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Korea Maritime University

The High Performance Liquid Chromatography (HPLC) Analysis of Polycyclic Aromatic Hydrocarbons (PAHs) in Mussels and Oysters from the intertidal zones of Chinhae Bay, Korea

Il Noh and Ki-Seok Lee*

The Department of Ocean Environmental Engineering, Korea Maritime University
*The Department of Ocean Engineering, Korea Maritime University

Abstract

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous contaminants in marine environments. PAHs enter estuarine and nearshore marine environment via several routes such as combustion of fossil fuels, domestic and industrial effluents and oil spills.

PAHs have been the focus of numerous studies in the world because they are potentially carcinogenic, mutagenic, and teratogenic to aquatic organisms and humans from consuming contaminated food. However, one can hardly find any available data on PAH content in marine organisms in Korea.

The present study was carried out in order to determine PAH content in mussels and oysters from the intertidal zones of Chinhae Bay, which is located in near urban center and industrial complex, and the bay is considered to be a major repositories of PAHs.

PAHs were analyzed by High Performance Liquid Chromatography (HPLC) with uv/vis and fluorescence detectors. 15 PAHs were analyzed in mussels and oysters, and they are Naphthalene (NPTHL), *Acenaphthylene* (ANCPL), *Acenaphthene* (ACNPN), Fluorene (FLURN), Phenanthrene (PHEN), Anthracene (ANTHR), Fluoranthene (FLRTH), Pyrene (PYR), *Benzo(a)anthracene* (BaA), Chrysene (CHRY), Benzo(b)fluoranthene (BbF), Benzo(k)fluoranthene (BkF), *Benzo(a)pyrene* (BaP), Dibenz(a,h)anthracene (DahA), and *Indeno*(1,2,3,-cd)pyrene ($I_{123cd}P$).

The PAH content in mussels ranged from < 0.1 to 1,600.5 ppb (mean 62.5 ± 10.7 ppb), and that in oysters ranged from < 0.1 to 992 ppb (mean 69.8 ± 9.85 ppb).

I. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) consist of hydrogen and carbon arranged in the form of two or more fused benzene rings in linear, angular, or cluster arrangements with unsubstituted groups possibly attached to one or more rings (Eisler, 1987). Of aromatic hydrocarbons, PAHs are now being of public concern, because they are potentially carcinogenic, mutagenic and teratogenic to aquatic organisms and humans from consuming contaminated food (Richards and Jackson, 1982; Mix, 1984).

However, we can't find any available data on the levels of PAHs concentrated in marine organisms in Korea, even though it is urgent to accumulate a database on spatial and temporal distributions of PAHs in marine flora and fauna for environmental and sanitary sense. Because PAHs are ubiquitous, humans are exposed to these chemicals as part of everyday living.

PAHs are also widespread in aquatic environment and enter marine environment via several route, domestic and natural sources such as biosynthesis by plant and microorganisms. However, oil spill and incomplete combustion of fossil fuels are major sources of PAHs (Neff, 1985; Rainio *et al.*, 1986). They typically adsorb to fine particulate material suspended in estuarine waters and sediment seafloor (Law and Whinett, 1992).

The purpose of this study is focused on the determination of PAH content in mussels (*Mytilus edulis*) and oysters (*Crassostrea gigas*) living in the intertidal zones of Chinhae Bay in Korea, with developing the methodology of HPLC analysis of PAHs in mussels and oysters in coastal areas of Korea.

II. MATERIALS AND METHODS

1. Study area

The study area, Chinhae Bay (Fig. 1) with a total area of nearly 637 km^2, includes several smaller bays such as Masan Bay, Haengam Bay, Chindong Bay, etc. the depths of the study area range from 5m to 20m.

Chinhae Bay is located in near urban communities of Masan and Changwon industrial complex. There are many small and medium industries of textile, metalworking, machine, electronics, petrochemical, automobile, shipbuilding, etc. Accordingly, vast amount of urban runoff, domestic and industrial wastewater has been discharged into the bay and subsequently contaminated the study area chronically

via several streams.

For many years, this area has been the recipient of various environmental injuries because of rapidly growing urban and industrial developments. Cities of Masan, Changwon and Chinhae surrounding Chinhae Bay to the north and east, are heavily populated areas. Total population around the Chinhae Bay is over 1.1 million, and approximately 3×10^5 tons/day of municipal and industrial wastewater currently are discharged into the Chinhae Bay (Ministry of Environment, 1991). The COD load of 45 tons/day was measured to be mainly due to discharges from Masan and Changwon City (Lee *et al.*, 1993).

As a matter of course, red tides and summer oxygen deficiencies have frequently occurred in this study area (Park, 1982; Hong, 1987).

2. Sampling of mussels and oysters

Mussel (Mytilus edulis) and oyster (Crassostrea gigas) samples were collected at 10 sites (Fig. 1) in the intertidal zones of the Chinhae Bay, during October of 1996. Much care was taken for collecting samples in the study area, to avoid contamination from the discharging water from the engine of the sampling boat used.

Samples collected in the study area were wrapped in aluminum foil, placed in an icebox and brought to the lab, and then stored frozen in a refrigerator at -20℃ for a couple of weeks at maximum prior to analysis.

3. The extraction of PAHs

HPLC grade of hexane, acetone, diethylether, petroleum ether, methanol, dimethylsulfoxide, cyclohexane, etc was used for all extraction procedures.

Glassware was washed in diluted detergent and rinsed twice with distilled water, followed by 90 min combustion at 560℃. Just before use, glassware was rinsed with the appropriate solvent (Smith *et al.*, 1987).

For analysis, the mussels and oysters were partially thawed, and the shells were removed. The tissue of mussels and oysters was respectively homogenized with macerator. 20g (wet wt) of each mussel and oyster homogenate was dried with anhydrous sodium sulfate ($NaSO_3$).

The hydrocarbons with the fats were Soxhlet-extracted with a mixture of hexane, acetone, diethylether, and petroleum ether (2.5 : 7.5 :1 : 9, v/v) for 6 hour at 40 ~ 60℃ (Rainio *et al.*, 1986). And then the solvent was evaporated to near dryness at 40℃ with Rotary Evaporator.

Mixed solvent, 150 ㎖ of methanol containing 7g of potassium hydroxide, was added to the fatty residue and the mixture was refluxed for saponification in darkness for 3 hours, and then 50 ㎖ of water added. After cooling for digestion, the methanol potassium hydroxide solution was separated with a separating funnel, and diluted solution was repeated three times with 50 ㎖ of cyclohexane (Smith *et al.*, 1984; Smith *et al.*, 1987).

