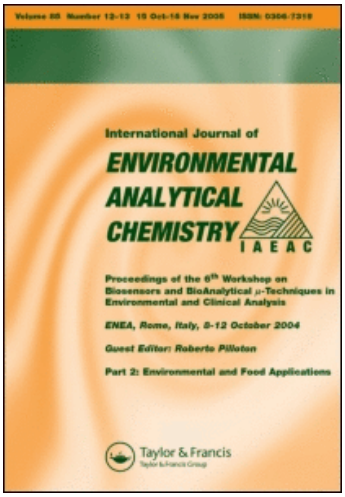

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Polycyclic Aromatic Hydrocarbons in Rock Oysters: A Baseline Study†

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Coral-rock oysters were collected in September 1982 from six locations in the area of Mermaid Sound in North-Western Australia. Analysis was carried out by digestion of the samples, followed by solvent extraction and analysis of the extracts using HPLC-UVF and GC-MS.

The levels of two- and three-ring aromatics ranged from very low for the site outside Mermaid Sound and for one site within the Sound, to low for the four other sites within the Sound. The PAH values at the latter four sites are attributed to occasional petroleum release episodes related to small boat activities and large-scale salt and iron ore shipping and general cargo activities of the Port of Dampier.

The levels of PAHs with four or more rings were found to be low or very low at all sites; in fact, in most cases values measured for specific PAHs were below the limits of detection of even the very sensitive methods used in this study. Samples from sites within Mermaid Sound closest to the town and port of Dampier showed noticeably higher levels than those from outside; the present study does not allow the source of the PAHs to be determined. It is interesting to note, however, that the parent PAHs appear to form a greater proportion of the total PAH assemblage in these cases, indicating contributions from material which has been subjected to high temperature processing prior to release into the Sound.

KEY WORDS: Coral-rock oysters, solvent extraction, HPLC-UVF, GC-MS, PAHs.

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INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are present in air, water, soils and sediments. The state of knowledge of the sources, fates and biological effects of PAHs in the aquatic environment has been reviewed by Neff.[1] They occur as very complex mixtures of homologues in crude petroleum where the two-ring naphthalenes and the three-ring phenanthrenes are usually well represented. They may be produced in biosynthetic processes from such naturally occurring compounds as terpenoids and large quantities are generated in natural, domestic and industrial pyrolysis and combustion processes. PAHs are present in bushfire smoke, automobile exhaust and used lube oils. They are produced during petroleum refining, coal coking, fossil power plants, and a variety of industrial processes. PAHs are biologically active, and some have been recognised as mutagens and carcinogens for many years.[2]

Polycyclic aromatic hydrocarbons may enter the marine environment in a number of ways which include: discharges from vessels such as ballast water, bilge pumping and exhaust; spills of crude oil or processed petroleum products, especially heavy refinery residues; effluents from coastal oil refineries and other similar heavy industries using fossil fuels; terrestrial runoff including sewage and urban storm water, particularly when it contains street dust derived from asphalt;[3] aerial fallout from domestic and industrial combustion processes; leaching of components of creosote from structures such as wharves and jetties, and the use and disposal of other coal-derived liquids.

Field and laboratory studies have demonstrated that filtering bivalves, particularly mussels and oysters, can accumulate high levels of PAHs.[4,5] These animals are unable to metabolise PAHs, apparently due to a lack of the enzyme, aryl hydrocarbon hydroxylase, and they therefore act as bioaccumulators of these compounds. For example, Erhardt and Heinemann[6] showed that mussels from the Kiel Bight concentrated aromatics from the water by a factor of 10,000. This raises the concern of public health risk and economic impact upon fishing industries based upon these species. Also the prospect of biomagnification of PAHs through the food chain impacting on other species or humans has not yet been set aside. As well as presenting these hazards, the potential of molluscs to act as integrators of inputs of PAHs was recognised over a decade ago, and their use as sentinel

organisms has become widespread.[7] The major programme of this type has been the Mussel Watch[8,9] involving monitoring at over 100 stations around the U.S. coast, and similar activities have been reported both in the U.S. and in many other places.[9–18]

The low concentrations of individual PAHs, the complexities of the mixtures due to the very large numbers of possible isomers, homologues, and sulphur and nitrogen analogues, and the complications of a biological matrix combine to make estimation of these compounds a formidable analytical problem. Two methods which combine the sensitivity and selectivity required for such analyses are widely used. The first method is reverse-phase high-pressure liquid chromatography which has largely replaced thin layer chromatography for analysis of PAHs, particularly in complex matrixes.[11,15,16,19,20,21] Two types of detection systems are commonly used. Ultraviolet absorbance detectors respond to all PAHs; that is, they are universal detectors. However, ultraviolet fluorescence (UVF) detectors, with careful selection of excitation and emission wavelengths, can typically offer an order of magnitude greater sensitivity: they can be used to selectively determine specific PAHs in many cases at the picogram levels.[22,23,24]

