Environmental assessment of Vancouver Harbour: The results of an International Workshop – trace metals

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Materials and methods

Sampling

Bottom sediments

Bottom sediment samples were collected by Van Veen grab from seven stations (Section I, Fig. 1.2). Three replicate samples were taken at each station. The surface layer of sediments was collected by plastic spoon in pre-cleaned Ziploc plastic bags. Samples were frozen after collection, then freeze-dried in the shore laboratory and transported to Russia for further analysis.

Mussels

Mussels (about 30 at each site) were collected from rocks and concrete piles during low tide at seven stations (Section I, Fig. 1.3). At all stations, mussels *Mytilus trossolus* were found. At station I-6, oysters *Crassostrea gigas* were also found. In the shore laboratory, soft tissues were removed, weighed, placed in pre-cleaned plastic containers and stored frozen. Then samples were freeze-dried and transported to Russia for further analysis.

Fish

Fish were collected by bottom trawl at 5 stations (Section I, Fig. 1.4). Fish muscle samples were taken from 5 individuals (English sole) at each trawling station and kept in pre-cleaned plastic bags on ice aboard the research vessel and then frozen in the shore laboratory. After freeze-drying, samples were transported to Russia for further analysis.

Analysis

In Vladivostok (Russia), samples of bottom sediments, mussel and fish tissues were homogenized and distributed for analysis in three laboratories:

- Pacific Research Centre of Fisheries and Oceanography (TINRO-Centre);
- Pacific Geographical Institute, Far East Branch, Russian Academy of Sciences (PGI FEB RAS);
- Pacific Oceanological Institute, Far East Branch, Russian Academy of Sciences (POI FEB RAS).

Analytical methods used at the TINRO-Centre are briefly described below.

Bottom sediments

After homogenization, about 0.4 g of dry sample was placed in a 50 ml Teflon beaker, $HClO_4$, HNO_3 and HF were added, the beaker was closed and heated to 50°C for 24 hours. Then HNO_3 and HF were added again and the beaker content was dried at 80°C. After that, 1 ml of concentrated HNO_3 and deionised water were added up to final volume of 20 ml. Concentrations of trace metals (Al, Fe, Co, Cr, Cu, Mn, Ni and Zn) were determined using the flame atomic absorption spectrophotometer NIPPON JARREL ASH, model AA-885, with D_2O background correction. For Al analysis, N_2O-acetylene mixture was used, for other metals – acetylene-air mixture. Contents of Cd and Pb were determined using a graphite furnace on atomic absorption spectrophotometer

HITACHI 170-70, with Zeeman background correction. Detection limits (ppm) were as follows: Al and Fe – 2, Cd – 0.0002, Cr – 0.02, Cu – 0.005, Pb – 0.04, Zn – 0.02.

Mussels

After homogenization, 1-3 g of dry sample were soaked in a Teflon beaker with concentrated HNO_3 (10 ml) for 24 hours, then the acid solution was heated to 120°C for 3 hours. After filtration, trace metal contents (Al, Fe, Co, Cr, Cu, Mn, Ni, Zn) were determined using the flame atomic absorption spectrophotometer NIPPON JARREL ASH, model AA-885. For Al analysis, N_2O-acetylene mixture was used, for other metals – acetylene-air mixture. Contents of Cd and Pb were determined using a graphite furnace on atomic absorption spectrophotometer HITACHI 170-70, with Zeeman background correction.

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Preliminary results and discussion

Metals in bottom sediments

Data on trace metal contents in bottom sediments are presented in the data section of this report. The results obtained in PGI and POI are in reasonable agreement with the TINRO-Centre data. According to the Fe content (from 2.3 to 4.4%), bottom sediment characteristics at sampling sites were quite different. Concentrations of total copper at all stations except B-50 (Howe Sound, reference site) were higher than 34 ppm (ERL, Long *et al*. 1995). Maximum concentration, 333 ppm, was observed at station B-3A (Sulfur Dock/Copper Ore Dock). On the contrary, contents of cadmium at all stations except B-3A were below ERL value, 1.2 ppm. Concentrations of Pb and Zn exceeded those criteria (46.7 ppm and 150 ppm respectively) at stations B-3A, B-38 (Port Moody, refinery) and B-41B (Port Moody, Ioco). For all these metals, maximum contents were observed at station B-3A (Sulfur Dock/Copper Ore Dock).

A large amount of data on trace metal contents in bottom sediments of Vancouver Harbour have been obtained by Canadian researchers (e.g., Goyette and Boyd 1989; Boyd *et al*. 1998). Data from these two reports for Cd, Cu, Pb and Zn are given in Table 1 along with the results from the PICES MEQ Practical Workshop. A similar comparison for the most polluted (in 1999) station B-3A is shown in Table 2. In both cases a decreasing trend in trace metal concentrations is evident.

Table 1. Trace metals in bottom sediments of Vancouver Harbour in 1985-87, 95 and 99 (ppm, dry weight).

Year	Cd	Cu	Pb	Zn	Reference
1985–1987	<0.3–10.2	48–9760	17–15420	88–2267	Goyette and Boyd, 1989
1995	0.1–3.6	31–1008	17–123	50–800	Boyd et al., 1998
1999	0.3–1.2	11–333	4–76	35–407	This work

Table 2. Trace metals in bottom sediments of Vancouver Harbour at station B-3A (ppm, dry weight).

Year	Cd	Cu	Pb	Zn	Reference
1985–1987	7.4	1200*	250*	1300*	Goyette and Boyd, 1989
1995	3.6	1008	123	800	Boyd et al., 1998
1999	1.2	333	76	407	This work

*approximate value from diagram

Metals in mussels

Data on trace metal contents in mussels *Mytilus trossolus* are presented in the data section of this report. The results obtained in PGI and POI are in reasonable agreement with the TINRO-Centre data. Concentrations of Al, Fe, Cd, Cu and Pb were maximum at station I-2A (Sulfur Dock/Copper Dock). Highest zinc content was registered at station I-3A (Longsdale Quay). Metal concentrations in soft tissues of mussels at other stations were comparable with values from reference stations (I-1, PEI, and I-7, Howe Sound).

A large amount of data on trace metal contents in mussels has been collected within US NOAA NS&T Program (e.g., O'Connor 1998). Data from this paper for Cd, Cu, Pb and Zn are given in Table 3 along with the results of the PICES MEQ Practical Workshop. It is necessary to take into account that NS&T sampling stations are situated outside the "hot spots". Therefore, contaminated sites in Vancouver Harbour (stations I-2A and I-3A) should not be considered as exceptionally polluted.