Soxhlet and refluxing apparatus were wrapped with aluminum foils, to prevent the compounds of hydrocarbons from being affected by light, because hydrocarbons can be altered by photochemical oxidation (Lee *et al.*, 1978; Tjessem and Palmork, 1984; Barth, 1984; Berthou *et al.*, 1985; Ducreux *et al.*, 1986).

To separate PAHs from the aliphatic hydrocarbons, the liquid-liquid extraction procedure developed by Natusch and Tomkins (1978) was applied. The dimethylsulfoxide (DMSO) layers, which contained the PAHs, were then combined. For clean up of PAHs, two volumes of water were added to the combined DMSO extracts.

The resulting solution was partitioned three times with equal volumes of cyclohexane. The cyclohexane layers were washed once with same volumes of water (Rainio *et al.*, 1986), and were dried nearly to a volume of 1.5 ㎖ with Rotary Evaporator at 40℃, and then the samples were filtered through a 0.45 ㎛ PVDF filter (Whatman).

Finally the sample was concentrated to a final volume of approximately 1 ㎖ under a stream of nitrogen gas, and the sample vials were stored in the freezer prior to analysis by HPLC.

4. HPLC system employed in the study

The analysis of PAHs was carried out by reverse phased HPLC (Linear Instruments co.) using a gradient elution. The HPLC system for the analysis of PAHs consists of binary solvent delivery system(Linear Instruments Model S-1100), an automatic gradient controller(Linear Instruments Model S-2000), an injector with a 20 ㎕ sample loop fitted with a Spherisorb S5 ODS 2 column (4.6 mm× 25 cm, 5 ㎛ particle size).

Solvent A of acetonitrile and solvent B of distilled water were utilized as mobile phases, and a binary gradient solvent system for the elution of PAHs employed in this study is as follows; solvent delivery was programmed at 70% solvent A for the initial condition, and then 80% solvent A at 12 min, 90% solvent A at 15 min, 100% solvent A at 20 min, followed by an isocratic hold (100% solvent A) until all the PAH peaks were eluted. The flow rate was held constant at 1.5 ㎖/min under the condition of 0.5 bar pressure.

Each sample was injected by 20 ㎕ syringe. Analytical blank tests were carried out between the each sample run and we found no analytical contamination for the HPLC system.

The peaks of PAHs were identified and quantified simultaneously using a fluorescence detector (Model LC 304 fluorescence detector) and uv/vis detector

(Model 200 uv/vis detector). The fluorescence detector was set up excited at 270 nm and emitted at 400 nm, and the absorption of uv/vis detection was set up at 254 nm.

The management of chromatograms, integration and calibration of data were carried out using Peaksimple Serial Data Program system (SRI Model 202).

III. RESULTS AND DISCUSSION

1. The analysis of PAHs in the standard solution and samples.

PAH assemblage in mussels and oysters analyzed by HPLC for this study consists of Naphthalene (NPTHL), Acenaphthylene (ANCPL), Acenaphthene (ACNPN), Fluorene (FLURN), Phenanthrene (PHEN), Anthracene (ANTHR), Fluoranthene (FLRTH), Pyrene (PYR), Benzo(a)anthracene (BaA), Chrysene (CHRY), Benzo(b)fluoranthene (BbF), Benzo(k)fluoranthene (BkF), Benzo(a)pyrene (BaP), Dibenz(a,h)anthracene (DahA), Benzo(g,h,i)peryline (BghiP) and Indeno(1,2,3,-cd)pyrene (I123-cdP). The chemical formula, structures, and retention times of each compound of PAHs were summarized in Table 1.

In the present study, certified reference material was used for verification of PAHs and each PAH was identified on the basis of retention time marked in the chromatograms.

The separation of individual PAH in the standard solution was satisfactory for the most part by the HPLC system; NPTHL, ANCPL, PHEN, ANTHR, FLRTH, PYR, BaA, CHRY, BaP and DahA were almost completely separated, while ACNPN, FLURN, BbF, BkF, BghiP, and I123cdP were not sharply separated with both fluorescence and uv/vis detection (Fig. 2 & 3).

When standard solution was analyzed with the uv/vis detector at 254 nm, the compound sets of ACNPN and FLURN, BbF and BkF, BghiP and I123-cdP were not sharply separated with each other, and even BaA compound could not be eluted. Nevertheless, uv/vis detector seems to be an useful tool for identification purpose of PAHs (Fig. 2).

ANCPL and BghiP were not eluted in the standard solution by the fluorescence detector under the wavelength set (Excitation: 270, Emission: 400), and the compounds of ACNPN and FLURN, BbF and BkF were not separated well likewise in uv/vis detection. However, the peaks of the rest of PAHs were could be clearly separated. BghiP is considered to co-elute with I123-cdP by fluorescence detection (Fig. 3).

The fluorescence detector was much sensitive than the uv/vis detector for the analysis of PAHs. Therefore, fluorescence detector was used for the purpose of quantification of each PAH. However, the peaks of ANCPL and BghiP were quantified using the uv/vis detector at 254 nm, because the fluorescence detector as mentioned before did not elute the two peaks.

The representative chromatograms of PAHs of mussels and oysters analyzed by fluorescence detector were presented in Fig 4. And the concentrations of PAHs in collected samples analyzed and quantified by the HPLC system were summarized in Table 2 and 3.

2. Mean concentrations of PAH compounds analyzed in mussels and oysters collected in the study area

1) PAHs in mussels

The predominant group of PAH compounds in mussels from the intertidal zone consisted of ACNPN (333.4 ± 34.2), NPTHL (96 ± 10.4), ANCPL (94.3 ± 14.3 ppb) and BaA (87 ± 16.2 ppb). The second dominant group of PAHs in mussels from the intertidal zone consisted of FLRTH (23.4 ± 2.0 ppb), BbF (13.1 ± 6.6 ppb), ANTHR (7.8 ± 1.7 ppb), PHEN (7.2 ± 0.6 ppb) and BkF (1.75 ± 0.75 ppb). And the least dominant group of PAHs in mussels from the same zone consisted of BaP (1.14 ± 0.09 ppb), PYR (0.6 ± 0.3 ppb), DahA (0.51 ± 0.33 ppb) , I123-cdP (0.26 ± 0.09 ppb), CHRY (0.18 ± 0.06 ppb) and FLURN (not detected).

2) PAHs in oysters

The predominant group of PAH compounds in oysters from the intertidal zone consisted of ACNPN (512.7 ± 63.1 ppb), NPTHL (105.2 ± 9.1 ppb), ANCPL (88.2 ± 12.2 ppb) and BaA (151.4 ± 57.9 ppb), just like in mussels. The second dominant group of PAHs in oysters from the intertidal zone consisted of FLRTH (25.9 ± 3.27 ppb), BbF (16.1 ± 6.1 ppb), ANTHR (8.6 ± 1.6 ppb), PHEN (7.61 ± 0.27 ppb) and BkF (10.7 ± 3.79 ppb). And the least dominant group of PAHs in oysters from the intertidal zone consisted of BaP (1.9 ± 0.39 ppb), PYR (0.2 ± 0.07 ppb), DahA (0.52 ± 0.14 ppb), , I123-cdP (0.61 ± 0.11 ppb), CHRY (0.58 ± 0.28 ppb) and FLURN (not detected).