The other analytical technique, capillary gas chromatography, offers the advantage of very high resolving power for the analysis of complex mixtures of PAHs.[13,14,25–27] This technique is particularly useful when coupled with the selectivity and sensitivity offered by a computer-operated mass spectrometer with a data system (capillary GC-MS-DS).[3,4,10,21,28–30] However, particularly for higher molecular weight PAHs, the sensitivity of this technique is typically well below that of HPLC-UVF.

In this report we present levels of a range of PAHs, including the 16 compounds listed by the U.S. Environmental Protection Agency, in coral-rock oysters (*Saccostrea cucculata*) from six sites in North-Western Australia measured by both GC-MS and HPLC-UVF. The study area (Figure 1) is significant because the Burrup Peninsula is the location of the on-shore facilities which are being constructed to handle the development of natural gas, condensate, and perhaps crude oil resources on the North-West Shelf. At present, PAH contamination of the Sound might arise from natural processes, shipping associated either with the construction project or the major iron ore port of Dampier at the foot of the peninsula, or perhaps from the Dampier townsite which has a population of 3,000 people.

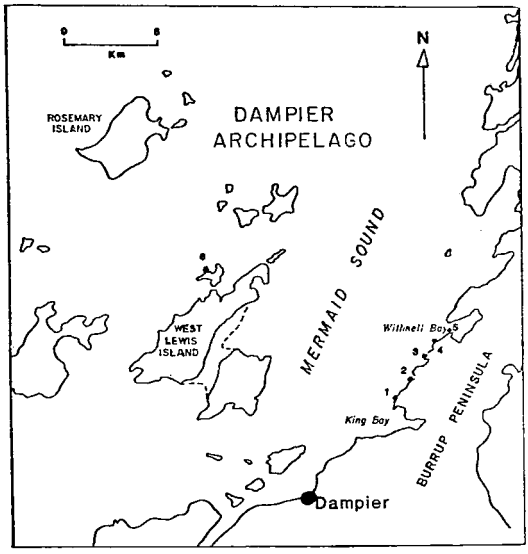


FIGURE 1 Location map showing sampling sites.

EXPERIMENTAL

Materials

Analytical grade pentane and dichloromethane were purified by fractional distillation. These were checked for purity by evaporating 100 ml to 100 μl and analysis of the residue by gas chromatography. The hexane and acetonitrile used for HPLC were Waters Associates HPLC-grade solvents. Water for HPLC was prepared by distilling rainwater twice, then refluxing for two hours with 0.1% w/w $KMnO_4$, followed by fractional distillation. All of these solvents produced a flat baseline at the sensitivity settings used for analysis. Finely ground analytical grade KOH was washed with dichloromethane, dried at 120°C and stored. Woelm super activity grade basic alumina and reagent grade anhydrous sodium sulphate were used as supplied.

Standards

Solutions of compounds for use as standards were prepared by

dissolving each of the compounds biphenyl (2.5 mg), benzanthracene (34 μg), and hexadecane (11 mg) in dichloromethane (100 ml). PAH standard mixture SRM 1647 was obtained from the United States National Bureau of Standards, Washington D.C. This mixture contains each of the 16 numbered PAHs in Table I, dissolved in acetonitrile at concentrations ranging from 4–22.5 ug/ml.

Sample collection and treatment

Samples were collected on 15th September, 1982 from five locations in the Mermaid Sound area and from a sixth location outside the Sound on the seaward side of a small island in the West Lewis Island group (see Figure 1). Great care was taken to avoid contamination: all utensils and containers were scrupulously cleaned (glassware with chromic acid) and rinsed with purified dichloromethane before use. Sample sites were approached on foot to avoid contamination from boat exhaust. Samples of approximately 50 oysters were collected, placed in sealed 5-litre cans, chilled on ice, and flow to Perth on the same day. Immediately upon arrival they were shucked, drained, and stored at -10°C.

