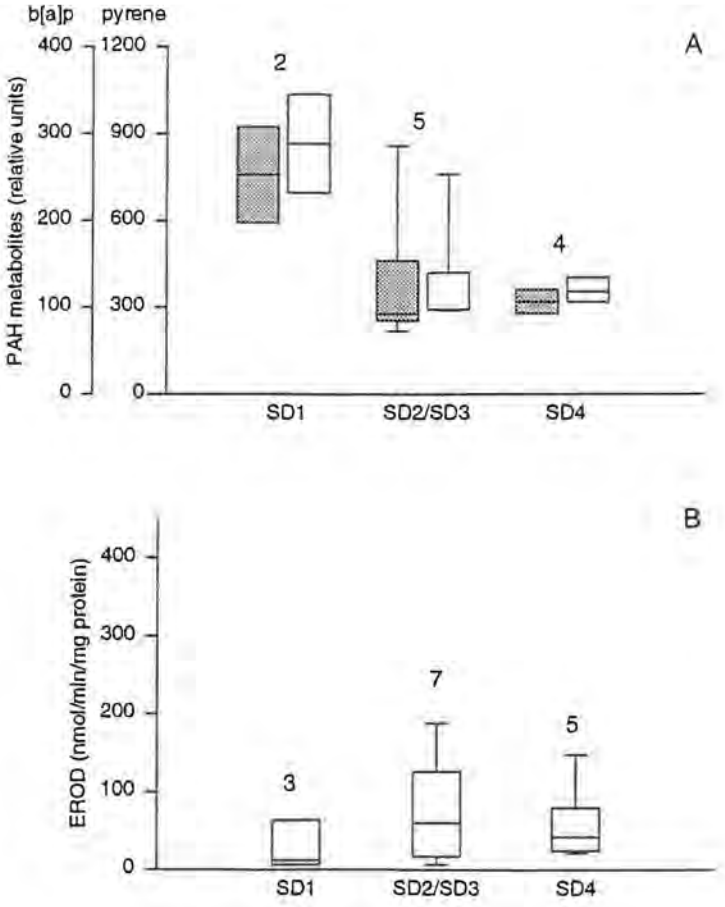

Fig 4. Biomarker responses in megrim (*Lepidorhombus whiffiagonis*). A. fluorescent bile metabolites; benzo(a)pyrene (379:425 nm) - grey, pyrene (341: 383 nm) - white. B. Cytochrome P4501A activity (EROD). Median with quartiles (box) and 10/90-percentiles (whiskers). Numbers indicate analysed individuals.

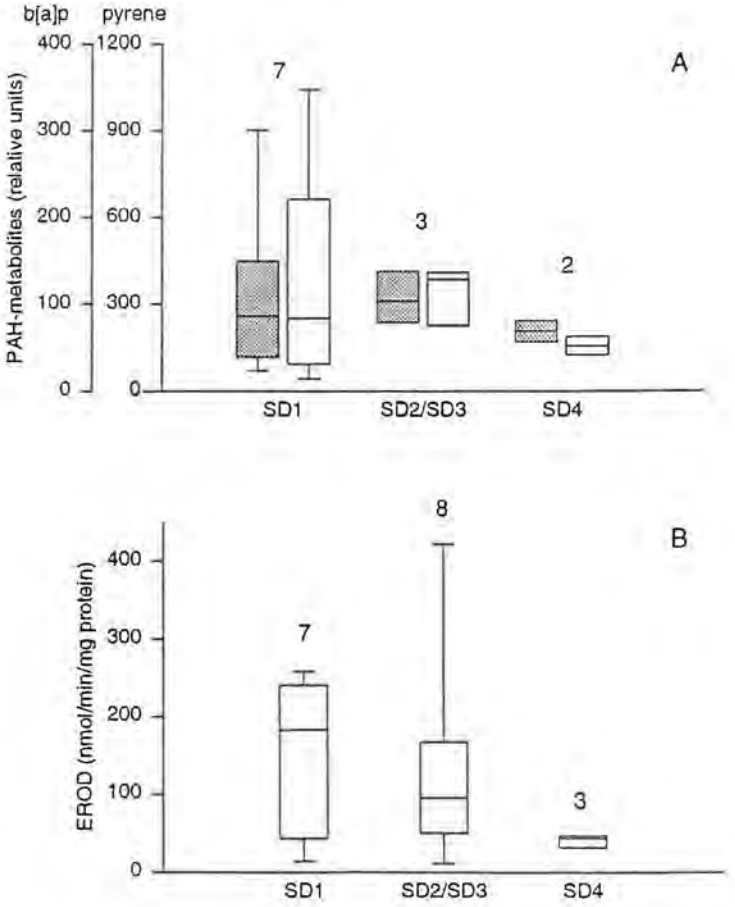

Fig. 5. Biomarker responses in witch (*Glyptocephalus cynoglossus*). A. fluorescent bile metabolites; benzo(a)pyrene (379:425 nm) - grey, pyrene (341: 383 nm) - white. B. Cytochrome P4501A activity (EROD). Median with quartiles (box) and 10/90-percentiles (whiskers). Numbers indicate analysed individuals.

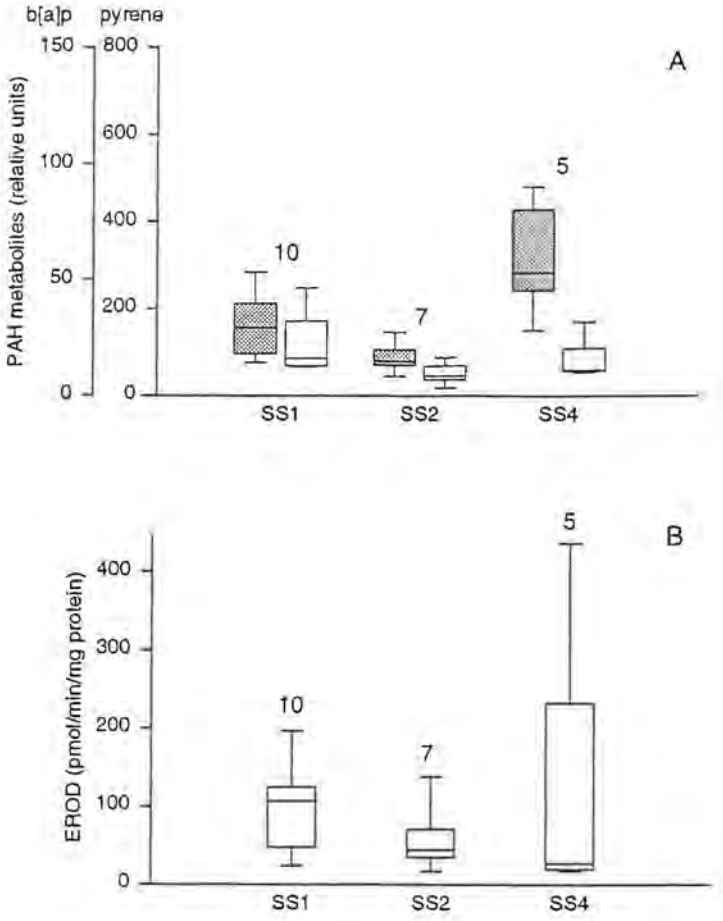

Fig. 6. Biomarker responses in flounder (*Platichthys flesus*). A. fluorescent bile metabolites; benzo(a)pyrene (379:425 nm) - grey, pyrene (341: 383 nm) - white. B. Cytochrome P4501A activity (EROD). Median with quartiles (box) and 10/90-percentiles (whiskers). Numbers indicate analysed individuals. Note different axes for bile metabolites compared with Figures 4 and 5.

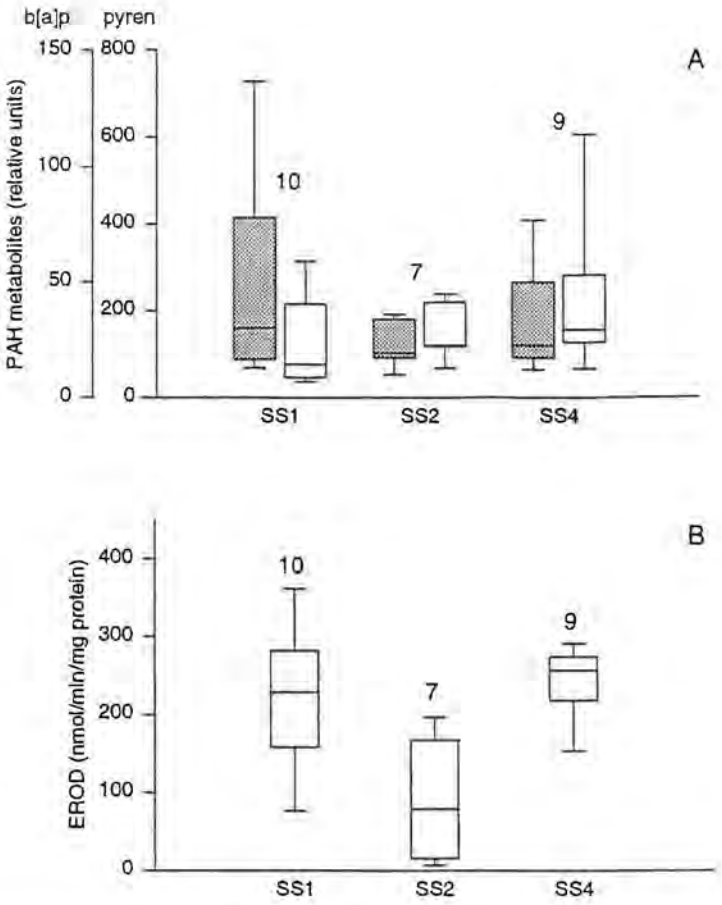

Fig. 7. Biomarker responses in Atlantic cod (*Gadus morhua*). A. fluorescent bile metabolites; benzo(a)pyrene (379:425 nm) - grey, pyrene (341: 383 nm) - white. B. Cytochrome P4501A activity (EROD). Median with quartiles (box) and 10/90-percentiles (whiskers). Numbers indicate analysed individuals. Note different axes for bile metabolites compared with Figures 4 and 5.

DISCUSSION

The concentrations at the two innermost stations sampled in the present study (SD1, SD2) fall between the guideline limits 'effect range - low' (ERL) and 'effect range - median' (ERM), which Long et al. [36] established for sediment quality assessments. Compiling data from a number of laboratory and field studies, they observed more than a 30 per cent incidence of adverse biological effects in the range between ERL and ERM (4000-44,000 µg/kg total PAH). Nevertheless, PAH contamination in the fjord did not seem to have adverse effects on the benthic biota. This is the main conclusion drawn from soft-bottom communities as well as from biomarker analyses. Studying biological effects near a Canadian aluminium smelter, Paine et al. [4] obtained corresponding results. In a synoptic study involving contamination, toxicity and fauna (Sediment Quality Triad, see e.g. [8]), they observed no sediment toxicity and minimal effects on benthic fauna despite extremely high sediment PAH concentrations.

The limited effects on soft-bottom macrofauna correspond with results from other Norwegian smelter-affected fjords. In most fjords, no correlation has been found between faunal diversity and PAH levels, except within restricted zones in the immediate vicinity of effluent outfalls. Knutzen [15] actually suggested that the physical damage done to the habitat from particles in the effluents, rather than the PAHs, was the main determinant factor for the benthic fauna in the most affected zones. However, Oug et al. (in prep.) identified species changes along PAH gradients in the fjords which could be associated with feeding habits and the behaviour of the species. They also found a significant correlation between echinoderms and low PAH values, suggesting that the echinoderms may generally avoid PAH-contaminated areas. The absence of echinoderms at the innermost

station (SD1) in Sunndalsfjord therefore coincides with results obtained elsewhere.