Metals in fish tissues

Data on trace metal contents in fish tissues (English sole, muscle) are presented in the data section of this report. Concentrations of Al, Cd and Cu were maximum at station T-48 (Cates Park, Indian Arm). Highest zinc content was registered at station T-38 (Port Moody, refinery) and maximum lead content at station T-11B (Longsdale Quay). Even the highest concentrations of copper, zinc and other metals in fish muscle were comparable with values from reference stations (T-49, PEI, and T-50, Howe Sound).

A large amount of data on trace metal contents in fish tissues have been collected by Canadian researchers (Goyette and Boyd 1989). Data from this report for Cd, Cu, Pb and Zn are given in Table 4 along with the results of the PICES MEQ Workshop. As in the case of bottom sediments, the decreasing trend in trace metal contents can be seen.

Conclusions

According to the results on trace metal contents in bottom sediments, station B-3A (Sulfur Dock/Copper Ore Dock) is the most polluted (among those sampled in May 1999). Comparison with data obtained in 1985-1987 and in 1995 revealed a decreasing trend in trace metal concentrations. To characterize temporal trends more precisely, analysis of dated sediment cores might be necessary.

Maximum contents of most metals (except Zn) in soft tissues of mussels were observed at station I-2A (Sulfur Dock/Copper Ore Dock). The highest concentration of zinc was determined at the Longsdale Quay (station I-3A).

In the case of trace metals in fish muscle, even the highest concentrations were comparable with values from reference sites. Contents of trace metals in 1999 were lower than in 1985–1986.

Table 3. Trace metal contents in mussels from Vancouver Harbour in 1986-1996 and 1999 (ppm, dry weight).

Year	Cd	Cu	Pb	Zn	Reference
1986–1996	2.0–3.2	7.2–10.0	0.6–1.1	104–143	O'Connor, 1998
1999	1.7–5.9	6.1–60.8	1.0–218.7	112–325	This work

Table 4. Trace metal contents in muscle of fish from Vancouver Harbour in 1985–1986 and 1999 (ppm, dry weight).

Year	Cd	Cu	Pb	Zn	Reference
1985–1986	0.04–0.51	0.4–4.6	0.08–1.59	5.1–39.8	Goyette and Boyd, 1989
1999	0.02–0.04	1.2–1.5	0.23–0.62	16.0–23.7	This work

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Assessment of chemical contaminant exposure and effects in English sole

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The Marine Environmental Quality Committee of PICES sponsored a Practical Workshop in Vancouver Harbour, Canada, during the summer of 1999. The goal of the workshop was to exchange information about approaches PICES member countries use to assess the biological impacts from marine pollution. To accomplish this, scientists from PICES member countries worked cooperatively to study the effects of chemical contaminants on marine organisms at several sites in Vancouver Harbour, British Columbia.

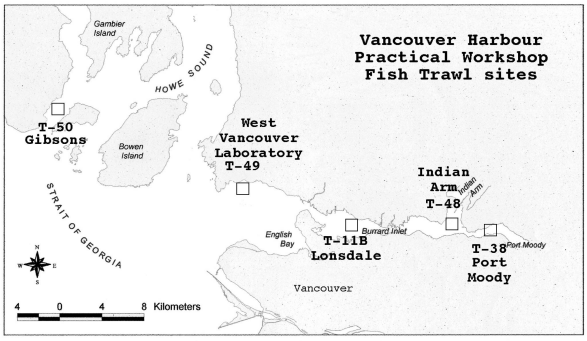


Fig. 1 Location of fish collection sites.

As part of this workshop, the Northwest Fisheries Science Center examined the relationship between liver lesions and chemical contaminant exposure in English sole (*Parophrys vetulus*). English sole is a benthic flatfish used extensively as a sentinel species for contaminant effects in North American west coast marine environments. English sole live in close association with bottom sediments, preying on clams, worms and other benthic invertebrates. This species of fish lives in nearshore environments that are often affected by urban activities, and are therefore at high risk of being exposed to chemical contaminants.

Fish were collected with a bottom trawl from a reference site outside the harbour (Howe Sound), a site near the entrance to the harbour (West Vancouver Lab), and three industrial sites (Lonsdale Quay, Indian Arm and Port Moody) within Vancouver Harbour (Fig. 1). Samples of fish liver, fish bile and sediment were collected, preserved, and returned to the laboratory for analyses. Liver and bile were collected from 30 fish at each site. A portion of the liver was preserved in Dietrichs fixative for histopathology. Paraffin sections were prepared and examined microscopically for non-infectious, toxicopathic lesions. Liver was also collected for chlorinated hydrocarbon analyses. Three composites of liver were analyzed. Each composite contained equal weights of liver from five fish. Bile from 10 individual fish was analyzed at each sampling site. Bile was analyzed for metabolites of aromatic hydrocarbons using HPLC as described by Krahn

et al. (1986). A Van Veen grab was used to collect sediment from each site.

After fishing operations were completed, the center of the trawl area was determined, the anchor was deployed to maintain position, and three grabs of sediment were taken from this area. At sites where no trawling was done, the site location was established by the location of the sediment sample. An equal amount of sediment from each grab was combined to form a sample for each site and analyzed for aromatic hydrocarbons (AHs) and chlorinated hydrocarbons (CHs). Sediment AHs and CHs, and liver CHs were analyzed by gas chromatography/mass spectroscopy as described by Sloan *et al*. (1993).