In summarizing this section, ACNPN was the most dominant PAH compound in both mussels and oysters in the study area.

3. Mussel versus Oyster

Mean concentrations of PAH compounds in mussels versus oysters were given in Fig. 5. Mean concentrations of all PAHs except PYR in oysters were slightly higher than those in mussels..

The reason for oysters in the intertidal zone to have slightly higher concentration of PAHs is not clear yet.

We need more knowledge of metabolic processes of a mussel and an oyster regarding PAH diagnosis to fully understand the fate of PAHs absorbed by mussels and oysters, as well as biochemical and microbial

degradation of PAHs in and out of tissues of mussels and oysters.

4. Total PAH concentrations in mussels and oysters in the study area.

Total PAH concentrations on the average in mussels and oysters were as follows: 576.6 ± 80.4 ppb in mussels, and 918.7 ± 120.2 ppb in oysters from the intertidal zones.

The highest of total PAH in mussels was detected at site 7. In case of oyster, the highest value was detected at site 10.

Although methodological differences make a comparison difficult, it is somewhat worth comparing our data with other parts of the world in order to diagnose the pollution level of PAHs in marine organisms in the study area. Unfortunately, there is no available data on PAHs in mussels and oysters in domestic marine environment.

A comparison table is shown in Table 4. The NPTHL concentration in blue mussel of the Finnish Archipelago Sea (Rainio, 1986) was reported to be 41 ppb, which is half as much as that in mussels in Chinhae Bay Korea.

Rainio (1986) also reported that ANTHL in blue mussel was 14 ppb, which is two times that in mussels in Chinhae Bay. The concentration of the reat of PAHs couldn't be compared because Rainio did analyze the concentration of a couple of PAHs.

Using HPLC fitted with uv/vis detector, Cocchieri *et al.* (1990) analyzed PAHs in mussels and razor fishes collected in the Gulf of Naples, which is located in Mediterranean coast, and thought to be much contaminated by anthropogenic activity. Comparing the PAHs in the Gulf of Naples with our work, ACNPN, ACNPL, NPTHL, PHEN, ANTHR and BaA were reported in lower concentrations in the Gulf of Naples than those in Chinhae Bay, while PYR, CHRY, BbF, DahA and I123-cdP in the Gulf of Naples were generally higher than those in Chinhae Bay. As a whole, however, mussels and oysters in Chinhae Bay generally showed to have very similar contents of PAHs to those in mussels and razor fishes in the Gulf of Naples.

According to Cocchieri, the total PAH content observed in common mussel in the Gulf of Naples is much higher than that in unpolluted areas (< 0.5 ~ 148.0 ppb), but relatively lower than those in heavily polluted areas (534 ~ 1,060 ppb wet wt). The total PAH content in mussels in Chinhae Bay falls in the criterion of the heavily polluted area.

Pendoley (1992) analyzed the concentrations of PAHs in oysters in Rowley Shelf, Australia by HPLC with fluorescence detection. The PAHs ranged from < 2 to 3 ppb (wet wt), which were two to three orders of magnitude lower than those in Chinhae Bay.

Pancirov and Brown (1977) analyzed PAH concentration in mussels and oysters from New Jersey. The concentration of PAHs in mussels ranged from < 0.2 to 2 ppb, and those in oysters, from < 2 to 58 ppb. The level of 4 PAHs (PYR, BaA, CHRY and BbF) analyzed in mussels and oysters in New Jersey were very similar to those in mussels and oysters in Chinhae Bay, although most PAHs including the predominant group of this study were not analyzed in mussels and oysters in New Jersey.

There are few data on PAHs in marine mammals. However, Law and Whinnett (1992) reported the mean concentration of PAHs in muscle tissue of harbour porpoises (*Phocoena phocoena*), and they are shown in Table 4. And the concentration of PAHs in muscle tissue of harbour porpoises ranged from 1.5 to 4.4 ppb. The level of NPTHL, PHEN, ANTHR, FLRTH and PYR analyzed were definitely lower than those in mussels and oysters in Chinhae Bay, even though content of a pollutant in mammal can't be directly compared with that in invertebrates.

In summary, the level of PAHs in mussels and oysters in Chinhae Bay is thought to be very much similar to that in severely polluted areas in other countries, as can be definitely seen in table 4.

5. The source of PAH compounds

The naphthalene to phenanthrene ratio is particularly diagnostic for inputs of fresh petroleum. While Phenanthrene compound may be of murogenic, petrogenic, or diagenetic in origin, Naphthalene compound are characteristic of fresh crude oil. The N/P (Naphthalene/Phenanthrene) ratio is much greater than 1.0 for most petroleums and decreases to between ≈ 0.2 and 1.5 in clean sediments. These parameters and ratios are useful in defining the hydrocarbon composition of the marine organisms and sediments, and in distinguishing the relative importance of petroleum-derived (petrogenic) hydrocarbons versus biologically derived (biogenic) or combustion-derived (pyrogenic) hydrocarbons (Steinhauer and Boehm, 1992).

Pyrogenic or combustion-derived PAH assemblages are relatively enriched in three- to five ring PAH compounds; uncombusted fossil fuels are highly enriched in the two- to three ring PAHs (Boehm and Farrington, 1984).

In this study, the N/P ration is observed between 7.3 and 18.8 from mussels and oysters in intertidal zone (Fig. 6). We, therefore, would suggest that the PAH contents in the present study area is petroleum-derived. Also the relative abundance of two- to three ring PAHs ranged from 62 % to 90 % from mussels and oysters except site 2 (Fig. 7). It has been confirmed that the major source of PAH is not combustion-derived hydrocarbons but uncombusted fossil fuels. Major source of PAH could be domestic and industrial effluents, and engine or fuel oil spilled from the vessels navigating. And subsequently there were very little affected by runoff from near road,

deposition of aerosols.

IV. CONCLUSION

Acenaphthene (ACNPN) was the most dominant PAH compound in both mussels and oysters in the study area, and all the PAH compounds analyzed in mussels and oysters from the intertidal zones can be grouped into three: predominant group [Acenaphthene (ACNPN), Naphthalene (NPTHL), Acenaphthylene (ANCPL) and Benzo(a)anthracene (BaA)], second dominant group [Fluoranthene (FLRTH), Benzo(b)fluoranthene (BbF) , Anthracene (ANTHR), Phenanthrene (PHEN) and Benzo(k)fluoranthene (BkF)] and least dominant group [Benzo(a)pyrene (BaP), Pyrene (PYR), Dibenz(a,h)anthracene (DahA), Indeno(1,2,3,-cd)pyrene ($I_{123cd}P$), Chrysene (CHRY) and Fluorene (FLURN)].