The CCA confirmed that species patterns in the fjord could be related to PAH distribution, but also suggested that factors associated with the freshwater inflow of carbon materials into the sediments might be important. In Sunndalsfjord, there are generally strong correlations between sediment PAHs, TOCs and C/N ratios [16]. The carbon material represents a mixture of soot particles from the effluents, river-borne terrestrial matter and detritus from plankton production. High C/N ratios and high correlations between C/N and PAHs suggest the importance of the soot particles, which are depleted in nitrogen [16]. However, terrestrial organic matter, which also has a low nitrogen content, is brought into the fjord close to the smelter discharge point and may form a distance gradient which parallels PAH distribution. The real influence of the various components may be difficult to assess owing to the intercorrelation, but terrestrial carbon does not normally delimit echinoderms and may be ruled out as an explanation for the lack of this group at the innermost station. Rather, some echinoderms, for instance the species of the brittle star *Amphiura*, are stimulated by organic input [37]. Essentially, the biomarker analyses suggest the possible effects of PAHs, considering that the sea star *Psilaster* further out in the fjord indicated a response to PAH.

The accumulation of PAHs and their biochemical effects on invertebrates are probably related both to their feeding mode and the ability of the organisms to metabolise PAHs. The different feeding modes may imply that the organisms are exposed to differences in ambient PAHs through preferential ingestion of sediment particles. This may, at least partly explain the difference in accumulation between the invertebrates, coinciding with findings elsewhere [38]. The increase in TSRs proportional to distance from

the smelter indicate that equilibrium is not reached between the PAHs in sediments and those in the tissues of epibenthic organisms. This is so because the use of bioaccumulation factors such as TSRs assumes, in addition to equilibrium, that the different PAHs are equally bioavailable, regardless of total concentration. PAH profiles were similar at all stations. It is therefore unlikely that a larger proportion of PAHs is bioavailable in sediments with low total levels compared with highly contaminated sediments. The increase in TSRs proportional to the molecular weight of the compounds in evidence could be explained by the relationship between bioconcentration factors and hydrophobicity [39, 40]. The decrease often observed in TSRs for the PAHs with the highest K_{ow} [38] was not clear in this study.

It is well known that the ability to metabolise and excrete PAHs differs among species [41], and this may be reflected in biomarker responses. Some echinoderms, e.g. sea stars, are known to metabolise PAHs [42, 43], thereby exposing themselves to increased levels of potentially deleterious metabolites. In the present study, the sea star *Psilaster andromeda* had significantly lower levels of PAHs compared with the other invertebrates, and it was the only invertebrate shown to make biomarker responses in relation to the PAH gradient. In particular, GST activity was as could be expected from metabolising PAHs. In contrast, the other echinoderm, the sea cucumber *Stichopus tremulus*, accumulated more PAHs and showed much lower activity and no trends in GST. Presumably, *Stichopus* has little ability to metabolise PAHs, as may also seem to be the case for tunicates and the scallop *Chlamys septemradiata*.

Perhaps surprisingly, there were no strong biomarker responses in the fish, although they have a well-defined capability to metabolise PAHs. To a certain extent, however, the results were bedevilled by unexpected results at

the shallow reference station (SS4). This station may have been affected by a local unidentified source of PAH. This is also supported by the ratio between benzo(a)pyrene and pyrene metabolites in flounder, which differed compared with the Sunndalsfjord proper. The presumed local source, however, apparently had no influence on DNA adducts. It may also appear that the deep reference station (SD4) was not influenced. The low levels of fish biomarker responses contrast with the results found for caged and free-living fish in the vicinity of smelters elsewhere [27, 28], and from experiments with controlled exposure to single PAHs [44]. However, the results from experiments using commercial PAHs are not necessarily transferable to *in situ* situations In the study performed by Beyer et al. [27], fish exposed directly to scrubber water showed effects, although not when exposed to contaminated sediments. The scrubber water contains a significant proportion of PAHs in the dissolved state.

Presumably, PAH speciation is important for explaining the restricted biological effects. In Sunndalsfjord, the PAHs from the aluminium smelter are probably to a great extend bound to oxides and soot particles [11]. This association is known to strongly restrict the bioavailability of the PAHs [45, 4]. Næs & Oug [11, 16] demonstrated that the PAHs from Norwegian aluminium smelters retained their source characteristics during transport in the fjord and after incorporation into the sediments, implying resistance to microbial transformations and very limited desorption. This coincides with the results of McGroddy et al. [46], who observed that only a small fraction of soot-associated PAHs were available for equilibrium partitioning. It is generally considered that the uptake of contaminants in marine organisms via the dissolved phase is a very important route, either directly via the gills or via desorption from particles in the intestinal tract [47]. The results for the fish imply that PAH concentrations in this phase were not high enough to cause significant effects.

In conclusion, sediments contaminated by PAHs generated by the production of primary aluminium appeared to have only minor biological effects, even at highly elevated sediment concentrations. This supports the assumption that PAHs associated with soot-like structures have limited bioavailability. The present study approach involving sediment chemistry, soft-bottom macrofauna and biomarker responses in invertebrates demonstrated clearly that elevated PAH concentrations do not necessarily elicit adverse effects. The biomarkers employed showed trends not necessarily consistent with the sediment impact or with the results from the community analyses. This further points to the need for linking various single-species approaches to measurements of effects on higher levels of organisation. Finally, to determine the cause, the speciation of the chemical contaminant must be understood.

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Paper VIII

Role of Soot Carbon and Other Carbon Matrices in the Distribution of PAHs among Particles, DOC, and the Dissolved Phase in the Effluent and Recipient Waters of an Aluminum Reduction Plant

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The speciation of polycyclic aromatic hydrocarbons (PAHs) in natural waters affects both the chemical fate and the bioavailability of these compounds. PAHs may be dissolved or sorbed to particles or dissolved organic carbon (DOC). Furthermore, soot carbon has recently been shown to control the sorption of PAHs onto particles in natural waters. The present study investigated the distribution of individual PAHs among these three phases by examining the effluent and recipient waters of a Søderberg aluminum plant and evaluating the importance of soot carbon partitioning. The results showed that soot carbon may control partitioning between the dissolved and particulate phases and that the partition coefficients were orders of magnitude higher than would be expected on the basis of organic carbon−water partitioning alone. The carbon-normalized partition coefficient for the distribution of individual PAHs between DOC and the dissolved phase (K_{DOC}) exhibited no more than a weak correlation with hydrophobicity (K_{ow}) as compared with what was found for the partition coefficients for particles. This indicates that there are different sorption mechanisms at work with DOC than with particulate organic carbon and soot. In the recipient water, up to 90% of the total concentration of individual PAHs was accounted for by a nonfilterable DOC-associated phase, which was not retained by the polyurethane foam (PUF) adsorbent.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous contaminants of urban coastal marine environments. PAHs originate from both natural and anthropogenic sources such as forest fires, releases of combusted and uncombusted petroleum products, and emissions from the metallurgical industry. Due to the carcinogenic and mutagenic potential of some PAHs (*1*, *2*), they are of serious environmental concern and are considered to be priority pollutants.

The source of a contaminant affects its environmental behavior. Specifically, the initial environmental fate of PAHs derived from oil or noncombusted petroleum products would be expected to differ in terms of speciation from that of PAHs occluded in combustion particles. The physicochemical speciation will ultimately control the transport, fate, and biological availability of PAHs and thereby their environmental effects. Hence, these factors will play a major role in setting PAH discharge limits and for the design and implementation of waste treatment technology.

The production of primary aluminum based on the Søderberg technology represents an important anthropogenic source of combustion-derived PAHs. For example, in Norwegian fjords and coastal waters, concentrations of more than 1000 times background levels have been observed in sediments and organisms in the vicinity of some of these plants (*3*). The PAH problem is mainly related to the use of coal tar pitch as a binder in the production of ramming paste and anode paste and in the fabrication of anode and cathode blocks. In some cases, it also stems from the production of anodes. The PAHs are released through ventilation air and pot fumes and enter the recipient waters through subsequent seawater scrubbing of the emissions.

Numerous studies have shown a hydrophobicity-dependent particulate partitioning of PAHs between the dissolved and particulate phases in different marine environments (*4*−*6*). The equilibrium partitioning of PAHs is generally described by normalizing the distribution coefficient to organic carbon (K_{oc}). However, field investigations have reported K_{oc} values for PAHs of pyrogenic origin to be far higher than predictions based on organic matter partitioning models (*6*−*9*). These results suggest the presence of a particulate phase, such as soot, to which PAHs sorb significantly more strongly than to natural organic matter, thereby affecting the environmental speciation and effects of the compounds.

Only a few studies on physicochemical speciation have been conducted in the immediate vicinity of an aluminum smelter. Bjørseth (*10*) and Thrane (*11*) suggested that PAHs in smelter effluents occurred both in solution and in association with particles. Næs and Oug (*12*, *13*) reported a seaward shift in the PAH profile in fjord sediments near an aluminum smelter that indicated different particulate size fraction associations of individual PAHs.

Furthermore, PAHs and other hydrophobic compounds have been observed to be sorbed not only by particles but also by nonfilterable organic carbon (*14*−*16*). This fraction is normally operationally defined as dissolved organic carbon (DOC) but may contain small molecules, such as amino acids and sugars, to humic acid macromolecular aggregates. The relative sorbing capacities of the different subfractions of DOC have so far only been speculated. In this study, only the entire nonfilterable sorbed fraction of PAH will be considered and will be denoted as the DOC-associated fraction.

The DOC-associated fraction will have different properties than PAHs in the dissolved and particulate forms. DOC-associated PAHs will not be directly subjected to sedimentation in the same way as particles, so they can be transported over longer distances. Furthermore, the DOC will not be bioavailable for direct gill uptake by aquatic organisms in the same way as the dissolved PAHs (*15*). The overall environmental fate and effect of the released PAHs will

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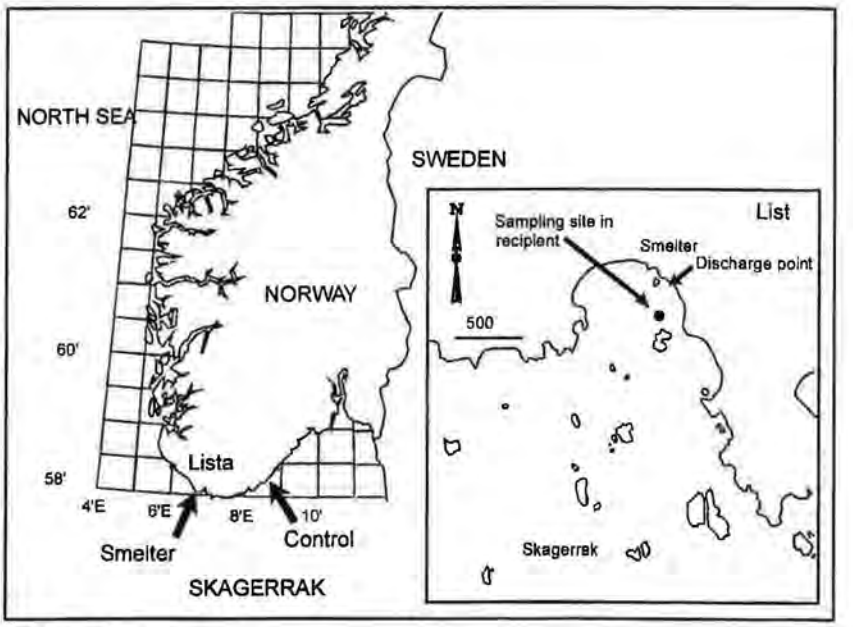

FIGURE 1. Map of the investigation area.

therefore be dependent on the distribution among all three phases.