Isolation of a PAH fraction from oyster tissue

Approximately 50 g of frozen oyster tissue was homogenised with purified water (100 ml) and KOH (7 g). The mixture was then transferred into a 500 ml round-bottomed flask, the internal standards biphenyl (500 ng) and benzanthracene (50 ng) were added, and the mixture was refluxed under a nitrogen atmosphere for a period of 4 hours. After cooling, the digest was transferred into a 1-litre separating funnel containing purified water (200 ml). This mixture was acidified to pH 3 with 3 M HCl, then it was extracted first with pentane (2×100 ml) and secondly with 60:40 pentane/dichloromethane mixture (2×100 ml). The extracts were combined, washed with 5% KOH solution (2×100 ml) and purified water (2×100 ml), dried with anhydrous sodium sulphate, and the solvent was evaporated.

Pentane (3 ml) was added to the residue, and this pentane solution was then applied to a column of basic alumina (20 g). Alkanes were removed by elution with pentane (30 ml), and the PAH fraction was eluted with 3:1 dichlomethane/pentane mixture (200 ml). The aroma-

TABLE I

Concentrations (ug/kg or ppb, wet weight) of polynuclear aromatic hydrocarbons and dibenzthiophenes measured in the oyster (*Saccostrea cucculata*) collected from fives sites in Mermaid Sound, and one site outside the Sound (Site 6) on 15th September, 1982.[a,b]

Compound Number	Compound Name	Percent[c] recovery	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6	
			GC–MS	HPLC	GC–MS	HPLC	GC–MS	HPLC	GC–MS	HPLC	GC–MS	HPLC	GC–MS	HPLC
1	Naphthalene	60	5	7	0.8	1	4	4	5	6	3	4	3	1
	C_1	—	1	—	<0.3	—	1	—	2	—	1	—	2	—
	C_2	—	6	—	<0.3	—	6	—	8	—	5	—	4	—
	C_3	—	9	—	<0.3	—	13	—	18	—	8	—	5	—
	C_4	—	0.3	—	<0.3	—	7	—	10	—	<0.5	—	<0.2	—
2	Phenanthrene	60	15	13	9	8	4	2	6	6	38	26	4	2
	C_1	—	9	—	4	—	7	—	7	—	25	—	2	—
	C_2	—	13	—	4	—	11	—	9	—	33	—	1	—
	C_3	—	11	—	2	—	8	—	6	—	18	—	<0.2	—
	C_4	—	12	—	<0.3	—	5	—	1	—	6	—	<0.2	—
3	Dibenzothiophene	60	3	—	4	—	2	—	3	—	6	—	2	—
	C_1	—	3	—	1	—	6	—	6	—	14	—	1	—
	C_2	—	11	—	2	—	10	—	12	—	24	—	1	—
	C_3	—	15	—	0.6	—	14	—	12	—	25	—	0.8	—
	C_4	—	13	—	<0.3	—	9	—	5	—	8	—	<0.2	—
4	Anthracene	60	<1	0.9	<1	0.4	<1	<0.08	<0.2	<0.2	<1	0.1	<1	<0.08
Approximate sum of identified PAHs with two or three rings			127		28		107		111		215		26	

5	Acenaphthene	65	1	<1	2	1	<0.4	<0.2	<0.4	<0.2	<0.4	<0.2	<0.4	<0.2
6	Fluorene	65	<0.5	0.4	<0.5	0.4	<0.5	0.1	<0.5	0.4	<0.5	0.8	<0.5	0.2
7	Acenaphthylene[d]	45	<0.4	3	<0.4	1	<0.4	<2	<0.4	<2	<0.4	<2	<0.4	<2
8	Fluoranthene	55	22	17	5	3	1	0.9	0.9	0.7	6	4	0.5	0.4
9	Pyrene	65	15	11	2	2	0.4	<0.4	0.3	<0.6	3	1	0.4	<0.5
10	Chyrsene	60	<3	<3	<3	2	<3	<0.2	<3	<1	3	<1	<3	<0.1
11	Benzo(b)fluoranthene[e]	60	}5	6	<3	0.3	<3	0.3	<3	<0.07	<3	<0.1	<3	<0.03
12	Benzo(k)fluoranthene[e]	2		<0.05	<3	<0.09	<3	<0.02	<3	<0.01	<3	<0.06	<3	<0.01
13	Benzo(α)pyrene	60	<4	5	<4	<0.2	<4	<0.06	<4	<0.01	<4	<0.02	<4	<0.02
14	Dibenz(ah)anthracene	45	<4	<0.5	<4	<2	<4	<0.2	<4	<0.01	<4	<0.1	<4	<0.2
15	Benzo(ghi)perylene	60	<4	5	<4	<0.3	<4	<0.1	<4	<0.07	<4	<0.1	<4	<0.05
16	Indeno(123-cd)pyrene	40	<4	5	<4	<1	<4	<0.2	<4	<0.01	<4	<0.1	<4	<0.05
Approximate sum of identified PAHs with four or more rings			57		11		2		3		7		1	

[a]Values quoted include correction for recovery factor.