Generally, mean concentrations of nearly all PAHs in oysters were higher than those in mussels, and the reason for that is not clear yet. We need more knowledge of metabolic processes of a mussel and an oyster regarding PAH diagenesis to fully understand the fate of PAHs uptaken by mussels and oysters.

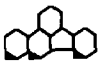
Total PAH concentration in mussels was 576.6 ± 80.4 ppb; oysters was 918.7 ± 120.2 ppb from the intertidal zones. These PAH contents generally seem to be higher than those in organisms living in polluted area like in mussel (*mytilus edulis*: 295 ppb) in the Gulf of Naples.

In conclusion, Chinhae Bay seems to be a heavily polluted area by PAHs, and further study about seasonal variations of PAHs in mussels and oysters as well as the PAH levels in various kinds of marine organisms would be needed for monitoring the area regarding PAH contamination.

According to N/P (NPTHL / PHEN) ratio and the ratio of 2 ~ 3 ring to 4 ~ 6 ring, we expect that the major sources of PAH compounds in this study area is petroleum-derived. Therefore domestic and industrial effluent and engine or fuel oil spilled from the vessels navigating in and out of the bay could be directly affected to the PAH level in the study, whereas it was little affected by runoff and combustion-fossil fuel.

Table 1. The chemical formula, structures and retention times (R*t*) of PAHs analyzed in the study

mw: molecular weight

	COMPOUND(ABBREV)	ALTERNATIVE NAME	FORMULAR (MW)	STRUCTURE	R*t* (min)†
1	Naphthalene(NPTHL)		$C_{10}H_8$ (128)		5.12
2	Acenaphthylene(ANCPL)		$C_{12}H_8$ (152)		5.46
3	Acenaphthene(ACNPN)		$C_{12}H_{10}$ (154)		7.35
4	Fluorene(FLURN)		$C_{12}H_{10}$ (166)		7.50
5	Phenanthrene(PHEN)		$C_{14}H_{10}$ (178)		8.45
6	Anthracene(ANTHR)		$C_{14}H_{10}$ (178)		9.35
7	Fluoranthene(FLRTH)		$C_{16}H_{10}$ (202)		11.24
8	Pyrene(PYR)		$C_{16}H_{10}$ (202)		12.32
9	Benzo(a)anthracene(BaA)	1,2 Benzanthracene	$C_{18}H_{12}$ (228)		13.21
10	Chrysene(CHRY)		$C_{18}H_{12}$ (228)		15.03
11	Benzo(b)fluoranthene(BbF)	3,4 Benzfluoranthene	$C_{20}H_{12}$ (252)		18.15
12	Benzo(k)fluoranthene(BkF)	11,12 Benzfluoranthene	$C_{20}H_{12}$ (252)		18.31
13	Benzo(a)pyrene(BaP)	3,4 Benzopyrene	$C_{20}H_{12}$ (252)		19.22
14	Dibenz(a.h)anthracene (DahA)	1,2,5,6 Dibenzanthracene	$C_{22}H_{14}$ (278)		20.13
15	Benzo(g.h.i)peryline (BghiP)	1,12 benzperylene	$C_{22}H_{12}$ (276)		21.12
16	Indeno(1.2.3-cd)pyrene($I_{123\ cd}$P)	o -Phenylenepyrene	$C_{22}H_{12}$ (276)		21.39

† The Retention times of PAHs analyzed were drawn from fluorescence detection, except ANCPL and BghiP, which were drawn from uv/vis detection.

Table 2. The concentration (㎍/㎏ wet wt) of PAHs in mussels from the intertidal zone determined by fluorescence detection (Excitation 270 nm : Emission 400 nm).

Site	NPTHL	ANCPL†	ACNPN	FLURN	PHEN	ANTHR	FLRTH	PYR	BaA	CHRY	BbF	BkF	BaP	DahA	I123-cdP	Total PAHs
Site 1	75.5	55.5	449.5	-	5	2	22.5	1	66.5	0.1	18	-	1	0.1	0.5	756.2
Site 2			-	-	-	-	-	0.1	143.5	0.1	2	1	1.5	0.3	0.2	148.6
Site 3	97.5	94	266	-	7	5	18	0.1	87	0.1	2	4	1	0.2	0.1	580.9
Site 4										Not Collected						
Site 5										Not Collected						
Site 6	101	57.5	310	-	5.5	6.5	18	-	74.3	0.3	-	1	1	-	0.1	584.1
Site 7	65.5	115	385	-	8.5	10	25	-	145	0.2	36.5	-	1	1.5	0.1	791.9
Site 8										Not Collected						
Site 9	137.5	145.5	226	-	8.5	13.5	27.3	1.5	33.5	0.1	-	1	1.5	-	0.5	596.4
Site 10	99	98.5	364	-	8.5	10	29.5	0.5	60	0.5	7	-	1	-	0.5	578.1

- : Concentration bellow the detection limits or not detected.
† : Concentration determined by uv/vis detection (uv = 254 nm).

Table 3. The concentration (㎍/㎏ wet wt) of PAHs in oysters from the intertidal zone determined by fluorescence detection (Excitation 270 nm : Emission 400 nm).

Site	NPTHL	ANCPL†	ACNPN	FLURN	PHEN	ANTHR	FLRTH	PYR	BaA	CHRY	BbF	BkF	BaP	DahA	I123-cdP	Total PAHs
Site 1										Not Collected						
Site 2	85	99.5	410	-	5	2.5	11.5	0.15	100.5	0.1	10.5	6	1	0.2	0.5	732.4
Site 3	155	38.5	586.5	-	9.5	5	32	0.1	146.5	0.2	2	15	3	0.2	0.5	993.8
Site 4	117	110	434	-	8.5	14	28.5	0.1	146	0.5	-	7.5	1.5	0.4	0.5	868.4
Site 5	92.5	142.5	667	-	8.5	11.5	29	0.05	102	2.5	-	16	3.5	1	0.5	1076.2
Site 6	97.5	47	294	-	6	6.5	19.5	0.5	53	0.2	23	-	2	0.5	0.5	550.2
Site 7	62.5	49.5	695		8.5	6	19	0.1	112.5	0.2	29	-	1	-	0.5	983.2
Site 8	93	125.5	400.5	-	7.5	4	17.5	0.2	50.5	0.1	-	1	0.5	0.5	0.5	702.3
Site 9	111.5	96.5	297.5	-	8	13	33.5	0.5	48.5	0.1	-	1	1	-	0.5	611.6
Site 10	132.5	84.5	830	-	7	14.5	43	-	603	1.5	-	28.5	3.5	1	1.5	1750

- : Concentration bellow the detection limits or not detected.
† : Concentration determined by uv/vis detection (uv = 254 nm).