Sediment concentrations of aromatic hydrocarbons were higher at the three industrialized sites in Vancouver Harbour (Fig. 2). Chlorinated hydrocarbons were higher at Indian Arm and Port Moody, the two industrial sites located farthest inside the Harbour (Fig. 2). Concentrations of PCBs and hexachlorobenzene in English sole liver

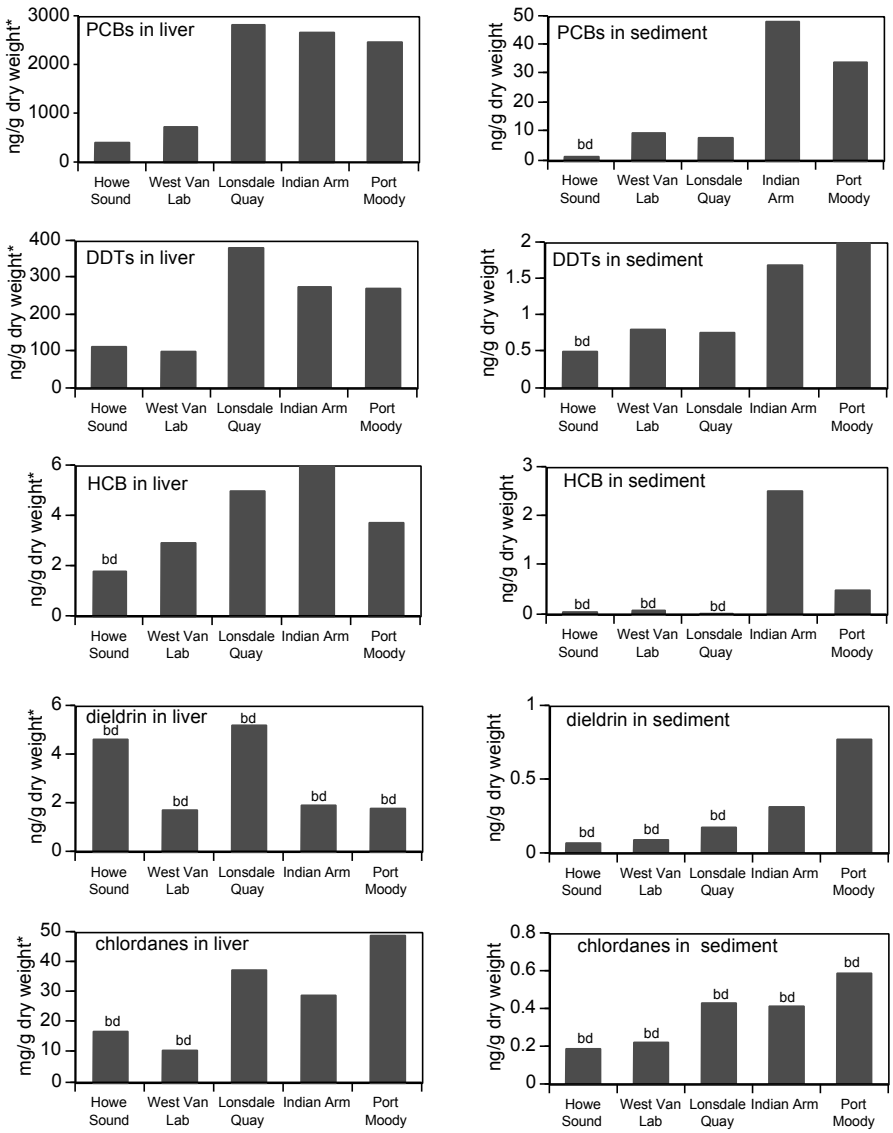
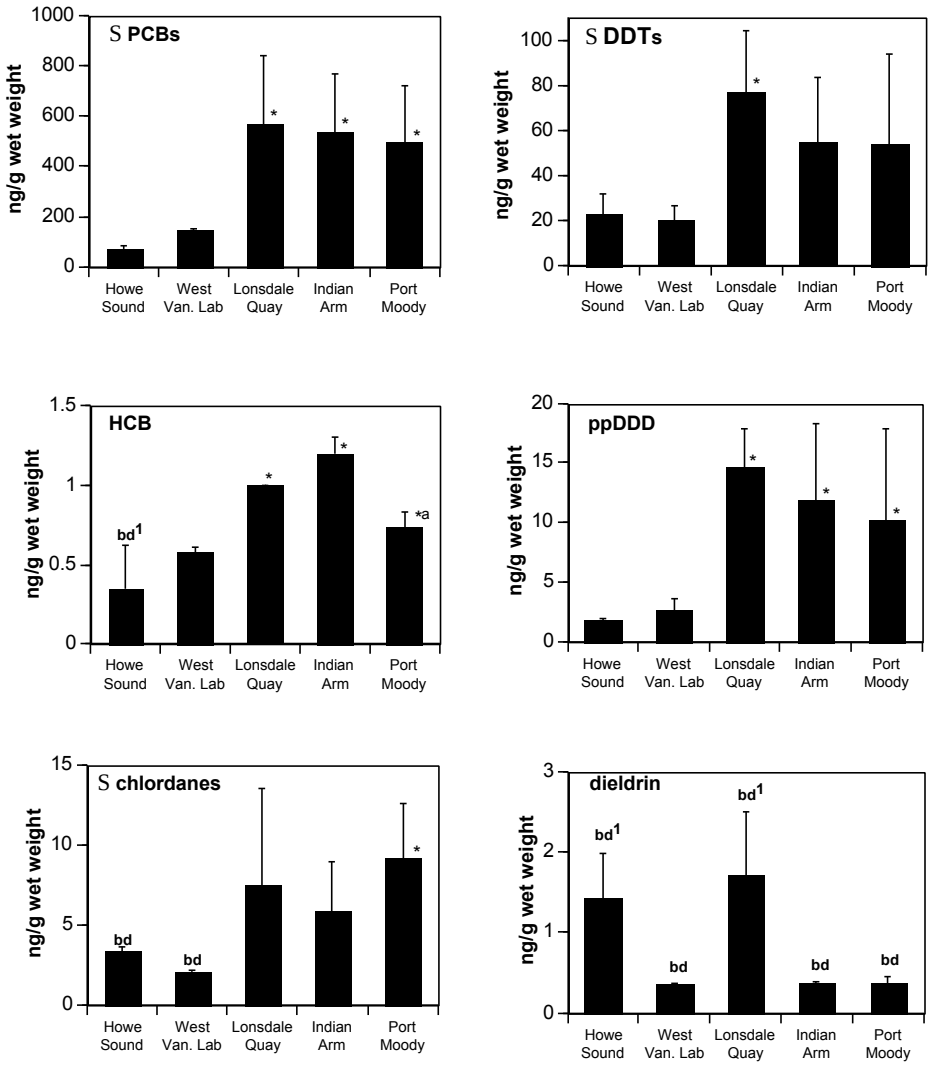


Fig. 2 Comparison of chemical concentrations in English sole and sediment.



Three samples were analyzed for each site. Each sample was a composite of 5 fish.

* indicates a significant difference from the reference sites (Howe Sound and West Vancouver Lab) as shown by the ANOVA statistical analysis.