[b]Upper Limits are quote for concentrations which were below the detection limits of the procedures used.

[c]Percentage recoveries were determined by addition of submicrogram quantities of a mixture of standard compounds to a pristine oyster sample; and comparison of the HPLC detector responses with those of the compounds in the standard mixture.

[d]Determined by UV absorbance at 254 nm.

[e]Benzo(b)fluoranthene and benzo(k)fluoranthene coelute under the GC–MS conditions used.

tic fraction was then reduced to 100 μl using a Kuderna–Danish apparatus. If the extract could not be reduced to the required volume due to the presence of fatty material, the chromatographic procedure was repeated using fresh alumina (5 g). Preparative chromatography to prepare samples for analysis was performed using a Merck Lobar Grobe A (240-10) Lichroprep Si 60, 40–63 μm column, attached to an Alltech single piston pump. With a flow rate of 4 ml min^{-1} of a hexane/dichloromethane solvent mixture (95:5), a PAH fraction was collected between 6 min (naphthalene) and 23 min (dibenz(ah)pyrene). The volume of the eluate was reduced to 100 μl by careful evaporation of the solvent using a Kuderna–Danish apparatus. A gas chromatogram was obtained, then 10 μl of the standard solution of *n*-hexadecane was added and the volume reduced to 100 μl in a stream of nitrogen. Analysis by GC-MS was then carried out on this sample. When this was complete, in order to minimise the risk of sample alteration during storage, a solvent exchange to prepare the sample for HPLC analysis was carried out immediately. Acetonitrile (100 μl) was added carefully to the solution (100 μl) so that a second lower phase was formed. Most of the hexane was then removed by evaporation in a gentle stream of nitrogen to leave a homogeneous residue which was made up to 100 μl with acetonitrile. A 5 μl sample of this solution was then used for HPLC analysis. All analyses were thus performed on the same day. A procedural blank was carried out in the absence of oyster tissue. Analysis by GC, GC-MS, and HPLC at sensitivity settings used for subsequent analyses revealed no detectable contamination.

Gas chromatography

Capillary gas chromatography (GC) was carried out using an HP Model 5880A gas chromatograph equipped with a Grob-type splitless injector and a flame ionisation detector. A 25 m × 0.25 mm ID SE-52 fused-silica column was used with a temperature program of 40°C to 260°C at 4°C min^{-1} and hydrogen as the carrier gas at a linear flow velocity of 30 cm sec^{-1}.

Gas chromatography—mass spectrometry

Capillary gas chromatography—mass spectrometry analysis was

performed using an HP 5985B system equipped with an HP 50 m × 0.2 mm ID OV-1 high performance cross-linked fused-silica column. A temperature program of 40°C to 280°C at 4°C min^{-1} was used in conjunction with a hydrogen carrier gas flow velocity of 30 cm sec^{-1}. The mass spectrometer was operated in the AQIRE mode with EI at 70 eV. Quantification was carried out using the parent ion for each compound. Detector response factors were determined for all compounds for which percentage recoveries are listed in Table 1. Detector response factors were also determined for 1-methylnaphthalene and 2,3-dimethylnaphthalene and found to be similar to that for naphthalene. In all other cases, in calculating values for Table 1, detector responses for methyl derivatives were assumed to be the same as for the parent hydrocarbons.

Liquid chromatography

Reverse-phase HPLC was carried out using a Varian 8500 series dual pump LC system equipped with a WATERS ASSOCIATES Radial Compression Module with a RADIAL PAK C_{18} cartridge. The solvent program used was 40% acetonitrile: 60% water for 10 min, then the water component was reduced by 3% min^{-1} for 20 min, and finally 100% acetonitrile for 10 min. The flow rate was 2 ml min^{-1}. A WATERS ASSOCIATES Model 420-AC Fluorescence Detector was used with a range of filter combinations: excitation 254 nm, emission 360 nm; excitation 254 nm, emission 425 nm; excitation 280 nm, emission 425 nm; and excitation 300 nm, emission 425 nm. Detector response factors were determined for individual PAHs at each pair of settings: these in turn were used to determine PAH concentrations giving an average value which is that presented in Table 1. A WATERS ASSOCIATES Series 440 UV absorbance detector at a wavelength setting of 254 nm was used for detection of acenaphthylene which has a weak fluorescence response.

Determination of recovery factors of individual PAHs from oyster tissue.