The objective of the present study was to quantify the distribution of PAHs in the dissolved, DOC-associated, and particulate phases in the effluent and recipient waters of a Søderberg aluminum plant. Recently, a method was developed to separate organic carbon from soot carbon in sediments (*17*). This method was applied to suspended particles in the present study, making it possible to assess the impact of a stronger sorbing phase than what occurs in natural organic matter in these waters. Thus, the partition coefficients between the dissolved phase and the two sorbed phases (particulate and DOC-associated) could be calculated, and the relative influence of the DOC on the speciation of PAHs in these waters could be shown.

Materials and Methods

Sampling Site Characteristics and Water Sampling. The aluminum smelter at Lista in southern Norway has a production capacity for primary aluminum of 80 000 ton per year based on Søderberg technology. The seawater scrubbers used to clean ventilation air produce an effluent flow of approximately 9000 m^3 h^{-1}. This volume also includes a small amount of water used for general purposes within the plant. The effluent is piped into the recipient water, an open coastal embayment (Figure 1), at a depth of 1.5 m. Water samples were drawn on three occasions in June 1996 from the effluent and recipient waters (58°04.0′ N, 06°46.3′ E) and from a control station (58°22.1′ N, 08°40.5′ E). The control station was situated in coastal waters influenced by general human activities, but by no point sources. The samples from the control and recipient waters were all drawn from a depth of 0.5 m. The samples of the effluent were drawn directly from the pipeline, immediately before the effluent entered the recipient water.

An on-line filtration system was used to collect PAHs (Figure 2). It consisted of precleaned 142 mm i.d. borosilicate glass microfilter (Whatman GF/F) in a stainless steel holder for the retention of particulate matter (nominal cut off 0.7 μm). It was followed by two polyurethane foam (PUF) adsorbents (30 mm diameter, 45 mm length) in stainless steel casing for the control and recipient sites and a glass casing for the effluent water. The PUF is assumed to retain PAHs in the dissolved fraction (*18*). The filtrate from the PUF adsorbent, containing the DOC-associated fraction, was collected in 5- (for effluent water) or 10-L glass containers, after which toluene (Burdick and Jackson, glass distilled quality) was immediately added to prevent loss of PAHs. Water supply to the on-line filtration system was provided at a flow of 1 L min^{-1} by a modified Flojet pump equipped with a silicone tube. Five liter of effluent water, 30 L of recipient water, and 40 L of control station water were pumped through the system at each sampling. The filters, casings, and containers were washed with toluene and heated to 480 °C prior to use.

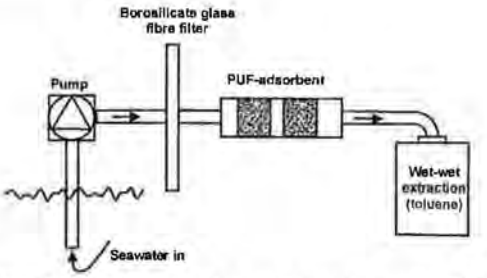

FIGURE 2. Flow diagram of the system used for sampling of the particulate, the dissolved, and the DOC-associated fraction of PAHs.

Water samples were kept dark in sealed containers. Immediately after collection, they were extracted with 50 mL of toluene per liter of sample and spiked with picene and perylene-d_{12} to serve as internal standards. They were subsequently shaken for 24 h and then left for phase separation for another 24 h. Most of the seawater was siphoned off, and the remaining solution was kept cool (4 °C) until analysis. Filters and PUF adsorbents were stored frozen at −20 °C prior to chemical analysis.

From each of the 142-mm filters, four subsamples (16 mm i.d.) were drawn to determine total particulate carbon

(PC), particulate organic carbon (POC), and particulate soot carbon (PSC). Samples were also taken to determine DOC concentrations. Filters were stored in a frozen state, and filtrates were stored at +4 °C.

Chemical Analysis. The following PAHs (abbreviations in parentheses) were analyzed: phenanthrene (Phe), anthracene (Ant), 3-methylphenanthrene (3-mePhe), 1-methylphenanthrene (1-mePhe), fluoranthene (Flu), pyrene (Pyr), 2-methylpyrene (2-mePyr), 1-methylpyrene (1-mePyr), benzo[*ghi*]fluoranthene (B[ghi]F), cyclopenta[*cd*]pyrene (Cp[cd]P), benz[*a*]anthracene (B[a]A), chrysene (Chr), benzo[*k*]fluoranthene (B[k]F), benzo[*e*]pyrene (B[e]P), benzo[*a*]pyrene (B[a]P), perylene (Per), indeno[1,2,3-*cd*]pyrene (Ind), benzo[*ghi*]perylene (B[ghi]P), and coronene (Cor). Filters and PUF adsorbents were spiked with two PAHs (perylene-d_{12} and picene) and then Soxhlet extracted for 24 h in toluene with a Dean–Stark trap for water removal. Water was removed from the liquid–liquid extracts with precombusted Na_2SO_4. The volume-reduced extracts of all the samples were treated by a dimethylformamide (DMF) cleanup procedure. The hexane solution containing the PAHs was then finally eluted on 100 × 10 mm columns containing deactivated SiO_2 (10% water w/w) with hexane as the mobile phase. The extracts were analyzed on a gas chromatograph/mass spectrometer (Hewlett-Packard 5890/5971A). Response factors and retention times were determined by analyzing a standard mixture containing all the PAH compounds analyzed, including the internal standards. This mixture was extracted and cleaned up following the same procedure as the samples. All the solvents used (toluene and hexane; Burdick & Jackson; pentane; Merck) were of glass distilled quality. Blanks were found to contain nondetectable amounts of high molecular weight (W_m) PAHs and negligible amounts of low W_m PAHs in relation to the amounts found in the samples.

Particulate Carbon and Dissolved Organic Carbon. Prior to the analyses, the filters for particulate carbon analyses were treated with HCl vapor to remove inorganic carbonates. Thermal oxidation in an excess of air at 375 °C for 24 h removed POC from the filters. The remaining carbon was thereby soot-like carbon (PSC). This method has been shown quantitatively to separate the two carbon fractions in particulate standard matrixes and in sediments (*17*). It has, however, not previously been applied on suspended particles. The filters were analyzed on a CHN element analyzer (Carlo Erba CHN). The total particular carbon (PC) was determined from the nontreated filters. POC was determined from the carbon content difference between the thermally treated filters and the nontreated filters. DOC samplers were acidified and determined using a Dohrman TOC analyzer.

Results and Discussion

Total Concentrations. The average sums of the concentrations in all three phases [filter (C_{filter}), PUF (C_{PUF}), and liquid–liquid extraction (C_{wet})], C_{tot}, at the three locations are presented in Figure 3. However, at the control location, the filtered volume was too small to accurately quantify the high molecular weight PAHs. The dilution factor from the effluent to the recipient was 34 ± 23 and did not correlate with hydrophobicity (log K_{ow} values taken from ref *19*) ($r^2 = 0.0408$, $p < 0.20$), indicating that the only process involved was the mixing of water, which is a process that affects all three phases equally. Any process significantly affecting a specific phase, such as the sedimentation of particles, would result in a dilution factor that correlates with hydrophobicity. The total concentrations at the control station were almost 3 orders of magnitude lower than in the recipient area, showing that the PAH load deposited in the recipient water by this aluminum smelter is substantial.

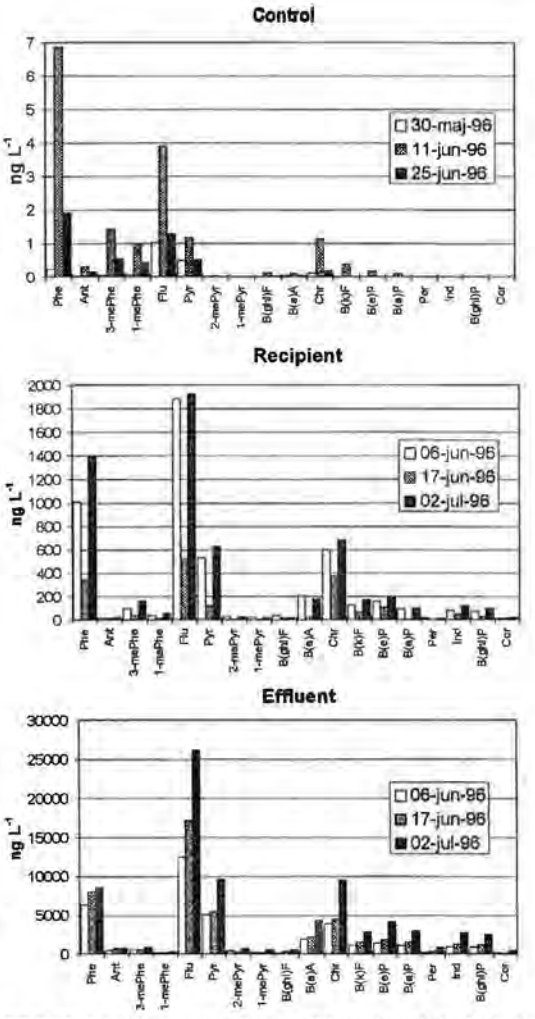

FIGURE 3. Total concentrations (sum of all three phases) of individual PAHs in the waters at the three different sampling locations.

Relative Distribution of the Three Phases Collected with Filters, PUF Adsorbent, and Wet Extraction. The relative distribution between particles, DOC, and the dissolved phase differed significantly between effluent and recipient waters. However, for each station individually the relative distribution between the phases was similar at all three sampling events (Figure 4). The variation in distribution during the different sampling occasions could not be evaluated at the control station because it was only possible to detect a few of the compounds in all three phases. The relative distribution for this station is therefore based on the total data available from all sampling occasions (Figure 4).

The pattern in the recipient waters differed from both the effluent and the control sites. It appears that the DOC in the recipient waters has a much higher sorbing capacity than the DOC at the other two locations (see also the section on partition coefficients below). The DOC concentrations were relatively similar at both locations (Figure 5), so the relative amount of the sorbing phase cannot account for the difference.