*a Significantly different from Howe Sound, but not West Van Lab

bd = all three samples were below detection limits

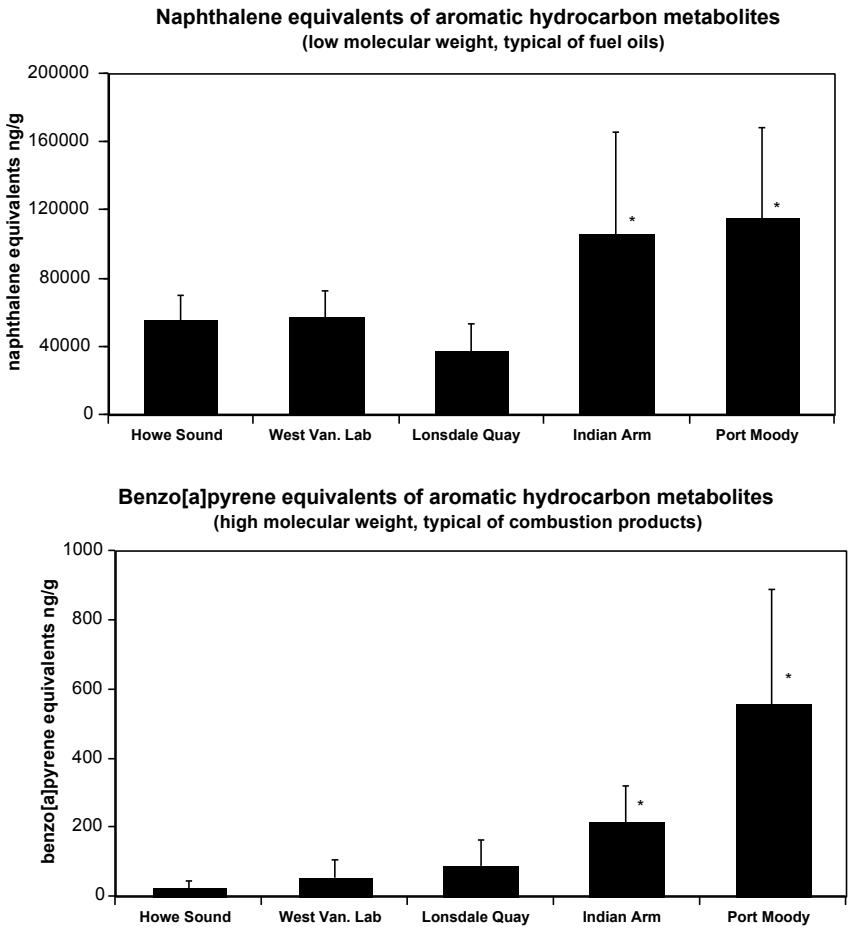
bd1 = two of the three samples were below detection limits.

The detection limit is different for each sample depending on sample size.

Fig. 3 Chlorinated hydrocarbons in English sole liver.

were significantly higher at all three industrial sites compared to the Howe Sound reference site (Fig. 3). Concentrations of aromatic hydrocarbon metabolites in English sole bile were significantly higher at the Indian Arm and Port Moody sites compared to the reference sites (Fig. 4).

Histopathology of English sole liver was examined as a biological marker of contaminant effects. Fish were examined for toxicopathic liver lesions including proliferative disorders (such as hepatocellular regeneration and cholangiofibrosis), specific degeneration/necrosis (including megalocytic hepatosis and hepatocellular nuclear pleomorphism), preneoplastic conditions (including eosinophilic, basophilic and clear cell foci), and neoplasms (including adenomas and carcinomas). Toxicopathic liver lesions were observed in 20 to 23% of the fish at each of the three industrial sites, while no lesions were observed at either of the reference sites (Fig. 5).



10 samples were analyzed at each site. Each sample was from an individual fish.

* indicates that the aromatic hydrocarbon metabolites at this site are signficantly higher than at the reference sites (Howe Sound and West Vancouver Lab), as shown by ANOVA statistical analysis.

Fig. 4 Aromatic hydrocarbon metabolites (fluorescent aromatic compounds) in bile of English sole.

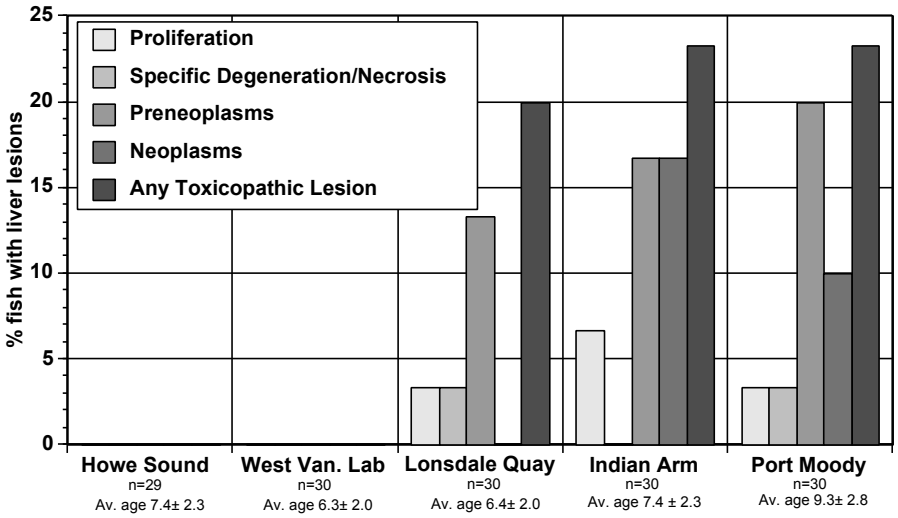


Fig. 5 Liver lesions in English sole.

Fish age data were provided by Colin Levings and colleagues at Fisheries and Oceans Canada in West Vancouver. Otoliths collected from English sole were aged as part of their fish community study that was conducted during the Practical Workshop. The average age of fish was 6 to 7 years at all sites except at Port Moody, where the average age was 9 years. Analysis of variance indicated that the mean age of English sole at Port Moody were significantly older than at other sites. It is important to account for fish age when evaluating prevalences of liver lesions, because the risk of developing these lesions increases with age (Rhodes *et al*. 1987). Therefore, the high prevalence of toxicopathic liver lesions in English sole from Port Moody may be occurring in part because these fish are older than those at the other sites. In other words, the prevalence of liver lesions at Port Moody would probably be somewhat less than 23% if only fish of comparable age were compared with the other Vancouver Harbour sites.