The recoveries of individual PAHs from oyster tissue were determined as follows. Dibenzothiophene (400 ng), biphenyl (500 ng), and 50 μl of the EPA standard PAH mixture (SRM 1647) were added to

oyster tissue (50 g). This mixture was then homogenised in a blender, and the PAH fraction was isolated and treated as described above. Percentage recoveries were determined firstly by GC-MS by comparison of the ion count for each component with that of the *n*-hexadecane internal standard, and making allowance for detector response factors. With the exception of dibenzothiophene and phenanthrene which co-elute, percentage recoveries were also determined for all compounds by comparison of HPLC peak heights with those of a trace of a mixture of authentic standards. Recoveries determined by the two methods agreed within $\pm 5\%$ in all cases: average values are presented in Table 1.

RESULTS AND DISCUSSION

Analytical protocols for determination of trace levels of PAHs in biota include three main steps, which are interrelated to some extent. The first step is isolation of a PAH fraction by solvent extraction to the sample. Secondly, there is the cleanup of the PAH isolate to remove material which would interfere with the subsequent analysis. Finally, measurement of the PAHs is carried out. Lee and his co-workers[26] have recently presented a comprehensive review of the state of the art, and both these workers and Warner, Riggin and Engel[21] have described the potential of the various analytical techniques in some detail.

The extraction procedure used to isolate the initial crude PAH fraction is probably the major area of variation between different laboratories carrying out PAH analyses. We heartily endorse the conclusions drawn by Lee and his co-workers[26] regarding the use of an aqueous digestion medium as opposed to alcoholic media; an alcoholic medium appears to offer no advantages but can cause considerable interference problems in subsequent analyses due to the formation of esters which are very difficult to remove in post-digest cleanup procedures. We found this to be a major difficulty in analysis of aromatics in biota from an area subject to chronic exposure to petroleum in a refinery effluent,[31] where the GC-MS analyses revealed complex mixtures of esters similar to phthalates.[32] We have also found that the acidification of the digest prior to extraction, as recommended by Lee and co-workers,[26] was very effective

in reducing the formation of stable emulsions. In our case, we acidified to pH 3 with 3 M HCl then extracted, firstly, with two aliquots of pentane followed by three aliquots of 60:40 pentane/dichloromethane, and this gave few emulsion problems and reproducible extractions. The use of the United States National Bureau of Standards mixture of EPA-designated PAHs made determination of recovery factors for the individual PAHs a comparatively simple matter. As is shown in Table 1, the recoveries are fairly uniform, typically in the range 45–65%, with the extraordinary exception of benzo(k) fluoranthene which is 2%. Values quoted in Table 1 for benzo(k)fluoranthene are consequently subject to a much greater error than those for other compounds. At present we can offer no explanation as to why this compound should behave so differently from its isomers and homologues. We are not aware of any published reports of recovery factors for such a range of PAHs, however, using a procedure involving extraction of an alkaline digestion mixture, Smith *et al.*[39] measured recovery factors of benzo(k)fluoranthene in excess of 100%; for four others the values were in the range 60–80%, but for perylene and benzo(ghi) perylene recoveries were only 13% and less than 20% respectively.

The cleanup procedure employed to prepare a sample for quantitative analysis depends to some extent on the selectivity of the analytical procedure used. Solvent partitioning procedures using dimethylsulphoxide (DMSO) and an alkane solvent have been widely used to remove biogenic materials,[16] but these now appear almost certainly to be replaced by gel permeation chromatography.[21,26] In our case, this was not considered necessary because our cleanup regime of removal of gross quantities of polar compounds on an alumina pre-column, followed by collection of a PAH fraction from a pre-calibrated Merck Silica MPLC column, gave fractions of the quality indicated by the capillary GC traces shown in Figure 2. Examination of mass spectral data of peaks labelled *a-j* in the GC traces indicated that they were a variety of compounds which were mainly long-chain oxygenated species, which did not cause interference with the selective detection methods used in this study.

The two methods of choice for quantitative analysis of PAH mixtures are capillary GC-MS-DS,[3,4,10,21,28,29,30] and reverse-phase HPLC-UVF using a range of excitation and emission wavelengths.[22,23,27] As can be seen from the results shown in Table