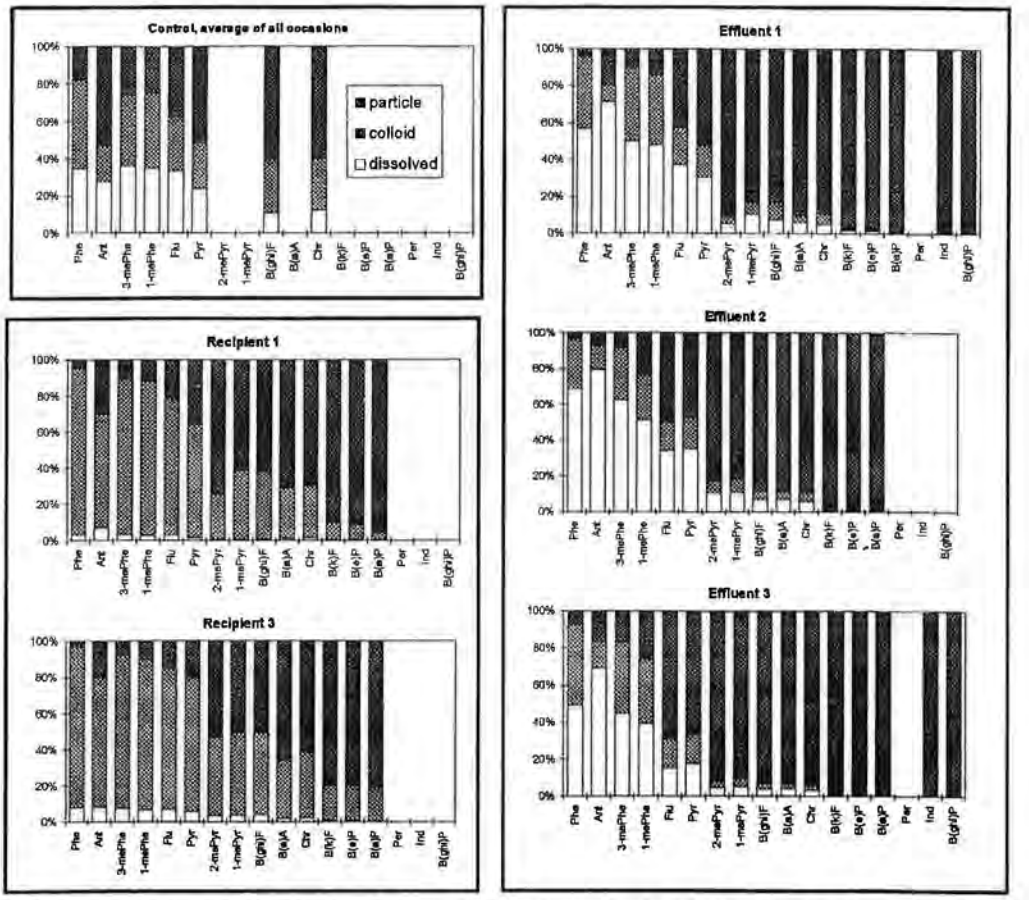

FIGURE 4. Relative distribution of individual PAHs between the three phases: dissolved, DOC-associated, and particulate. Only a few PAHs could be detected in all three phases at the control site, so all three sampling occasions were combined. On sampling occasion 2, the filter at the recipient station was damaged, so a significant portion of the particulate fraction was retained by the PUF adsorbent. These data were therefore excluded from this graph.

In regard to the recipient water, the results also indicate that the DOC-bound fraction is the dominating pool for PAHs with low W_m. A total of 60−90% of the low W_m PAHs and 10−30% of the high W_m PAHs passed through the filters and PUF adsorbent. This may have significant implications for the choice of sampling methods used to determine contaminant concentrations. In the event that only a few low W_m PAHs are selected for screening and conventional filter and PUF sampling techniques are used, C_{tot} could be underestimated by an order of magnitude.

Partition Coefficients: Dissolved Phase−Particles. It has been suggested that the partitioning of PAHs between particles and water in the aquatic environment is controlled by soot carbon rather than by organic carbon. This implies higher partition coefficients than those observed between organic carbon and water (*9*). In the present study, the partition coefficients were orders of magnitude higher than would be expected from the empirically derived relationship between log K_{ow} and log K_{oc}. The use of coal tar pitch in the Søderberg process implies that soot-like structures, i.e., large aromatic structures (*20*), may be released with the effluent water. Although the variation is high in the PSC analysis of the effluent and recipient waters, the results still imply that a large fraction of the total particulate organic carbon (PC) in these waters is not POC. Karickhoff (*21*) derived an empirical relationship between log K_{ow} and log K_{oc}:

$$\log K_{oc} = \log K_{ow} - 0.39 \quad (1)$$

A method to estimate the partition coefficient of individual PAHs for the water to soot carbon partitioning (K_{sc}) has been described by Gustafsson and Gschwend (*9*). This method takes the specific molecular sorbing mechanisms between soot and PAHs into account:

$$\log K_{sc} = \log \gamma_w - \log \gamma_s + \log (V_w/A_s) + \Delta S_\Phi /2.3R(T_m/T - 1) \quad (2)$$

where γ_w and γ_s are the activity coefficients of the molecule in water and in the sorbed state at the soot surface, respectively; V_w is the molar volume of water; A_s is the specific area of soot carbon; ΔS_Φ is the entropy of the phase transfer of the molecule; R is the gas constant; T_m is the melting point of the molecule; and T is the temperature of the system (approximately 10 °C in this study). γ_w is dependent on the subcooled liquid solubility in water ($^{sat}C_L$). The sorption of a PAH onto soot is assumed to be thermodynamically similar to the association of a PAH with a pure PAH crystal (*9*). γ_s is therefore 1. K_{sc} was calculated for all PAHs in this study except B[ghi]F and Ind, for which no estimates of T_m and

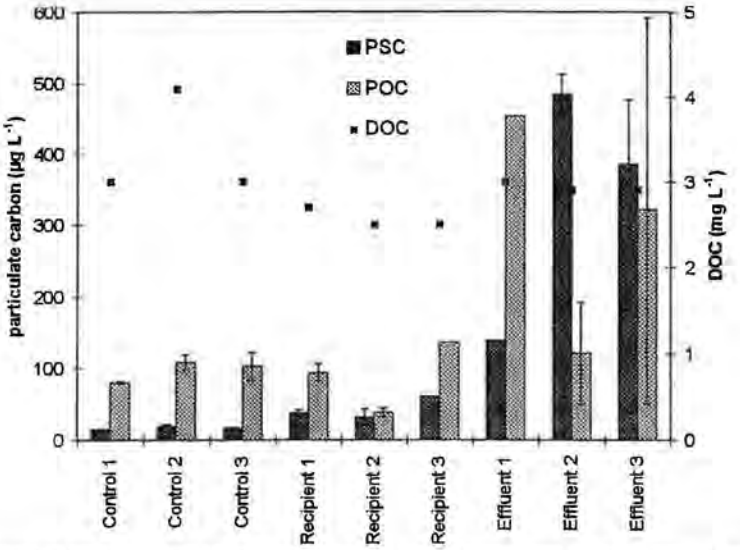

FIGURE 5. Concentrations of particulate soot-like carbon, particulate organic carbon, and dissolved organic carbon at the different locations on all three sampling occasions. The error bars indicate standard errors.

$^{sat}C_L$ was found. Literature values for $^{sat}C_L$ and T_m was taken from Miller et al. (*22*), and literature values for PAHs not included in that study were from Mackay et al. (*18*). The addition of a methyl group (in the 9 position) to anthracene was found to give a 70% decrease in $^{sat}C_L$ and a 135 K decrease in T_m (*23*). These effects were applied to the literature values of $^{sat}C_L$ and T_m for Phe and Pyr to roughly calculate K_{sc} (also for the methylated PAHs included in this study).

Assuming that Karickhoff's relationship was derived for organic carbon, it is possible to assess the relative influence of the partitioning of these two phases. Using the fractions of PSC (f_{PSC}) and the fraction of POC (f_{POC}) of the total sorbing matrix PC, the total partition coefficient ($^{est}K_c$), may be calculated by the following equation (*17*):

$$^{est}K_c = f_{POC}K_{oc} + f_{PSC}K_{sc} \quad (3)$$

The observed partition coefficient (log $^{obs}K_c$) exhibits a strong positive linear correlation with log K_{ow} in the effluent and recipient waters, with slopes of 1.8 for the effluent ($r^2 = 0.87$, $p < 0.001$) and 1.4 for the recipient ($r^2 = 0.62$, $p < 0.001$). The slope was <1 in the control water. However, this estimate was uncertain ($r^2 = 0.30$, $p < 0.10$). Both the slopes of the regression lines and $^{obs}K_c$ values themselves were significantly higher than would be expected from the findings of Karickhoff (*21*) and Gustafsson et al. (*17*). An underestimated dissolved fraction could have caused such an effect. However, the PUF adsorbent has been thoroughly evaluated and found to efficiently trap dissolved hydrophobic compounds from seawater. The volumes sampled in the present study were far too small for any filtration breakthrough to occur (*18*).

The $^{obs}K_c$ values of all PAHs lie much closer to $^{est}K_c$ than to the coefficient based solely on organic carbon partitioning (eq 1; Figure 6). The term f_{sc} K_{sc} in eq 3 is the largest, and considering the observed partition coefficients, it appears that eq 3 is better suited than eq 1 to the partitioning of the particulate matter in these waters. The $^{obs}K_c$ values are lower in the effluent water than in the other two types of water. The differences in average f_{PSC}, which ranged from 0.15 to 0.63, are not large enough to explain this discrepancy. It is evident from the dilution factors that most of the PSC in the recipient water did not originate from the effluent water. On the contrary, it is most probably long-range, air-transported soot carbon and/or air emissions of coal tar volatiles that have emanated from the plant. The PSC measured in the effluent water might therefore differ in terms of sorbing capacity from the atmospherically deposited PSC at the recipient and control stations.

Partition Coefficients: Dissolved Phase—DOC. By normalizing the fraction of PAHs to the DOC concentrations at the various locations, it is possible to determine the partition coefficients for the distribution between the truly dissolved fraction and the DOC-associated fraction, K_{DOC}. Plots of the observed log K_{DOC} vs log K_{ow} are given in Figure 7. Using new techniques that avoid the problems of phase separation, Gustafsson (*23*) recently showed that, based on carbon mass, the capacity of marine DOC to sorb hydrophobic compounds is lower than that of other carbon matrixes. Gustafsson (*23*) reported a log K_{DOC} of 5.3 for methylperylene (a PAH component not included in this study), which has a log K_{ow} of 6.9 (based on ref *24*). The present study's measurements of benzo[*a*]pyrene (log $K_{ow} = 6.20$) gave an average log K_{DOC} of 5.1 in the effluent water and 7.1 in the recipient water. Benzo[*a*]pyrene was not detected in any of the three phases at the control station. The most hydrophobic PAH for which a log K_{DOC} could be estimated at the control station was chrysene (log $K_{ow} = 5.86$) for which the average log K_{DOC} was 5.7. If the PUFs also trap the DOC-associated fraction of PAH, the true K_{DOC} values would be significantly higher than the measured ones. Considering that our measured K_{DOC} values were equal to or even higher than those reported by Gustafsson (*23*), this appears unlikely. This supports the assumption that PUFs exclusively trap the dissolved phase.

Interestingly, no correlation could be detected between hydrophobicity and K_{DOC} in the effluent and control waters (Figure 7). On the other hand, a significant correlation was found in the recipient water ($r^2 = 0.58$, $p < 0.001$), although the slope of the regression line was only 0.15 (Figure 7). If the sorption mechanism for DOC is the same as has been observed for organic pools of particles, it would be reasonable to expect a positive correlation between log K_{ow} and log K_{DOC} with the slope of the regression line near 1 (*21*). In this study, all log K_{DOC} values for the control and effluent waters were within 1.5 log units (4.5–5.9), except for a single value of 6.3.