Spearman-Rank correlations showed that the prevalence of toxicopathic liver lesions was significantly associated with low and high molecular weight aromatic hydrocarbons in sediment, and with aromatic compounds fluorescing at Benzo[a]pyrene wavelengths measured in the bile. This is consistent with the hepatocarcinogenicity and hepatotoxicity of high molecular weight polycyclic aromatic hydrocarbons that has been observed in fish, including English sole, from other contaminated sites along the northeastern Pacific coast.

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Organochlorine and polyaromatic hydrocarbon residues in English sole, *Pleuronectes vetulus*, at Vancouver Harbour

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Introduction

The processes for intake of the contaminants by aquatic animals are roughly classified. One is bioconcentration or the intake of dissolved chemicals in water through the gills, and the other is biomagnification through the food web (Herbert 1986). In general, most of the lipophilic compounds such as PCBs and DDTs are accumulated due to biomagnification. Therefore, these chemicals are accumulated at much higher concentrations in the upper trophic level of the

food chain (Bentzen *et al*. 1996; Campfens and Mackay 1997; Morrison *et al*. 1997). The lipophilic contaminants could cause negative effects on reproduction and individual health. As a result of regulations by the governments of advanced countries, use of some persistent organo-chlorine chemicals has declined, and their residual levels in the environment have been decreasing. But these organochlorine chemicals have been detected in wildlife, and contamination has continued at low levels. Benthic fish have been good indicators of coastal pollution in the water column and sediments, although the bioaccumu-lation patterns of the different chemicals varied substantially among species (Pastor *et al*. 1996).

Usually, soxhlet and ultrasonic extraction methods are used for the extractions of PCBs and polyaromatic hydrocarbons (PAHs) from biological samples. These are fine methods, but they are time consuming and also need complicated preparation, such as hydrolysis of lipids by saponification. In addition, many pesticides are broken down during saponification.

Supercritical fluid extraction (SFE) is performed with carbon dioxide at temperatures and pressures above critical point. The SFE extraction is completed in a shorter time compared to the usual method, and it is possible to simultaneously perform the rough clean-up using alumina and silica. Therefore sample preparation such as the removal of the lipids is expected to be simplified. In addition, SFE is able to extract PCBs, PAHs and organochlorine pesticides simultaneously. Although the application of SFE is increasing for real environmental samples, presently there are only few reports that have examined biological samples from the field (Chester *et al*. 1998).

The PICES Practical Workshop was held from May 24 to June 7, 1999, in Vancouver Harbour, Canada. In this study, English sole, (*Pleuronectes vetulus*), which occupies the upper trophic level in the food chain, were collected from 5 sites within Vancouver Harbour (sampling sites are shown in Section I, Fig. 1.4). English sole tissues were analyzed to determine concentrations of PCBs, organochlorine pesticides and PAHs. SFE was used to extract the contaminants.

Methods

English sole were collected at 5 sites within Vancouver Harbour. Fish were immediately dissected on the ship, and tissues were frozen. Before analysis, the tissue samples were homogenized, and freeze-dried for 24 - 48 hours. The freeze-dried samples were cut into pieces with scissors.

PCBs, PAHs, and organochlorine pesticides in the organs were extracted by SFE using carbon dioxide and a 1% modifier of methanol. The temperature and pressure of SFE were 50°C and 214 bar, respectively (carbon dioxide density, 0.80 g/ml). Before the extraction, the supercritical carbon dioxide was equilibrated in the sample for 10 min, and the extractions were performed for 40 min. The flow rate of supercritical carbon dioxide was 3.0 ml/min. Each sample was put into a stainless steel tube, and 1 g-alumina was packed on the sample. The extracts were adsorbed to a florisil trap which was kept at 65°C. After the extraction, the extracts were eluted first with 3 ml hexane and then with 3 ml acetone. The elutions were combined, the solvent was exchanged to hexane completely, and the hexane solution was concentrated to 1 ml under a nitrogen stream. The concentrated extract was loaded on a florisil column (packed in a Pasteur pipette), washed with 5 ml hexane, eluted with 10 ml hexane, and then with 10 ml - 5% diethyl ether – hexane. Each eluent was concentrated to 0.1 ml under a nitrogen stream and quantified by GC/MS. The analytical assurances were certified by standard reference material 2974 (organics in freeze-dried mussel tissue (*Mytilus edulis*) of the National Institute of Standards and Technology.

Results and discussion

PCBs

Figure 1 shows total PCBs (ΣPCBs) in organs. The concentration distributions were different for muscle, ovaries, testes, and liver among each sampling point. The total PCB concentrations in liver were remarkably high, about 3 - 670 times higher compared to other organs, at all sampling sites. Total PCBs were detected at relatively high levels in organs at sites T-11B, T-38, and T-48.

The ratios of the concentrations were different among the sampling sites, but the order of the concentration was liver > testis > ovarian > muscle.

Seventy PCB congeners were measured. The ratios of distribution for individual PCB congeners in organs were similar. As an example, at site T-11B, the concentration of PCB 153 was highest for all organs (muscle: 0.96 ng/g, ovaries: 1.08 ng/g, testes: 8.92 ng/g, and liver: 27.65 ng/g (Fig. 2)). In addition, the concentrations of PCB 138, 187, 180, 110, and 99 were high in organs. Although fewer congeners were detected in testes than in other organs, the individual concentrations were higher than in muscle and ovary. The concentrations of individual congeners in muscle and ovaries were similar, but these concentrations were 1/20 - 1/400 of that in liver.

PAHs

The concentrations of naphthalene, and 1- and 2-methylnaphthalene were relatively high in all organs. But these chemicals are easily introduced as contaminants during the extraction process, therefore these quantities could be overestimated. In this investigation, because only a small amount of testes were gathered at all sampling sites, naphthalene and methylnaphalene could influence the concentration of total PAHs (ΣPAHs). Except for naphthalene, the concentrations of ΣPAHs in testes and liver were roughly similar at each site. In addition, PAH concentrations in testes and liver were higher than in muscle and ovaries, although the differences were smaller than those found in the case of PCBs. Concentrations of PAHs were lower and less variable among organs than PCBs. This suggested that PAHs were metabolized considerably more than PCBs (Krahn *et al*. 1993) and the parent compounds of PAHs showed less bioaccumulation in lipids than PCBs. The highest concentration of total PAHs in organs was detected at site T-11B. Concentrations of PAH at sites T-50, T-49, T-38, and T-48 were similar, with a slightly higher concentration at site T-50.