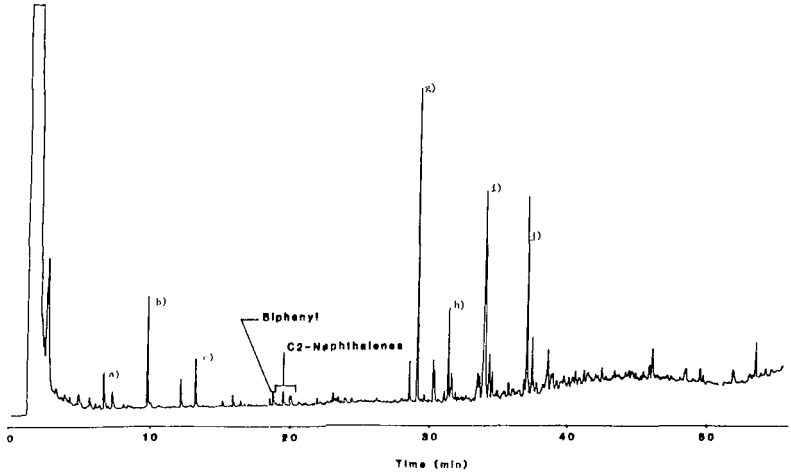
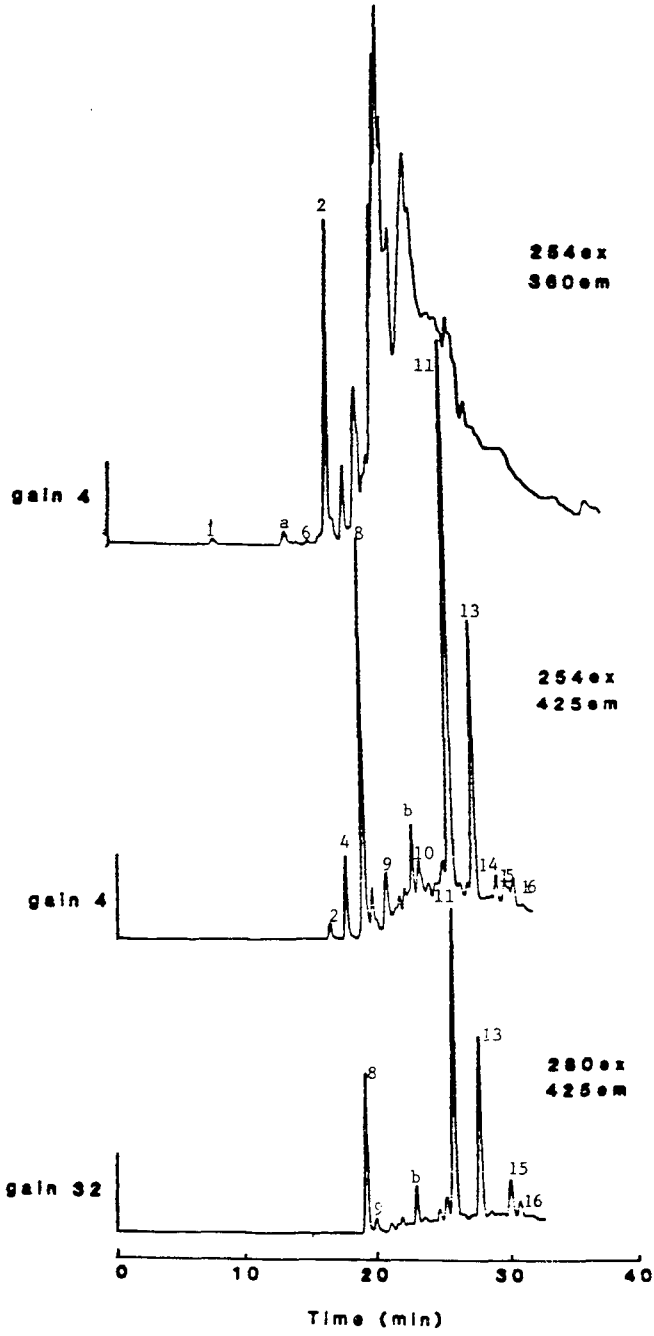


FIGURE 2 Capillary gas chromatogram of the PAH fraction obtained from oysters (*Saccostrea cucculata*) from Site 1. The peaks labelled *a–j* are long chain oxygenated species as described in the text.

1, the two techniques are to some extent complementary; however, we are aware of other reports[33,34] where both procedures were used to determine PAHs. Difficulties were experienced with HPLC in resolving the very complex mixture of isomers found in petroleums, but as is evident from the HPLC traces shown in Figure 3 and Figure 4, the very high sensitivity of the procedure and the selectivity available from the use of a range of wavelengths make it the method of choice for determination of higher molecular weight PAHs. Capillary GC-MS-DS is the preferred method for analysis of lower molecular weight PAHs. Fused-silica capillary columns of 4,000 or more theoretical plates per metre enable separation of the complex mixtures of isomers and homologues, and a mass spectrometer with a computer and a data system acts as a very selective detector. Injector frationation, decomposition, broadening of longer retention time peaks, and lower sensitivity for some PAHs than HPLC-UVF make GC-MS less attractive for analysis of higher molecular weight PAHs, although most of these drawbacks might be largely overcome with the use of an on-column injector and a careful choice of GC and MS conditions.