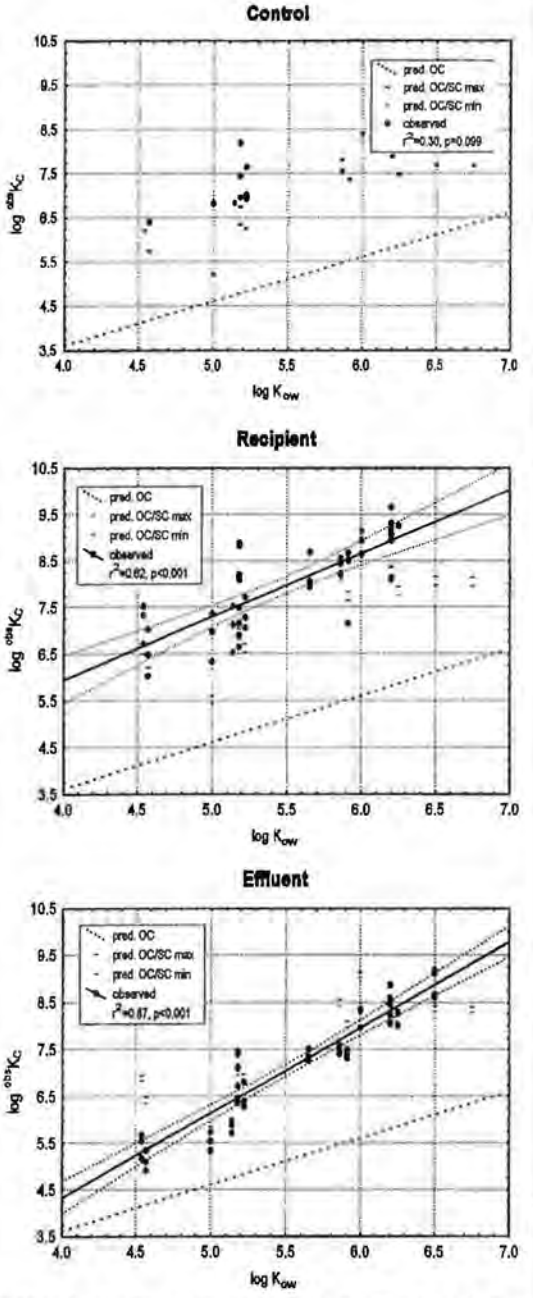

FIGURE 6. Logarithm of the observed partition coefficients (log $^{obs}K_C$) of individual PAHs plotted against the respective log K_{ow}. The linear regression lines with 95% confidence bands have also been plotted. The predicted relationship between log K_{ow} and log K_C based solely on organic carbon partitioning (predicted OC) and based on both soot carbon and organic carbon (predicted SC and OC). The SC and OC prediction is stated as a range that takes the uncertainties in the PSC and POC measurements into account.

Similarly, there was a narrow range for all observed K_{DOC} values (6.6−7.7) in the recipient waters. In other words, the low dependence on hydrophobicity was similar for all locations, but the absolute values differed greatly. As pointed

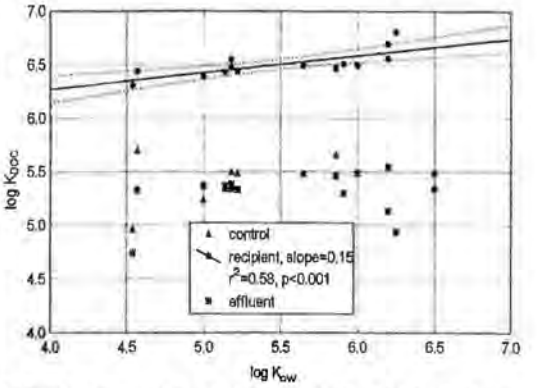

FIGURE 7. Observed K_{DOC} for individual PAHs at the three different sampling locations. The K_{DOC} values of the individual PAHs are averages from the different sampling occasions.

out, in the recipient water the DOC appears to have a very high sorbing capacity for PAHs. The reasons for these differences can only be speculated. The difference for the effluent can possibly be explained by the fact than there were very high concentrations of PAHs in the effluent water. The concentration of dissolved ΣPAH was 10−15 $\mu g\ L^{-1}$, which in fact is as much as 0.5% of the total concentration of dissolved organic carbon. Considering that only part of the DOC is available for partitioning, it is possible that saturation effects may occur. The lower K_{DOC} in the effluent water could thus be caused by the fact that the most favorable sorbing sites at the DOC are saturated. However, this would still leave the difference between the recipient and control site unexplained.

The low dependence observed in this study of log K_{DOC} on hydrophobicity indicates that DOC and particles have different sorption mechanisms. One possible explanation is as follows: The molecules in natural DOC are often small, with the largest mass fraction having molecular weights lower than 30 kDa, of which a large part are W_M < 1 kDa (*25*). This may imply that the sites at which the PAHs can associate are also small in size. The decrease in aqueous solubility with increasing W_m that normally is accompanied by increasing hydrophobicity could, in the case of DOC, thus lead to a decreased compatibility with the small binding sites. As a result, an increase in log K_{ow} will not result in the expected increase in log K_{DOC}.

Again, there is a possibility that an unknown volume of the very small particles containing PAHs pass through the GF/F filters and are collected by the PUF. If there actually were a significant relationship between log K_{ow} and log K_{DOC}, these particles would lower the slope, since the particle's relative content of PAH would increase with increasing log K_{ow}. In that case, the observed noncorrelation would be the result of a sampling error. However, that would mean that the $^{obs}K_C$ in the previous section, which also was based on C_{PUF}, would then also be too low. Moreover, the slope of the correlation between log K_{ow} and log K_C in the effluent and recipient waters would be higher. Considering that the observed slopes were already higher (1.8 and 1.4, respectively) than the frequently observed one-to-one relationship (e.g., ref *21*) and that the partition coefficients were also high, a significant sampling error of that kind appears unlikely. Since the partition coefficients for the two phases were calculated from the same dissolved concentrations, the differences in terms of sorption mechanisms between particles and DOC would still be present in all three locations, regardless of the accuracy of the C_{PUF}.

Acknowledgments

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Paper IX

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PAH accumulation in SPMDs and caged mussels (*Mytilus edulis* L.), and comparison with PAH distribution in dissolved, colloidal and particulate size fractions in the water column

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Abstract-Semipermeable membrane devices (SPMDs) and blue mussels (*Mytilus eduls* L.) were deployed at a location contaminated by discharges of polycyclic aromatic hydrocarbons (PAHs) from an aluminium reduction plant, and at a control location. The accumulation of PAHs in SPMDs versus mussels, along with the ability of the two matrices to predict contaminant concentrations in the ambient environment, were evaluated through concurrent measurements of PAHs in the particulate, colloidal and dissolved fractions in the water column. The results showed that blue mussels were more efficient at sequestering PAHs than the SPMDs were. The PAH profile (i.e. the relative distribution of PAHs among individuals) in the two matrices were similar, but

differed from the PAHs in the dissolved phase. Further, back-calculation of the ambient dissolved concentrations from SPMDs indicated systematic overtrapping with increasing hydrophobicity. The calculation of bioconcentration factors (BCFs) for the blue mussels indicated the desorption and uptake of colloidal-bound PAHs. Hence, using mussels to predict ambient concentrations would require that site-specific BCFs be applied.

Keywords-PAHs SPMDs *Mytilus edulis* Bioconcentration factors

INTRODUCTION

Bivalve molluscs have been used over the past 20 years to monitor contaminant levels in aquatic systems [1-4]. The organisms most commonly used can accumulate contaminants without being adversely affected. It is generally considered that the levels in the organisms represent a time-integrated picture of the concentrations in the ambient environment. The monitoring of contaminant levels in organisms is therefore used as an alternative to water sampling, for which labour-intensive and costly programmes would be needed to describe the most important trends.

The blue mussel *Mytilus edulis* L. is among the molluscs most commonly used in monitoring programmes. In some areas, for example in Norway, the physical and chemical conditions are such that no single species is found along the entire coast. Although the choice of monitoring organisms might be standardised insofar as possible among the various areas investigated, the choice of organism is inevitably dependent on local availability and occurrence. Further, the detection of spatial trends may be hampered by the use of different organisms since they may differ in their contaminant accumulation characteristics. To counterbalance the limitations of using living organisms as measures of contaminant exposure, *in situ* passive sampling devices such

as lipid-filled semipermeable membrane devices (SPMDs) have been developed and used extensively in recent years [5-8]. These devices are designed to sample the truly dissolved fraction exclusively, and thereby to mimic the bioconcentration process in aquatic organisms [5, 9, 10]. It might therefore be prudent to use man-made devices instead of living organisms. However, before SPMDs can be used to replace living organisms, their performance must be tested relative to the organisms in question.

Mussels are used not in temporal programmes alone, but also as a tool to compare contaminant levels at different locations. However, this ability is predicated on the assumption that bioconcentration factors (BCFs), i. e. the relationship between the concentrations in the mussel and in the ambient waters, does not differ significantly between locations subject to different conditions. This study investigated the accumulation of polycyclic aromatic hydrocarbons (PAHs) in SPMDs and blue mussels (*Mytilus edulis* L.) deployed at a contaminated site and at a control location. The ability of the membrane devices and the mussels to predict contaminant concentrations in the ambient environment was evaluated through concurrent measurements of PAHs in the particulate, colloidal and dissolved fractions in the water column.

MATERIALS AND METHODS

Sampling

Sampling at a contaminated site was performed in the waters affected by discharges from the aluminium reduction plant at Lista in southern Norway (Figure 1). The recipient received PAH-carrying effluents from the seawater scrubbing of furnace off-gases and ventilation air from the plant. The effluent is piped into the recipient water, an open coastal embayment, at a depth of 1.5 m. The control site was in coastal waters

approximately 200 km from the contaminated site. The control site is influenced by general human activities, but not by any point sources.

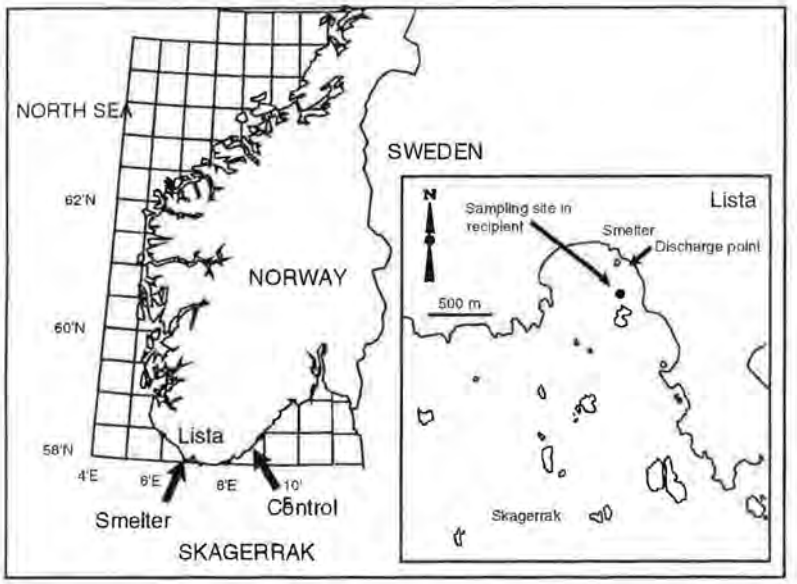

Fig. 1. Map of the investigation area.

A standardised set-up of SPMDs and caged blue mussels (*Mytilus edulis* L.) was deployed in June 1996 at each location at a water depth of 0.5 meter. Both monitoring matrices were attached to the same deployment device and spaced closely together (1 meter apart). The semi-permeable membrane devices consisted of commercially available 45.7×2.54 cm (surface area 232 cm^2) layflat polyethene tubes filled with 0.455 g of triolein and held in a stainless steel container. Twenty-five mussels ranging in size from 4 to 7 cm were put in a plastic mesh and transplanted from a clean site. For each station, two replicates of mussels and SPMDs were held for 34 and 26 days for recipient and control, respectively, and analysed separately.