Of the individual PAHs measured in fish tissues at the sampling sites (Fig. 3.), phenanthrene was found at the highest concentrations. Furthermore, concen-trations of pyrene, dibenzothiophene, and

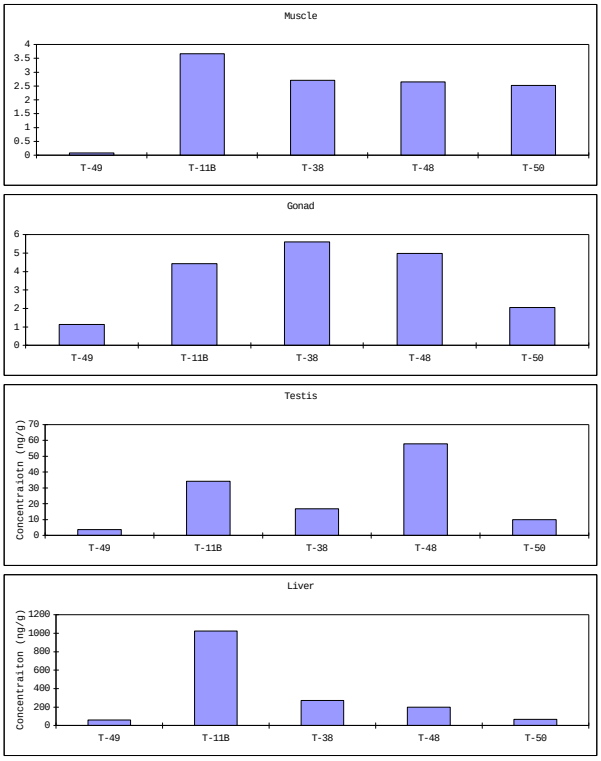

Fig. 1 Concentration of total PCBs in the tissue of English sole from Vancouver Harbour.

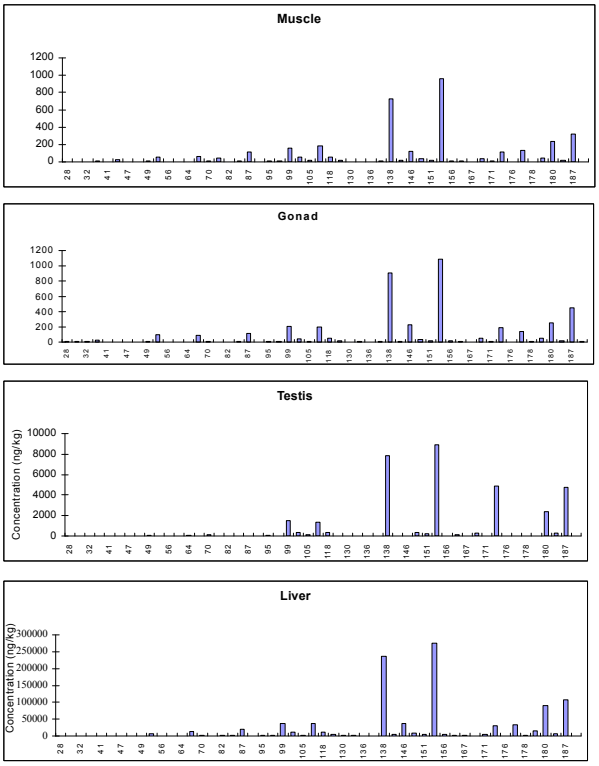

Fig. 2 Concentration of PCB congeners in tissues of English sole at site T-11B.

fluoranthene were slightly elevated. In the mussel investigation portion of the Vancouver Harbour Practical Workshop (see Mussel report, this publication), mussels (*Mytlilus trossulus*) were collected from intertidal sites within Vancouver Harbour and whole body tissue was analyzed for PAHs. The distribution pattern of PAH concentrations in mussels were different from that of fish. Namely, fluoranthene concentrations were higher in mussel than phenanthrene, and concentrations of phenanthrene and pyrene were similar. In addition, dibenzothiophene concentrations were only a small proportion of total PAHs in mussels. These differences may be caused by dissimilar methods of PAH uptake by English sole and mussels.

Organochlorine pesticides

The primary organochlorine pesticides detected in English sole were DDTs (*p,p'*- and *o,p'* -) and its metabolites (*p,p'*-, and *o,p'*-DDDs and DDEs). Other pesticides were found at considerably lower levels. The concentrations of DDTs and its metabolites were highest in liver, and second highest in ovaries. Concentrations of DDTs and its metabolites were higher in liver of fish from sites T-49, T-11B and T-38 (33.36 ng/g, 66.28 ng/g, and 44.36 ng/g, respectively) compared to other organs (Fig. 4). Relatively low concentrations of DDTs were detected in testes, even though PCB and PAH concentrations were high.

The concentration of DDE was higher than DDT and DDD for all organs. Concentrations of DDT metabolites accounted for 40 - 90% of total DDTs (Fig. 5). Concentrations of DDT metabolites in muscle, testis and liver accounted for more than 80% of total DDTs. However, at all sites, DDT concentrations in ovaries were higher than in other organs, and ovaries accumulated parent DDTs at much higher concentrations compared to other organs. The concentration of *o,p*'-DDT was lower in all organs, and it was detected much less frequently than *p,p*'-DDT.

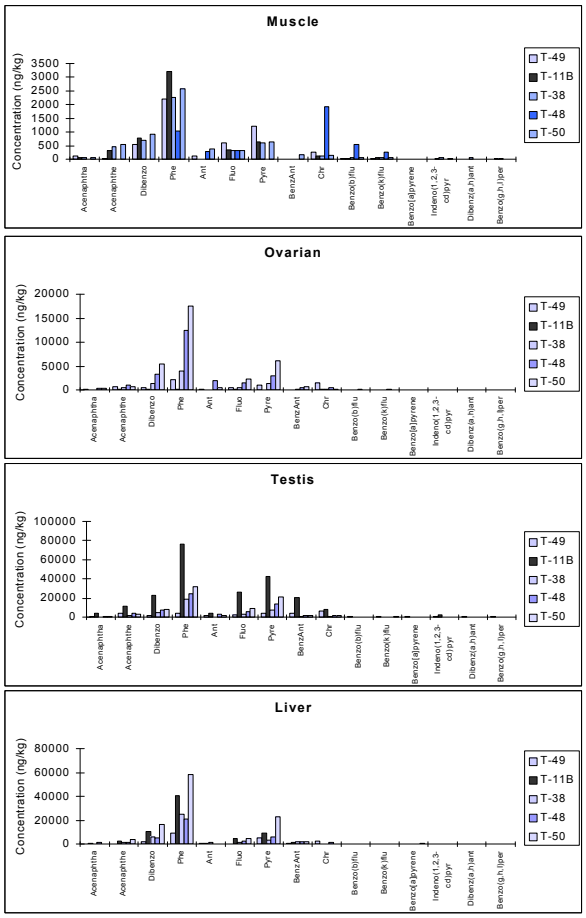

Fig. 3 Concentrations of individual PAHs in tissues of English sole from Vancouver Harbour.