SITE 1

FIGURE 3 HPLC traces of the polynuclear aromatic hydrocarbon fraction from oysters (*Saccostrea cucculata*) from Site 1. Detection by UVF using the three different filter combinations shown. Peak numbers are those shown in Table 1, and the peaks labelled *a* and *b* are due to the internal standards biphenyl and benzanthracene.

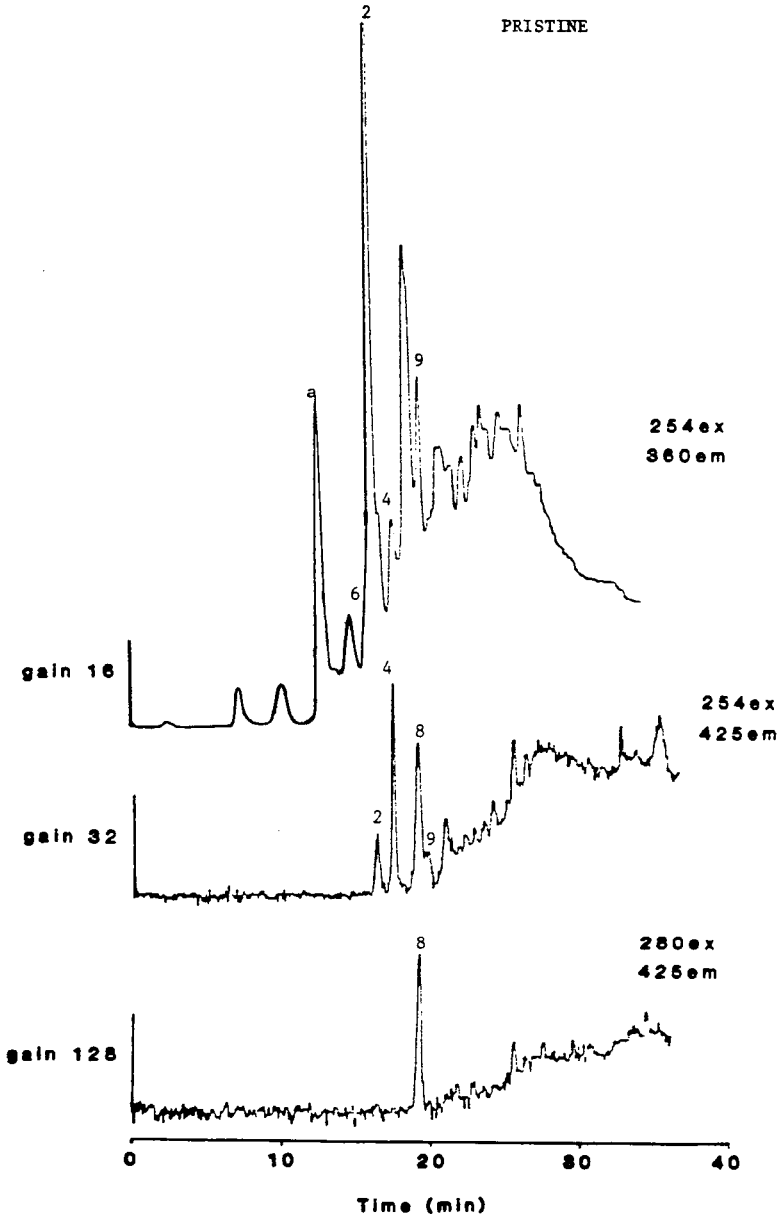


FIGURE 4 HPLC traces of the polynuclear aromatic hydrocarbon fraction from oysters (*Saccostrea cucculata*) from Site 6. Detection by UVF using the three different filter combinations shown. Peak numbers are those shown in Table I, and the peak labelled *a* is due to the internal standard biphenyl.

The values for PAHs shown in Table 1 are best considered in two broad groups. The two- and three-ring species are major components of crude petroleums which typically find their way into the lighter refined products such as gasoline, kerosene and gas oil.[35] These compounds are lost from spill slicks by evaporation at rates which reflect their relative vapour pressures. In cases of acute spills, they can be rapidly depurated by molluscs, with the two-ring compounds tending to be depurated faster than three-ring compounds.[4] In situations where there has been repeated exposure to petroleum for longer periods of time depuration occurs much more slowly.