Water samples from a depth of 0.5 m were drawn on three occasions from both locations during the deployment period. An on-line filtration system was used to collect PAHs. It consisted of a pre-cleaned 142 mm borosilicate glass microfilter (Whatman GF/F) in a stainless steel holder for the retention of particulate matter (nominal cut-off 0.7 μm). That was followed by two polyurethane foam (PUF) adsorbents (30 mm diameter, 45 mm length) in stainless steel or glass (effluent water) casing. The PUF is assumed to retain PAHs in the dissolved fraction. The filtrate from the PUF adsorbent, containing the colloidal fraction, was collected in 10 l glass containers and immediately preserved with toluene (Burdick and Jackson, glass distilled quality). Water supply to the on-line filtration system was provided at a flow of 1 l $min.^{-1}$ by a modified Flojet® pump equipped with a silicone tube. Thirty litres of recipient water and 40 l of control station water were pumped through the system at each sampling. The filters, casings and containers were washed with toluene and heated to 480 ^{o}C prior to use.

Water samples were kept dark in sealed containers. Immediately after collection, they were extracted with 50 ml toluene per litre of sample, and spiked with picene and D_{12}-perylene to serve as internal standards. They were subsequently shaken for 24 h, then left to stand for another 24 h for phase separation. Most of the seawater was siphoned off and the remaining solution was kept cool (4 ^{o}C) until analysis. Filters and PUF adsorbents were stored frozen at -20 ^{o}C prior to chemical analysis, and filtrates were stored at +4°C.

Chemical analysis

The following PAHs (abbreviations in brackets) were analysed: fluoranthene (Flu), pyrene (Pyr), 2-methylpyrene (2-mePyr), 1-methylpyrene (1-mePyr), benzo[ghi]fluoranthene (B[ghi]F), cyclopenta[cd]pyrene (Cp[cd]P), benzo[a]anthracene (B[a]A), chrysene/triphenylene (Chr), benzo[k]fluoranthene (B[k]F), benzo[e]pyrene (B[e]P), benzo[a]pyrene (B[a]P), perylene (Per),

indeno[1,2,3-cd]pyrene (Ind), benzo[ghi]perylene (B[ghi]P) and coronene (Cor). Mussels, glass fibre filters and PUF adsorbents were spiked with two PAHs (D_{12}-perylene and picene), then Soxhlet extracted for 24 h in toluene with a Dean-Stark trap for water removal. The amount of extractable organics (lipids) in the mussels was determined by evaporating the solvent from the extracts and continually weighing the extract until a constant weight was obtained. Biofouling was mechanically removed from SPMDs by means of facial tissues and rinsing in tap water. The contents of two SPMDs from each location were extracted in capped glass containers using 100 ml pentane for 3×24 h. Water was removed from the liquid-liquid extracts with pre-combusted Na_2SO_4. The volume-reduced extract from the PUFs was first eluted on 100×10 mm columns containing deactivated SiO_2 (10 per cent water w/w) with toluene as the mobile phase. The volume-reduced extracts of all the samples were then treated by a dimethylformamide (DMF) cleanup procedure. The hexane solution containing the PAHs was then finally eluted on 100×10 mm columns containing deactivated SiO_2 (10 per cent water w/w) with hexane as the mobile phase. The extracts were analysed on a gas chromatograph/mass spectrometer (Hewlett Packard 5890/5971A). Response factors and retention times were determined by analysing a standard mixture containing all the PAH compounds analysed, including the internal standards. This mixture was extracted and cleaned up following the same procedure as the samples. All the solvents used (toluene and hexane; Burdick & Jackson; pentane; Merck) were of glass distilled quality.

Statistical analyses

Principal component analysis (PCA) was used to examine PAH profile patterns between the different matrices, and to apportion the variance in the data. This was performed using standardised PCA on log-transformed data (i.e. eliminating the effects of scale, thus focusing on relative patterns). PCA represents the patterns and trends by arranging the PAHs (variables) and stations along axes (principal components) which are assumed to represent basic factors or relationships. The first axis (PC1) illustrates

the most prominent trend, while the successive axes (PC2, PC3, etc.) represent secondary trends in decreasing order of importance. The axes are uncorrelated. The amount of variance explained by each axis, stated as a per centage of the total variance, may be taken as a measure of the relative importance of the axis. The results are depicted in combined plots (bi-plots) of variables and samples with the variables represented by arrows running from the origin of the plot to the position of the variable scores (loadings). The arrows point in the direction of increasing variable concentrations, while the length of the arrow represents the strength of the increase.

To test the significance of observed differences in PAH profiles between different matrices, the data were used in redundancy analyses (RDA) applying the 'forward selection' procedure and Monte Carlo permutation tests. RDA is a technique related to PCA, but it includes an additional data set of explanatory variables. The axes represent linear combinations of the explanatory variables. Thus the analysis will detect specific variation patterns among PAHs, which correlate with the explanatory variables. In the RDA, each sample was assigned to an appurtenant matrix, which were entered in the analyses as explanatory variables. The PCAs/RDAs were performed using the software CANOCO version 3.10 software [11, 12]. Plots were made using CanoDraw 3.0 [13].

RESULTS AND DISCUSSIONS

Characteristics of PAHs in the water phase

The average sums of the concentrations in all three phases (particulates ($C_{part.}$), colloids ($C_{col.}$) and dissolved ($C_{dis.}$)) at the three locations are presented in Table 1. In all phases and at both locations, phenanthrene, fluoranthene, pyrene and chrysene were quantitatively most important. At the control location, the filtered volume was too small to accurately quantify the high molecular weight (W_m) PAHs. The relative distribution between particles, colloids and the dissolved phase differed between

effluent and recipient waters. The colloids in the recipient waters appear to have had a high sorbing capacity. This fraction was the dominating pool for PAHs with low molecular weights (W_m). Sixty to 90 per cent of the low W_m PAHs and 10 to 30 per cent of the high W_m PAHs passed through the filters and PUF adsorbent.

Accumulation in SPMDs and mussels

Both matrices reflected the considerable difference in concentrations between the control and the recipient location. At the control location, the total PAH concentration in the mussels was equal to or slightly higher than what has been denoted as a background level in uncontaminated areas, according to Norwegian criteria for the assessment of environmental quality [14]. Mussels from the recipient location were classified as extremely contaminated. As for the three water phases, phenanthrene, fluoranthene, pyrene and chrysene were quantitatively most important in the membrane devices and in the mussels. However, their accumulation efficiency differed considerably with the highest concentrations in the blue mussels, Table 1. At the control site, the ratio between the accumulation in mussels and in SPMDs the total PAHs expressed was 2.2 to 2.4 on a lipid basis, and ranged from 3.6 to 6.7 in the recipient waters of the reduction plant. The magnitude of the increased sequestering efficiency of the mussels compared with SPMDs coincides reasonably well with findings elsewhere [4, 8, 15].

A marked difference in concentrations was also observed between parallel samples of mussels at both locations and between parallel SPMD samples at the control location. For the SPMDs, this was surprising since identical tubes were closely spaced in the same steel container. However, accumulation differences between parallel SPMDs have been reported previously, for instance, Moring and Rose [16] observed a coefficient of variation of up to 50 per cent for many PAH compounds. One explanation for the difference in accumulation at the control location may be related to differences in biofouling on the membranes. It is known that biofouling can

substantially diminish membrane permeability and lead to poor reproducibility [5, 7]. In contrast to the control location, there was an excellent agreement between the SPMD parallels at the recipient location.

In the mussels, an accumulation difference of a factor of approximately two, expressed on a lipid or dry weight basis, was observed at both the recipient and the control location. The mussels were transplanted from the same uncontaminated site. Differences in body size and physiological state have been shown to have a marked influence on the rate of contaminant uptake in the blue mussel [17]. However, an effort was made to minimise this by using mussels of roughly the same size. Further, algal food concentrations may influence the contaminant accumulation in mussels [18]. The parallels were closely spaced (approx. two metres), so the food supply should have been the same. However, the mussels were set out in plastic mesh. This means the food supply of the sample specimens could theoretically differ from that of the individuals sitting on the outside of the mesh.. The results therefore indicate substantial differences in PAH accumulation between individuals.

Table 1. Concentrations of PAHs in the dissolved, colloidal and particulate size fractions and accumulation in semipermeable membrane devices (SPMD) and in *Mytilus edulis* L. Dry-weight based concentrations are derived by multiplying values for *Mytilus* 1 and 2 by factors of 0.23 and 0.19, respectively. A dry weight percentage of 15 is used to calculate concentrations on a wet-weight basis. For SPMDs, triolein-based concentrations equal dry-weight concentrations.