Conclusion

In this study, the contaminant concentrations in muscle, ovaries, testes, and liver of English sole were investigated at Vancouver Harbour, Canada, during the PICES Practical Workshop. The concentrations of PAHs in testes and liver were higher than in muscle and ovaries. The highest concentration of ΣPAHs in organs was detected at site T-11B. Concentrations of PAHs in tissue of English sole from sites T-50, T-49, T-38, and T-48, were similar to each other. Of the individual PAHs measured, phenanthrene was found at the highest concentrations in all tissues. Pyrene, dibenzothiphene, and fluoranthene were also found at detectable levels.

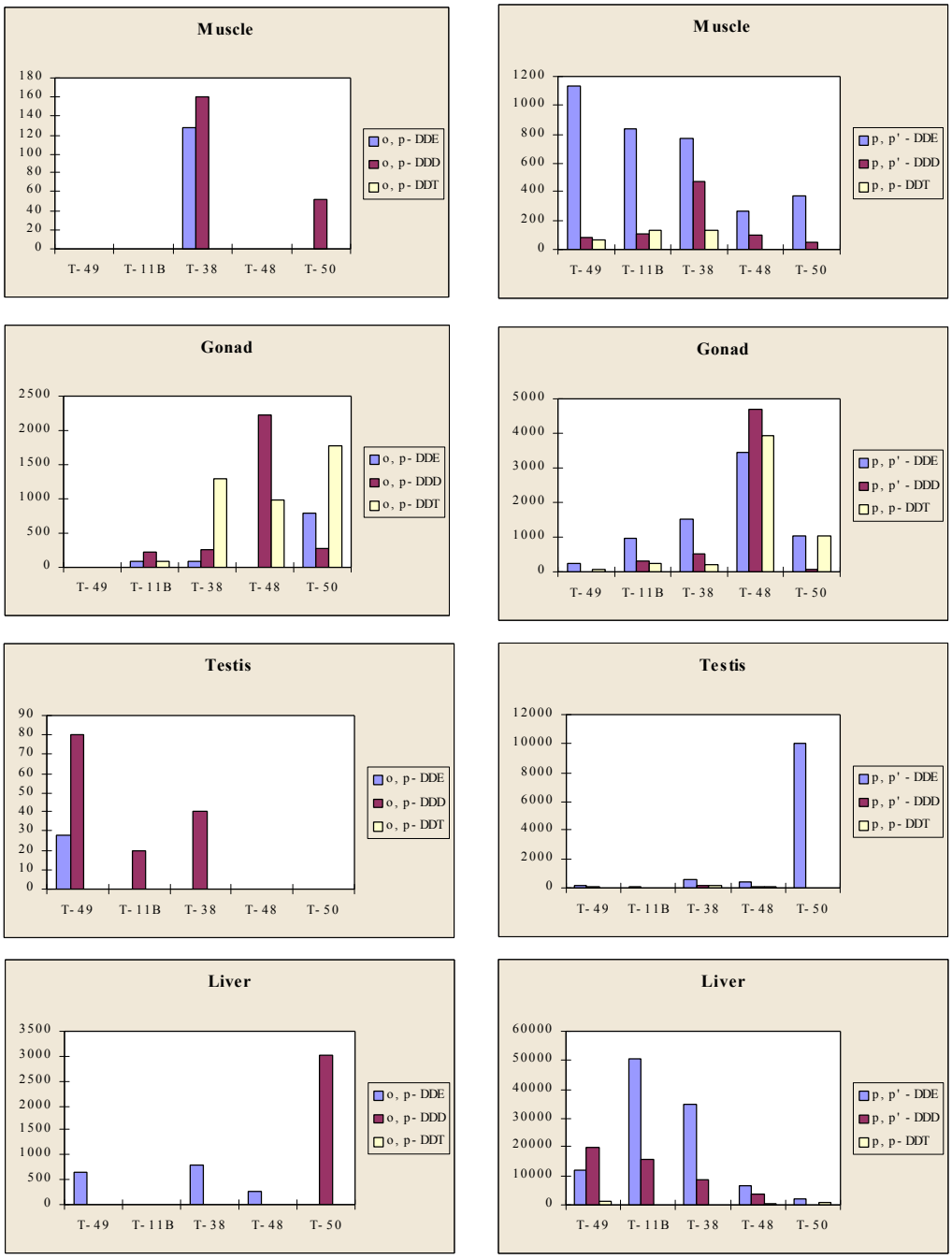

Fig. 4 Concentrations of DDT and its metabolites in tissues of English sole, Vancouver Harbour.

The concentrations of DDT and its metabolites were highest in liver and second highest in ovaries. The concentrations of DDT and its metabolites in liver were relatively high in fish from sites T-49, T-11B and T-38. The concentration of DDE was higher in all organs compared to DDT and DDD. DDT metabolites accounted for 40–90% of total DDT concentrations. However, DDT concentration in ovaries of fish from all sites were higher than in other organs, and ovaries accumulated DDT parent compounds at much higher levels than other organs.