Table 4. Comparison of PAH contents in mussels and oysters in Masan Bay with other studies.

N A : Not analyzed, - : Not detected, Units in ppb

PAHs	Masan Bay		Finland [A]	Italy [B]		Australia [C]	U.S.A [D]		England [E]	Antarctic [F]	
	Mussel*	Oyster*	Mussel	Mussel	Razor fish	Oyster	Mussel	Oyster	Porpoise	Seawater	Sediment
NPTHL	96	105	41	-	20	3	N A	N A	4.4	-	-
ANCPL	94	88	N A	60	25	-	N A	N A	N A	N A	N A
ACNPN	333	512.72	N A	35	27	0.4	N A	N A	N A	N A	N A
FLURN	-	-	N A	5	17	0.2	N A	N A	N A	N A	N A
PHEN	7.1	7.61	-	4	2	2	N A	N A	2.1	136.7	9.0
ANTHR	7.8	8.55	14	5	2	0.3	N A	N A	6	10.8	3.9
FLRTH	23.4	25.94	-	21	22	0.2	N A	N A	1.5	4.3	18.6
PYR	0.64	0.2	-	24	-	0.2	2	58	1.9	-	4.2
BaA	87	151.38	-	29	4	< 1	< 0.2	8	N A	0.3	4.7
CHRY	0.18	0.57	-	13	13	< 1	< 0.2-0.3	< 2-15	N A	1.3	20.6
BbF	12.6	16.12	N A	46	26	-	< 0.2-0.3	< 2-15	N A	1.1	3.7
BkF	2	10.71	N A	4	25	< 0.02	N A	N A	N A	-	5.4
BaP	1.16	2.2	-	5	5	< 0.01	< 0.5	2	N A	0.1	1.3
DahA	0.39	0.51	N A	20	-	< 1	N A	N A	N A	N A	N A
BghiP	N A	N A	N A	22	7	< 0.05	< 0.2-0.3	< 2-15	N A	-	7.4
I123-cdP	0.26	0.61	N A	2	4	< 0.1	N A	N A	N A	N A	N A

[A] Rainio *et al.*, (1986) in Finnish Archipelago Sea. [B] Cocchieri *et al.*, (1990) in Gulf of Naples. [C] Pendoley (1992) in Rowly Shelf. [D] Pancirov & Brown (1977) in New Jersey. [E] Law & Whinnett (1992) at UK waters. [F] Cripps (1992) in Antarctic.

*represented as mean concentrations in the intertidal zone.

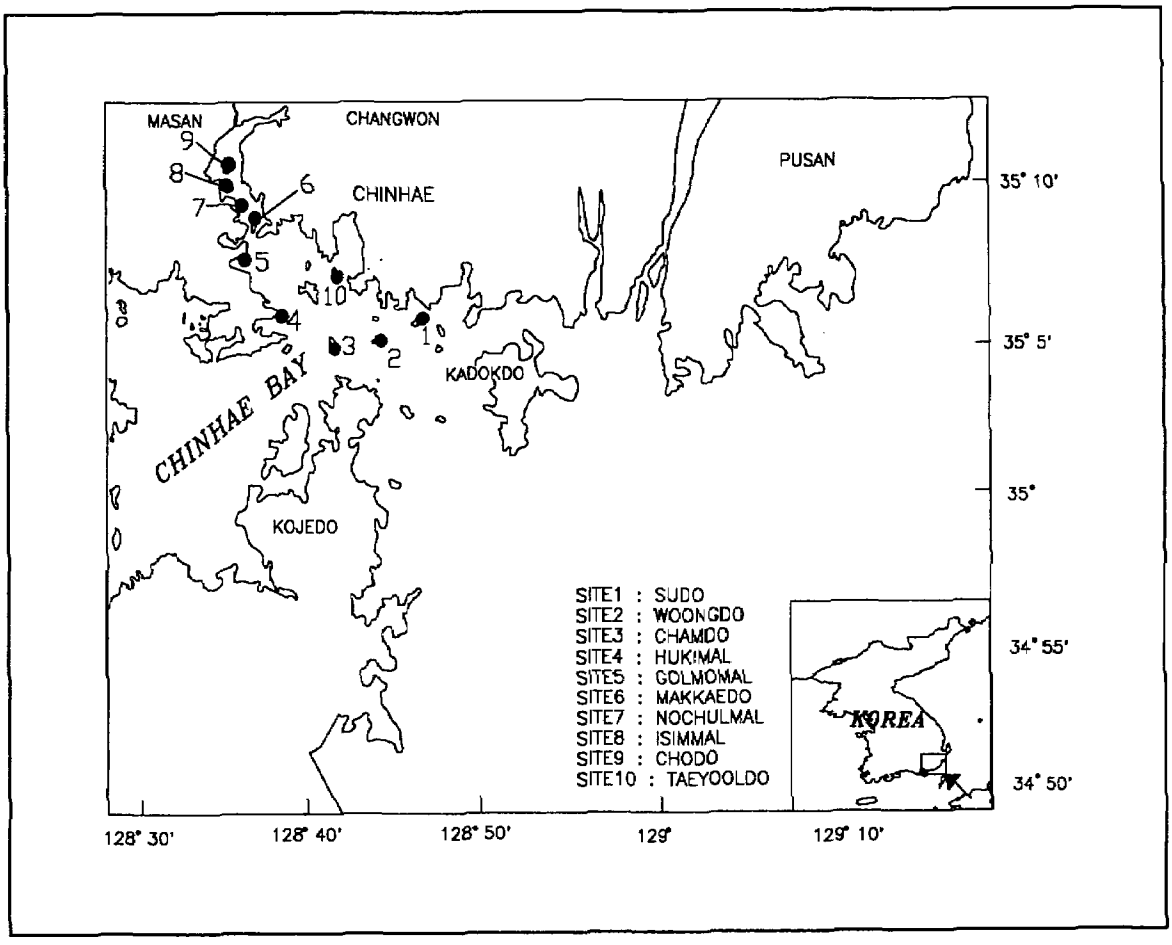


Fig. 1. Location of sampling sites in Masan Bay.