The second group of compounds in Table 1 are those with four or more rings. These compounds occur in crude petroleum, where typically alkyl substituted homologues predominate.[1] They are also produced in processes where organic material undergoes pyrolysis, thus they may occur at substantial levels in materials such as coal tar, asphalt, residual oils, and many other materials. In these cases, the parent, unsubstituted compounds tend to be a significant proportion of the PAH assemblage.[1] When an oil slick is weathered and the more volatile, water-soluble, and biodegradable components are progressively removed, the proportion of the higher molecular weight components must necessarily increase, so that the PAH composition in exposed oysters will be affected accordingly.

Taking the two- and three-ring PAH results first, we find that Site 2 and Site 6 show very low levels. We suspect that the values for Site 6, which is outside Mermaid Sound, reflect exposure to minor quantities of small boat fuel; there were boats at the island at the time of sampling, and a major route to a fishing and recreational resort passes nearby. The low levels found at Site 2 compared with those found at Site 3 and Site 4 are interesting. At the time of sampling, there was a high level of construction activity, and heavy earthmoving equipment was operating in the vicinity of both Site 3 and Site 4. The most obvious source of the higher levels of these PAHs observed in the oysters from these sites would therefore appear to be diesel fuel or similar material used in these operations. Another plausible, but perhaps less likely, explanation of these observations lies in the fact that all of these sites are close to each other on the shoreline of Mermaid Sound, facing the shipping lane servicing the iron ore and salt ports at Dampier. It is possible

therefore that Sites 3 and 4 may have been exposed to a fresh slick of a light material such as gasoline or diesel fuel, and with the prevailing south to north current in the Sound, Site 2 may have escaped.

The relative concentrations of individual two and three-ring PAHs in oysters from Site 1 and Site 5 are broadly similar. The proportions of three-ring PAHs is greater at these sites than it is at Sites 3 and 4, an observation which may be explained in two ways. Firstly, the oysters may have been exposed to a material with a composition similar to weathered diesel fuel from which the lower molecular weight material such as the naphthalenes had been lost. Secondly, the observed distributions may have arisen from exposure of the oysters to a material like fresh diesel fuel, with subsequent loss of the more rapidly depurated lower molecular weight material. This latter explanation is perhaps most likely, as a temporary workboat refuelling station had been located near to Site 5 but had ceased operation some months prior to sample collection. Further, Site 1 is located immediately adjacent to the wharf facilities and supply base located on the north shore of King Bay.

In broad terms, the levels of two- and three-ring PAHs can be described as follows. The low levels at Sites 2 and 6 are comparable with those reported for clean sites,[9,10] and those for the other sites are similar to those found at sites with low levels of contamination.[9,10,38] The higher values are a factor of approximately 20–200 lower than levels found in mussels 250 metres from a refinery outfall[31] (Table II).

The values for PAHs with four or more rings show that levels in oysters at Sites 3, 4, and 6 are extremely low: in most cases the level of individual compounds is below the detection limit of the procedure. The levels at Site 5 are marginally higher, perhaps reflecting the exposure to gas oil. The levels at Sites 1 and 2 are noticeably higher than those at the other sites; further, there is a significant difference between the HPLC traces for these sites and those for the other sites. At Sites 1 and 2, the parent PAHs appear to form a substantially greater proportion of the total PAH assemblage than they do at the other sites, although there is a typical unresolved hump due to complex mixtures of alkyl PAHs at all sites. The fact that these two sites are closest to the port and town of Dampier may be significant; however, we can find no really satisfactory explanation

TABLE II

Levels of identified two- and three-ring polynuclear aromatic hydrocarbons in the oyster (*Saccostrea cucculata*) from the six sites in Mermaid Sound compared with those found in the mussel (*Mytilus edulis*) from the BP refinery outfall, Cockburn Sound.[31]

Sample location	Concentration (μg/kg)
Mermaid Sound Site 1	127
Mermaid Sound Site 2	28
Mermaid Sound Site 3	107
Mermaid Sound Site 4	111
Mermaid Sound Site 5	215
Mermaid Sound Site 6	26
Cockburn Sound BP refinery outfall	5000

for these observations. They do not appear to be attributable to the more obvious potential sources such as cresoted pilings, run off from roads or asphalted areas, or similar common sources.[1,10] In broad terms, compared with values reported from other areas,[1,9,10,11,37,39] the levels at Sites 2–6 of this group of PAHs with four or more rings would have to be described as very low: in most cases the levels were close to or below the limits of detection for the procedure. At Site 1 the levels are clearly higher, but still well below those found in polluted areas.

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