	Mean of 3 samplings, ng l^{-1}						ng g^{-1} triolein $^{-1}$		ng g^{-1} lipid $^{-1}$		ng g^{-1} triolein $^{-1}$		ng g^{-1} lipid $^{-1}$	
Location Type	**Control Colloids**	**Control Dissolved**	**Control Particles**	**Recipient Colloids**	**Recipient Dissolved**	**Recipient Particles**	**Control SPMD 1**	**Control SPMD 2**	**Control *Mytilus* 1**	**Control *Mytilus* 2**	**Recipient SPMD 1**	**Recipient SPMD 2**	**Recipient *Mytilus* 1**	**Recipient *Mytilus* 2**
Phe	1.6	1.2	$6.0*10^{-1}$	$7.8*10^{2}$	$9.9*10^{1}$	$3.3*10^{1}$	$4.8*10^{2}$	$2.3*10^{2}$	$4.5*10^{2}$	$4.6*10^{2}$	$6.1*10^{4}$	$6.8*10^{4}$	$2.4*10^{5}$	$5.9*10^{5}$
Ant	$6.0*10^{-2}$	$1.4*10^{-1}$		$1.1*10^{1}$	2.7	4.2	$1.3*10^{1}$	$1.3*10^{1}$	$2.5*10^{1}$	$2.1*10^{1}$	$3.3*10^{3}$	$4.7*10^{3}$	$1.7*10^{4}$	$4.7*10^{4}$
3-mePhe	$3.0*10^{-1}$	$4.0*10^{-1}$	$2.9*10^{-1}$	$7.7*10^{1}$	$1.1*10^{1}$	8.4	$1.9*10^{2}$	$1.0*10^{2}$	$2.0*10^{2}$	$1.9*10^{2}$	$1.2*10^{4}$	$1.2*10^{4}$	$6.4*10^{4}$	$1.7*10^{5}$
1-mePhe	$2.4*10^{-1}$	$2.8*10^{-1}$	$2.0*10^{-1}$	$2.8*10^{1}$	3.6	3.9	$1.1*10^{2}$	$5.3*10^{1}$	$1.0*10^{2}$	$1.1*10^{2}$	$4.8*10^{3}$	$4.6*10^{3}$	$2.2*10^{4}$	$4.9*10^{4}$
Flu	$5.8*10^{-1}$	$6.3*10^{-1}$	$8.6*10^{-1}$	$1.0*10^{3}$	$1.2*10^{2}$	$2.8*10^{2}$	$6.5*10^{2}$	$2.4*10^{2}$	$1.2*10^{3}$	$4.5*10^{2}$	$4.8*10^{5}$	$4.5*10^{5}$	$8.8*10^{5}$	$1.6*10^{6}$
Pyr	$1.6*10^{-1}$	$1.4*10^{-1}$	$4.2*10^{-1}$	$2.8*10^{2}$	$3.3*10^{1}$	$1.1*10^{2}$	$1.0*10^{2}$	$5.3*10^{1}$	$3.5*10^{2}$	$1.3*10^{2}$	$7.2*10^{4}$	$7.1*10^{4}$	$3.6*10^{5}$	$8.9*10^{5}$
2-mePyr	$2.0*10^{-2}$			6.2	$7.2*10^{-1}$	$1.1*10^{1}$	6.1	1.3	$3.7*10^{1}$	7.8	$2.8*10^{3}$	$2.5*10^{3}$	$3.9*10^{4}$	$9.9*10^{4}$
1-mePyr				6.1	$6.9*10^{-1}$	8.0	5.8	1.6	$2.8*10^{1}$	$1.1*10^{1}$	$2.3*10^{3}$	$2.1*10^{3}$	$2.8*10^{4}$	$6.9*10^{4}$
B(ghi)F	$2.0*10^{-2}$	$1.0*10^{-2}$	$7.0*10^{-2}$	8.2	$9.5*10^{-1}$	$1.4*10^{1}$	$2.5*10^{1}$	$1.3*10^{1}$	$8.0*10^{1}$	$6.1*10^{1}$	$3.7*10^{3}$	$3.3*10^{3}$	$3.4*10^{4}$	$6.2*10^{4}$
B(a)A	$5.0*10^{-2}$	$3.0*10^{-2}$		$4.1*10^{1}$	4.1	$9.0*10^{1}$	$1.9*10^{1}$	$2.5*10^{1}$	$3.3*10^{2}$	$1.3*10^{2}$	$3.9*10^{4}$	$3.4*10^{4}$	$2.2*10^{5}$	$4.3*10^{5}$
Chr	$2.0*10^{-1}$	$9.0*10^{-2}$	$5.6*10^{-1}$	$1.5*10^{2}$	$1.7*10^{1}$	$3.8*10^{2}$	$2.0*10^{2}$	$1.9*10^{2}$	$9.2*10^{2}$	$3.5*10^{2}$	$1.4*10^{5}$	$1.3*10^{5}$	$5.6*10^{5}$	$1.1*10^{6}$
B(k)F	$2.3*10^{-1}$		$1.5*10^{-1}$	$1.6*10^{1}$	1.3	$1.0*10^{2}$	$2.0*10^{1}$	$1.7*10^{1}$	$1.8*10^{2}$	$5.3*10^{1}$	$1.9*10^{4}$	$1.6*10^{4}$	$1.3*10^{5}$	$2.4*10^{5}$
B(e)P	$1.7*10^{-1}$			$1.8*10^{1}$	1.2	$1.3*10^{2}$	$1.8*10^{1}$	$1.6*10^{1}$	$2.8*10^{2}$	$6.4*10^{1}$	$2.1*10^{4}$	$1.8*10^{4}$	$1.8*10^{5}$	$3.7*10^{5}$
B(a)P	$1.0*10^{-1}$			7.8	$2.6*10^{-1}$	$5.7*10^{1}$	$9.3*10^{-1}$	1.9	$1.4*10^{2}$	$2.2*10^{1}$	$9.4*10^{3}$	$7.6*10^{3}$	$1.1*10^{5}$	$2.3*10^{5}$
Per				2.4	$7.0*10^{-2}$	$1.5*10^{1}$	$8.3*10^{-1}$	1.7	$2.6*10^{1}$	4.5	$1.5*10^{3}$	$1.9*10^{3}$	$1.9*10^{4}$	$4.1*10^{4}$
Ind(1,2,3-cd)P				9.1		$7.3*10^{1}$	$4.4*10^{-1}$	1.0	$7.9*10^{1}$	$1.3*10^{1}$	$5.6*10^{3}$	$4.8*10^{3}$	$4.0*10^{4}$	$9.6*10^{4}$
B(ghi)P				8.0		$6.1*10^{1}$	$3.7*10^{-1}$	$9.0*10^{-1}$	$1.0*10^{2}$	$3.5*10^{1}$	$5.2*10^{3}$	$4.2*10^{3}$	$3.8*10^{4}$	$8.8*10^{4}$
Cor						$1.5*10^{1}$			$1.0*10^{1}$	$8.8*10^{-1}$	$3.6*10^{2}$		$5.7*10^{1}$	$1.7*10^{3}$
Total	3.8	2.9	3.2	$2.5*10^{3}$	$3.0*10^{2}$	$1.4*10^{3}$	$1.8*10^{3}$	$9.6*10^{2}$	$4.5*10^{3}$	$2.1*10^{3}$	$8.9*10^{5}$	$8.3*10^{5}$	$3.0*10^{6}$	$6.1*10^{6}$

PAH profile comparison

The PCA performed to evaluate differences in relative accumulation revealed that the main trend in the data from the recipient was the difference in PAH profile between the dissolved fraction sampled by PUF on the one hand and the other sample matrices on the other, see Figure 2. PAHs in the dissolved fraction were dominated by the lower W_m compounds phenanthrene, metylphenanthrene, flouranthene, anthracene and pyrene. This trend accounted for 93 per cent of the total variance, see Table 2A. The second most important trend (PC2) was represented by a difference in profile between the particulate and the colloidal fraction, which captured five per cent of the variance in the data set. The PAH profiles from the SPMDs and *Mytilus edulis* were quite similar, although they differed on PC3, but this accounted only for one per cent of the total variance. It should be noted that although considerable differences were observed in total concentrations for parallel samples from SPMDs and mussels, the PAH profiles within each matrix were very similar.

The redundancy analyses (RDA) based on Monte Carlo permutations showed that there were significant trends in the data set. Applying the procedure of "forward selection" in the RDA, PUF as a group accounted for as much as 89 per cent of the variance, see Table 2B, and was the only variable significant at $p<0.01$. This implies that the profile in the four other matrix groups: colloids, SPMDs, *Mytilus edulis* and particles, could not be significantly ($p>0.05$) differentiated internally. However, it must be pointed out that the data set is limited. The same pattern that emerged in the data set from the recipient was also generally evident at the control station (results not shown). However, due to the number of higher W_m compounds that were below the detection limit in the dissolved and colloidal fractions, the analysis had to be restricted to fewer individualPAHs. The results showed that SPMD did not mimic the relative PAH distribution in the dissolved phase. However, the PAH profiles in the SPMDs and *Mytilus edulis* were similar, falling between the profiles seen for particles and for colloids.

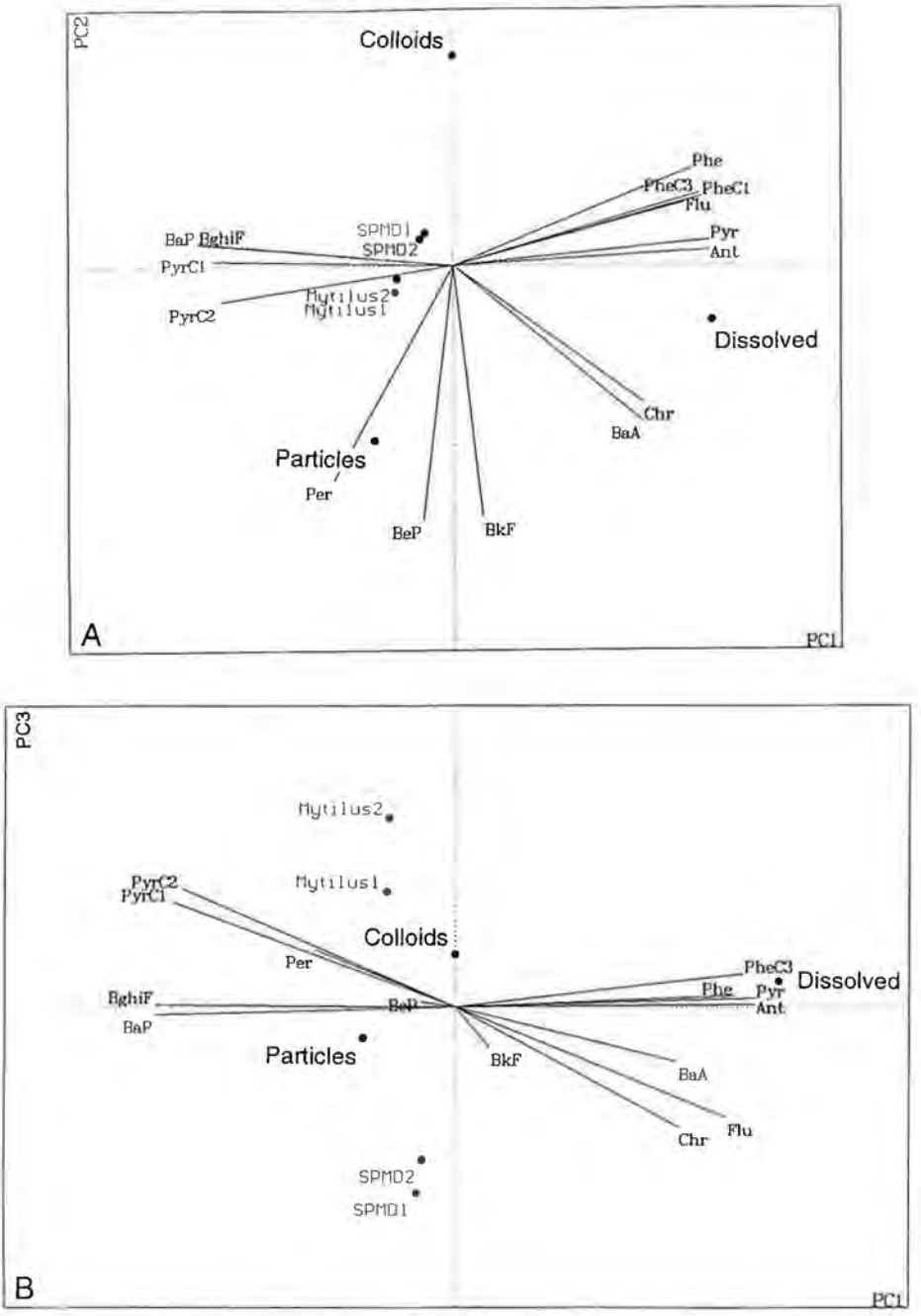

Fig. 2. Standardised PCA for comparing the profiles of different sampling matrices. A: Principal component 1 and 2. B: Principal component 1 and 3. Indeno(1,2,3-cd)pyrene, benzo(ghi)perylene and coronene were excluded from the analyses due to concentrations below detection limit.

Table 2. **A**: Data for standardised PCA of PAHs in different matrixes showing variances explained by the first three principal components. **B:** Data for RDA stating the explained variance attributable to each individual sample matrix by applying the "forward selection" procedure.

A:

Variance explained (%)		
PC1	**PC2**	**PC3**
93	5	1

B:

Matrix	**Variance explained (%)**
Dissolved	89
Mytilus	12
Particulates	11
SPMDs	4
Colloids	3

Prediction of ambient concentrations

SPMDs. The dissolved phase was sampled in two different ways: by PUF adsorbent after GF/F filters, and by SPMDs. The PUF adsorbents provide "snapshots", while the SPMDs show integration over time. Using laboratory-calculated rates for initial linear uptake (Table 3), the concentration of individual PAHs in the dissolved phase can be retro-calculated on the basis of the PAH content in the SPMDs at the end of the sampling period. The concentrations measured by the two different methods represent average concentrations in similar volumes of water, that is, 30 to 40 l of water were passed through the PUF adsorbents while the SPMDs sampled water for 26 and 34 days, respectively, for control and recipient waters. Multiplying 26 and 34 days times general uptake rates of 1 to 3 l per day^{-1} (Table 3) equals 30 to 100 l. Unfortunately, few uptake rates are available in the reference literature for the PAHs included in this study, especially for the high W_m PAHs. This, in turn, limits the applicability of the following discussion on these compounds.

Table 3. The uptake rates used for the calculation of dissolved concentrations of PAHs from the contents in the SPMDs from ref. [28].