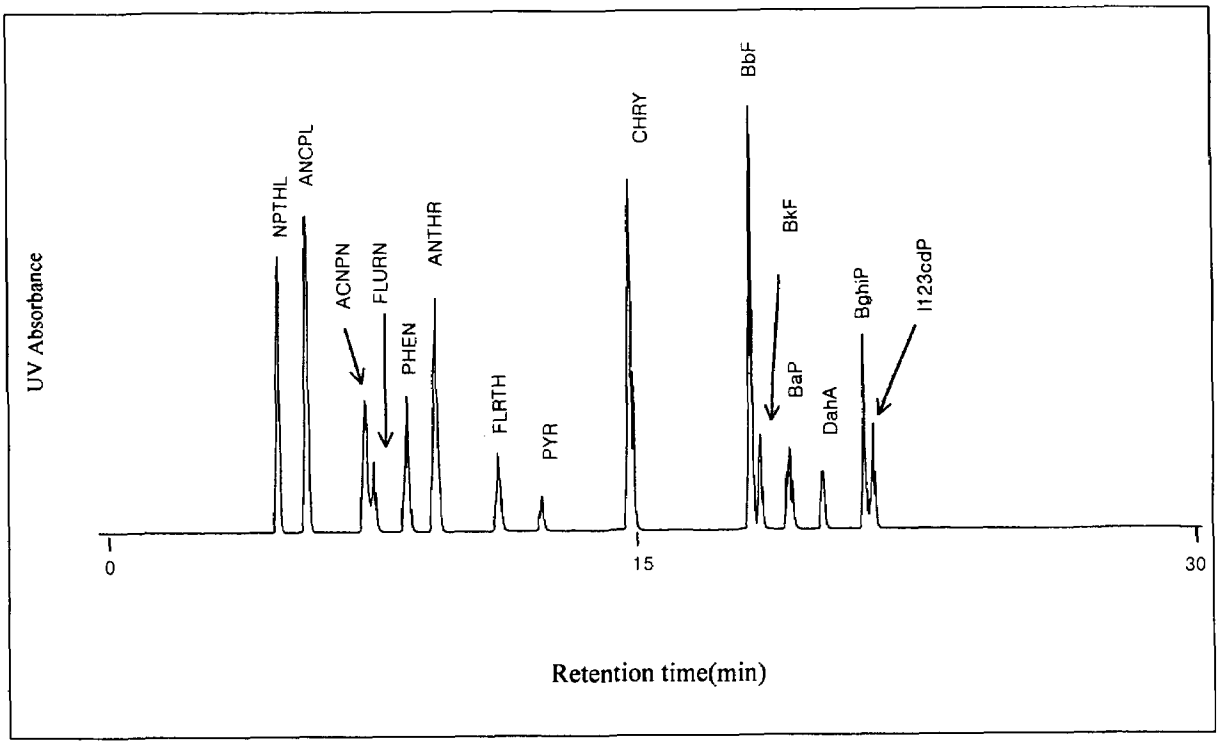


Fig. 2. Chromatogram of PAH standard solution by HPLC with uv/vis detection. BaA was not eluted at 254nm.

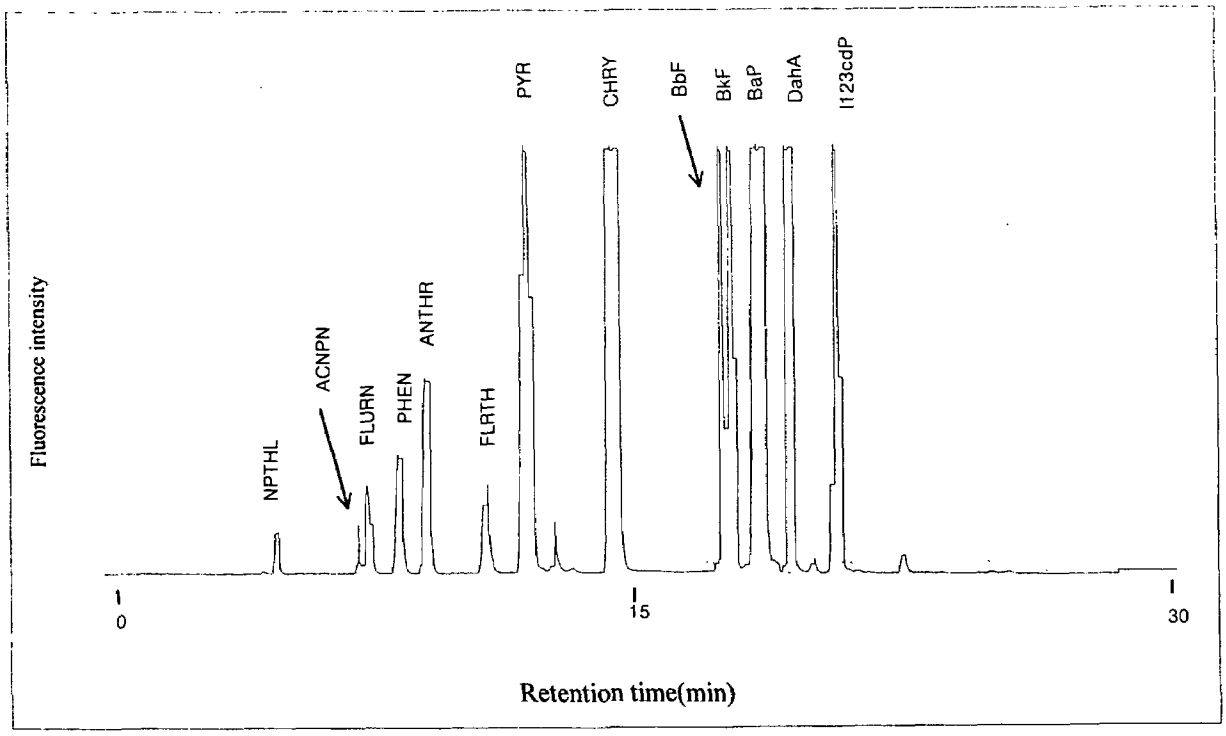


Fig. 3. Chromatogram of PAH standard solution by HPLC with fluorescence detedtion. ANCPL and BghiP was not eluted under the wavelength setting (excitation 270nm ; emission 400 nm).

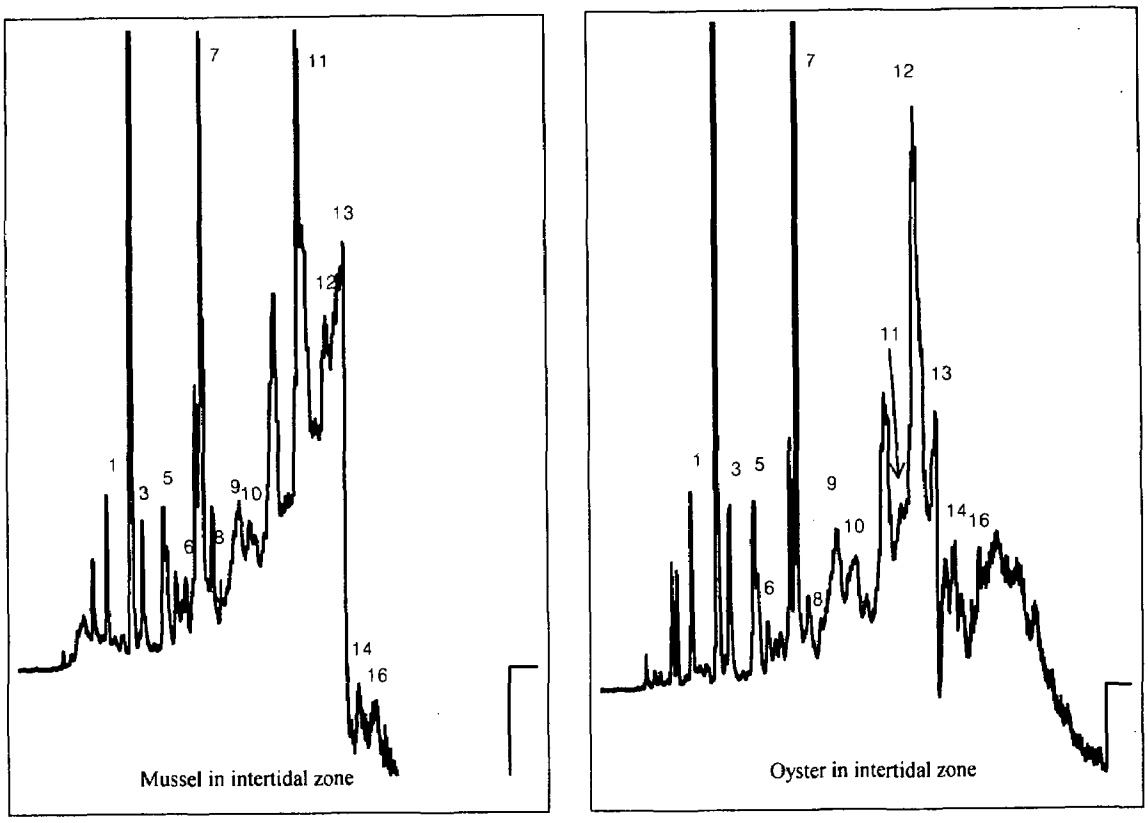


Fig. 4. Chromatograms of PAHs in mussels and oysters by HPLC with fluorescence detector at site 3.

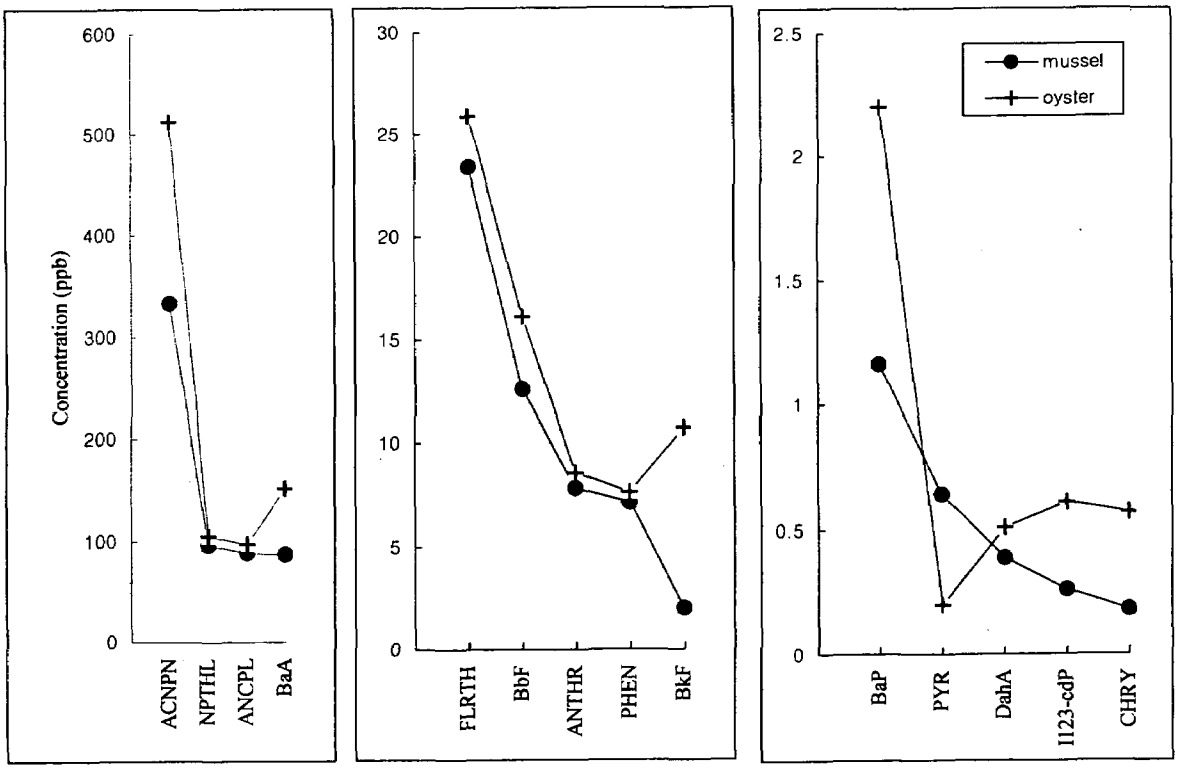


Fig. 5. The mean concentrations of PAH compounds in mussels versus oysters from the intertidal zone.