Compound	Uptake rate	
	1 ng l^{-1}	100 ng l^{-1}
Phe	2.2*	2.4
Ant	1.84	1.8
B(a)A	2.7	3
Chr	1.4	1.9
B(k)F	1.9	1.5
B(a)P	1.9	2
Ind	1.8	1.6
B(ghi)P	1.2*	1.3

* determined for 10 ng l^{-1}

Dissolved concentrations of individual PAHs at the recipient and control stations were calculated using the uptake rates for 100 ng l^{-1} and 1 ng l^{-1}, and comparing them with those measured by the PUF absorbent (Figure 3). Interestingly, although the estimated concentrations are on the same order of magnitude, the results indicate a systematic difference between the two methods. The ratio between the concentrations determined by SPMD (C_{SPMD}) and by PUF adsorbent ($C_{dis.}$) for individual PAHs (R) is plotted against log K_{OW} in Figure 4. There is a significant positive correlation between log R and log K_{OW} for the recipient water. However, this correlation was not significant (i.e. p=0.08) at the control station, probably because of the limited number of observations. Although the slopes were similar for the two locations, the intercepts were different. The recipient R values for some of the low W_m PAHs were less than 1, while, for the control station, all R values were >1 (Figure 4). In the recipient water, the PUF adsorbent tended to overtrap (R<1) low W_m PAHs and undertrap (R>1) high W_m PAHs, relative to SPMDs. One explanation for R<1 for low W_m PAHs may be that they are rapidly desorbed from the organic colloids and subsequently trapped by the PUF adsorbent. Another explanation, which is more likely, is that the linear uptake

phase of SPMD for low W_m has been exceeded, resulting in a back flux of these PAHs from the SPMDs during the latter part of the sampling period. This is not applicable for PAHs with higher W_m since their depuration rates are substantially lower.

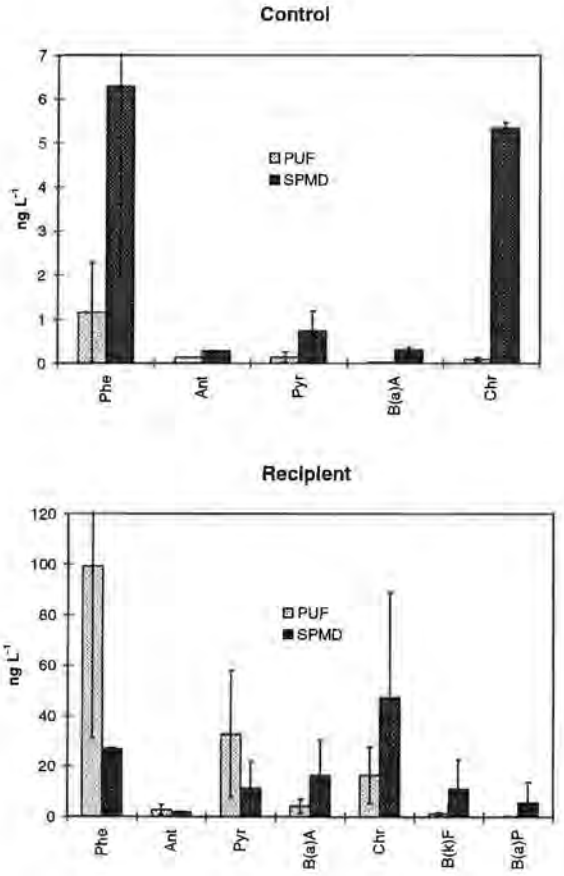

Fig. 3. A comparison of the estimates of the dissolved concentrations of PAHs as determined by PUF and SPMD at the two sampling locations: control and recipient. The bars indicate a standard error of n=2 or 3, depending on the number of sampling occasions on which the compound was detected.

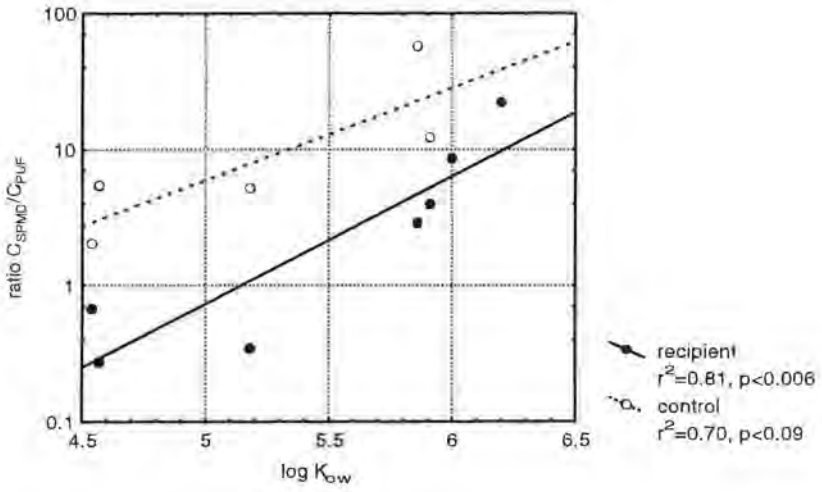

Fig. 4. The ratio (R) between the estimates of the dissolved concentrations, using the SPMD (C_{SPMD}) and PUF ($C_{dis.}$) of individual PAHs plotted against log K_{ow}.

It is more difficult to explain the undertrapping of high W_m PAHs by the PUF adsorbent in relation to SPMD (R>1). The PUF adsorbent has been thoroughly evaluated and found efficient in trapping dissolved hydrophobic compounds from seawater, although the possible effect of colloids as a sorbing matrix has not been evaluated [19]. The volumes sampled in the present study were far too small for any filtration breakthrough to occur [19]. Consequently, the problem appears to be related to the SPMD sampling methodology. There seems to be a systematic increase in overtrapping with increasing PAH hydrophobicity.

The SPMD methodology requires that algae and other fouling organisms be removed from the surface of the membranes prior to extraction. This was done by means of rinsing in tap water and mechanical removal using a facial tissue. The total amount of benzo(a)pyrene (B[a]P) extracted from the two SPMDs in the recipient was 4.3 and 3.7 μg. In the recipient water, the carbon-normalised concentration of B(a)P in suspended particles ranged from 170 to 370 μg gC^{-1}. A residue of 10 mg C on the

SPMD surface after cleaning could therefore account for an amount of B(a)P corresponding to the amount found in the SPMD. Accordingly, further attention should be devoted to the cleaning method. The explanation that residual organic film or soot particles can affect total amounts is supported by the finding that the C_{SPMD} of chrysene, the most hydrophobic PAH detected by both PUF and liquid-extraction on all three occasions at the control station, was actually eight to nine times higher than the sum of $C_{dis.}$ and $C_{col.}$.

Blue mussels. Several studies have reported that accumulation of contaminants in aquatic organisms can be described by the bioconcentration factor (BCF) which is an equilibrium partitioning between the organism and the dissolved phase in the water column [20, 21]. The driving force for this partitioning is the hydrophobicity of the contaminant, often expressed as the partition coefficient in an octanol/water system (K_{OW}).

The relationship between log BCF and log K_{OW} at the control and recipient location is presented in Figure 5 on a wet weight basis. There was good linear correlation with K_{OW}. The slopes of the regression lines for the two locations were not significantly different, although the BCFs in the recipient were one order of magnitude higher. The results reported by Geyer et al. [20], Pruell et al.[22] and Murray et al. [23] are also included in Figure 5. Compared with Geyer et al. and Pruell et al. (op. cit.), the BCFs at the control location are almost an order of magnitude higher, even more so if compared with Murray et al. (op. cit.). It is difficult to compare BCFs with previously published values because of differences in experimental conditions and reporting formats. Many of the values have been derived from laboratory experiments where conditions are not necessarily comparable to *in situ* situations. Further, the calculation of BCFs requires determination of the truly dissolved fraction, which is complicated, analytically speaking. For example, the inclusion of a significant dissolved organic-bound fraction will result in lower BCFs.

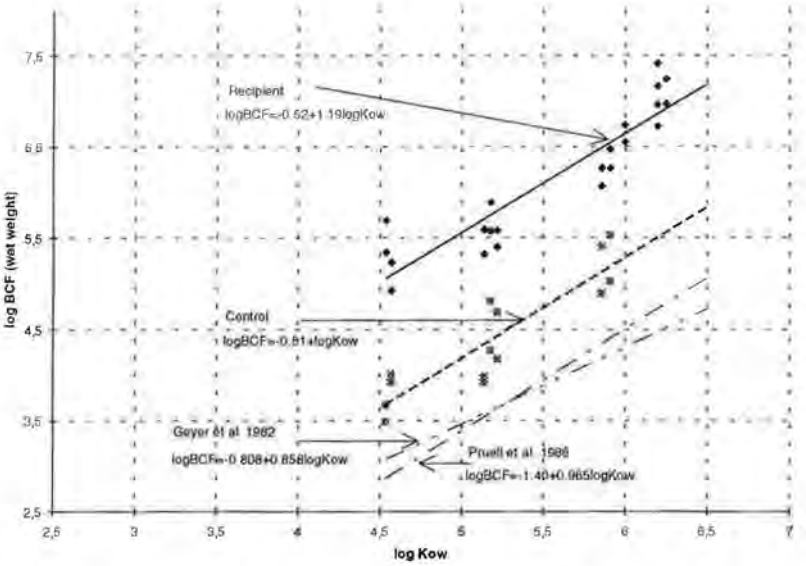

Fig. 5. Biocencentration factors for blue mussels on Day 26 and Day 34 for control and recipient locations, respectively. Results from Geyer et al. (1982) and Pruell et al. (1986) are included for comparison.

Ideally, and at equilibrium, log BCF_{Lipid} equals log K_{OW}. However, deviations from this are observed often and may be attributed to the fact that, as a sorbing matrix, octanol solubility does not represent lipids completely. At the control location, the ratio between log BCF_{Lipid} and log K_{OW} indicates that the concentrations are at or near equilibrium. However, at the recipient location, the results indicate additional uptake. This could be from the colloidal or particulate size fractions, although combustion-derived, particulate-bound PAHs are considered to have low bioavailability [24, 25]. An allometric relationship was used to estimate approximate filtration rates for the individual mussels [26]:

$$F = 3.900W^{0.60}$$

F where is the filtration rate (l day^{-1}) and W the weight (g dw). The filtration rates were estimated to 2.6-3.0 l hr^{-1} and 1.8-2.1 l hr^{-1} for the mussels at the control and recipient sites, respectively. The dissolved phase concentrations in the water column from only 1 to 10 days of exposure will account for the amounts of PAHs observed in

the mussels at the control site. The higher values refer to the high W_m PAHs. As the mussels were deployed for 26 days, less than a 30 per cent uptake from the dissolved phase could account for the observed concentrations in the mussels. Applying the same calculations at the recipient location, up to one year of deployment would be needed to account for the concentrations in the mussels, even if the uptake efficiency from the dissolved phase was 100 per cent. The analyses of the PAH distribution in the water column showed that colloidal-bound PAHs were a very important fraction in the recipient, and the dominating pool for PAHs of low W_m. The results therefore indicate the uptake of colloidal-bound PAHs. It has been shown that no correlation existed between hydrophobicity and the partition coe.fficients for distribution between the truly dissolved phase and the fraction bound to colloids at the recipient location [27]. Desorption from colloids would therefore result in a parallel displacement of the regression line for the recipient compared to the control, as observed. In conclusion, to calculate ambient PAH concentrations from BCFs, the results indicate that *in situ* determined, site-specific values are required.

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