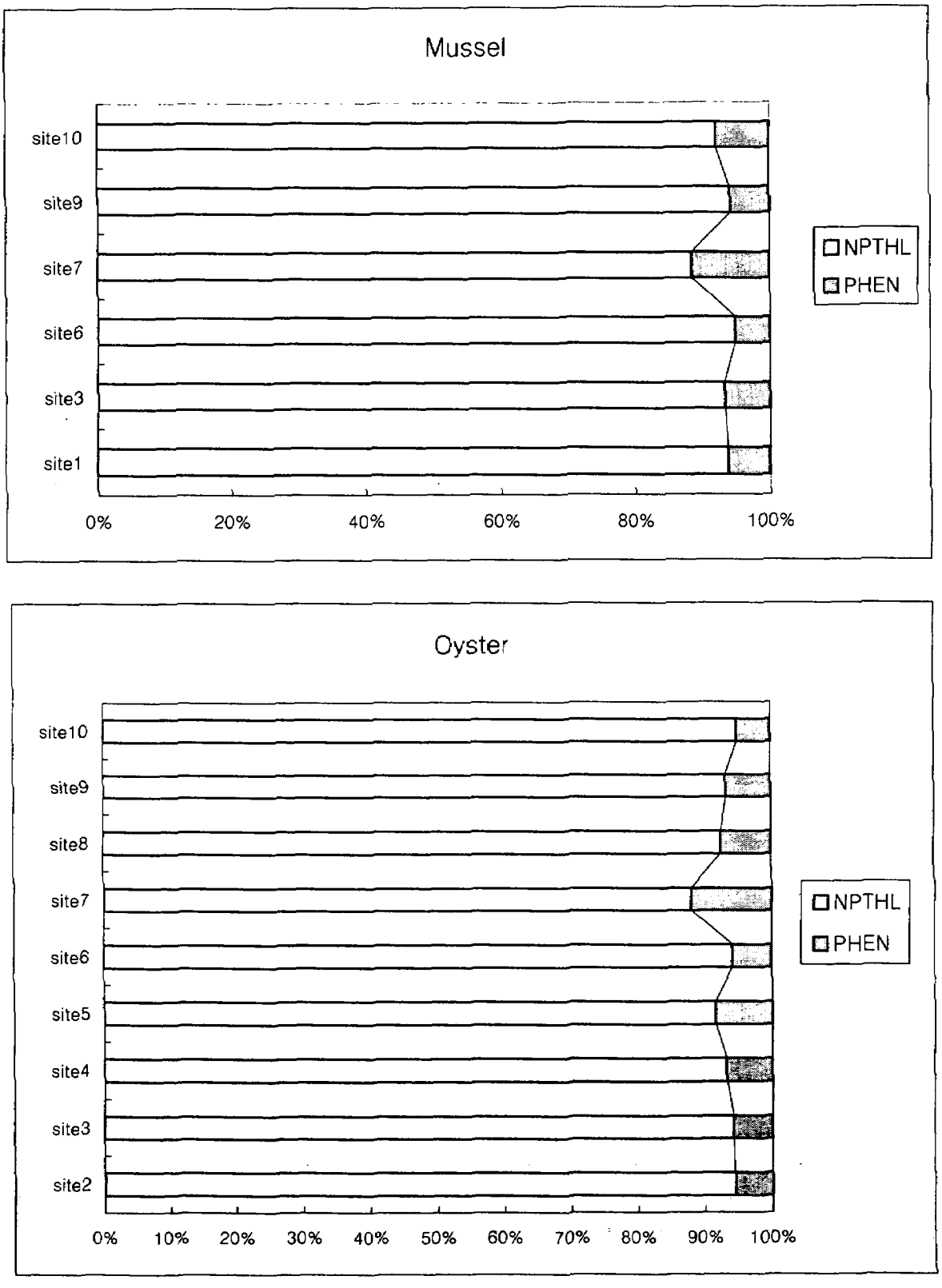


Fig. 6. NPTHL/PHEN (N/P) ration of each sites in mussels and oysters.

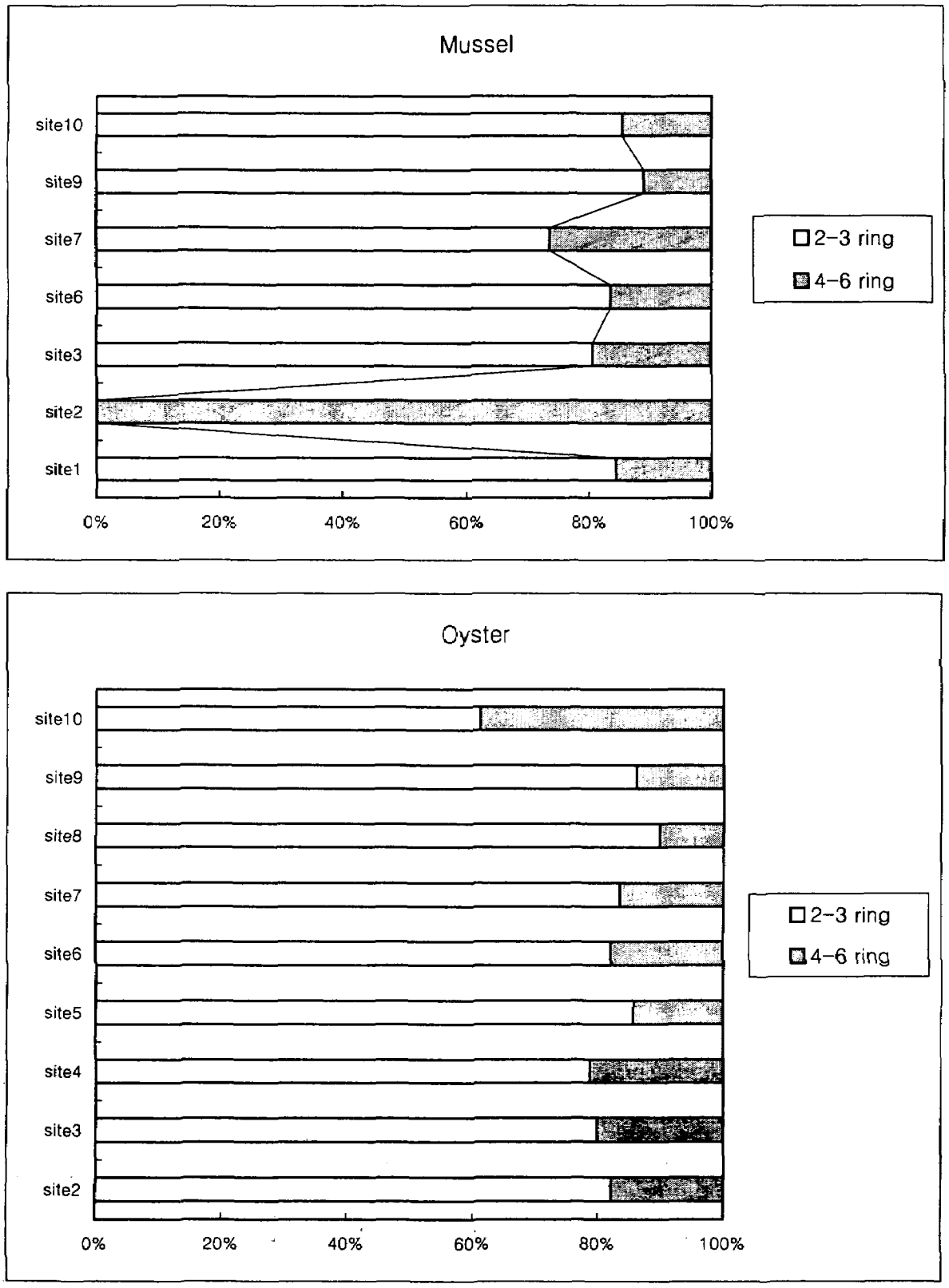


Fig. 7. The relative abundance of 2 and 3 ring vs. 4- and 6 ring in mussels and oysters.

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