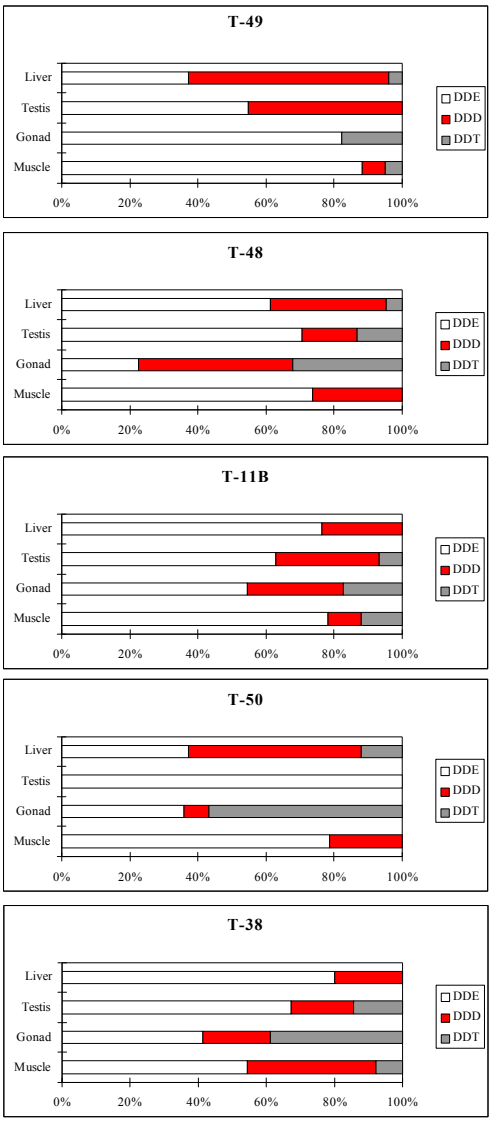

Fig. 5 Concentrations of DDT and its metabolites in tissues of English sole.

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Organochlorine and polyaromatic hydrocarbon residues in bivalves at Vancouver Harbour

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Introduction

Since bivalves have a wide distribution, extensive populations, filtering habits, and ability to accumulate organic contaminants, analysis of chemicals in the soft tissue of bivalves is useful as an index of contamination in the aquatic environment. In general, invertebrates have low

metabolic abilities and the contaminants are accumulated and remain in them for a longer period than in vertebrates. This is especially true for the lipophilic compounds such as organochlorine contaminants (e.g. PCBs and DDT), and polyaromatic hydrocarbons (PAHs) (Connell 1995). These contaminants could cause negative effects for reproduction and individual health. As a result of regulations for the use of some persistent organochlorine chemicals by the governments of advanced countries, residual levels of these chemicals in the environment have declined. But organochlorine chemicals have been detected in wildlife, and contamination has still continued at low levels. Therefore, bivalves have been used by a number of investigators to study the contamination of wildlife (Tanabe *et al*. 1987, Colombo *et al*. 1995, Hofelt and Shea 1997).

Supercritical fluid extraction (SFE) is performed with carbon dioxide under temperatures and pressures above a critical point. The SFE extraction can be completed in a shorter time compared to the usual method, and it is possible to simultaneously perform the rough clean-up using alumina and silica. Therefore sample preparation for GC/MS and HPLC can be simplified. In addition, SFE is able to simultaneously extract PCBs, PAHs and organochlorine pesticides from biological samples. In this decade, the application of SFE is increasing for real environmental samples. However, only few studies of biological samples from the field have been reported so far (Chester *et al*. 1998).

The PICES Practical Workshop was held from May 24 to June 7, 1999, in Vancouver Harbour, Canada. In this study, the contamination levels of PCBs, organochlorine pesticides, and PAHs in mussel, *Mytilus trossulus*, were investigated at West Vancouver Harbour, Canada. Furthermore, 6 species of bivalves were also sampled at a few sampling sites where mussels were collected. Chemical concentrations were also determined in these bivalves and the inter-species differences were examined.

Methods

Mussels were gathered at 9 sampling sites in Vancouver Harbour (these I-sites are shown in Fig. 1). Before analyses, the soft tissue of bivalve samples were shelled, homogenized, frozen at –20°C for a day, and then freeze-dried for 24 - 48 hours. The freeze-dried samples were broken to pieces with scissors.

PCBs, PAHs, and organochlorine pesticides in the bivalves were extracted by SFE using carbon dioxide with a 1% modifier of methanol. The temperature and pressure of SFE were 50°C and 214 bar, respectively (carbon dioxide density, 0.80 g/ml). Before the extraction, the supercritical carbon dioxide was equilibrated in the sample for 10 minutes, and the extractions were performed for 40 minutes. The flow rate of supercritical carbon dioxide was 3.0 ml/min. The sample was put into the stainless steel tube, and 1 g alumina was packed on the sample. The extracts were adsorbed to a florisil trap which was kept at 650°C. After the extraction, the extracts were eluted first with 3 ml hexane, and then with 3 ml acetone. The elutions were combined, the solvent

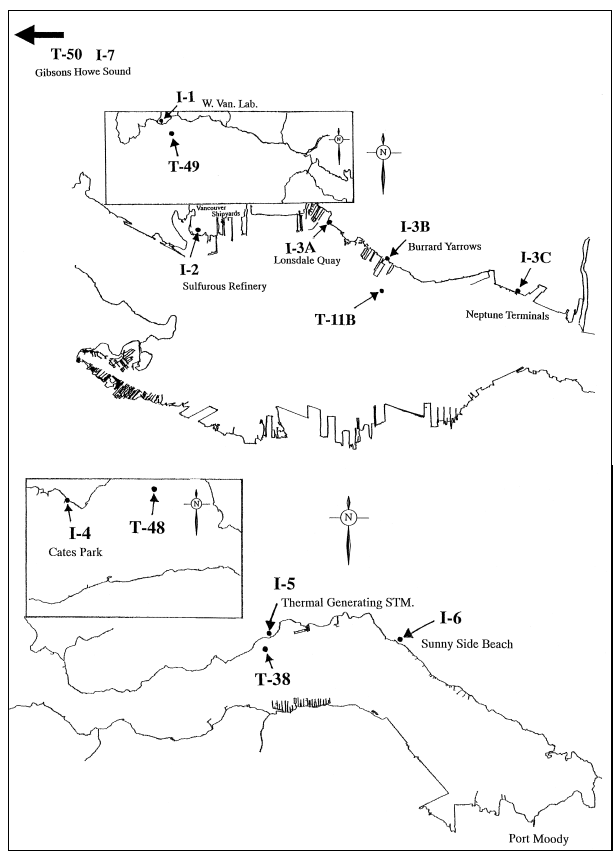

Fig. 1 Sampling sites in Vancouver Harbour.

was exchanged to hexane completely, and the hexane solution was concentrated to 1 ml under a nitrogen stream. The concentrated extract was loaded on a florisil column (packed in a Pasteur pipette), washed with 5 ml hexane, eluted with 10 ml hexane, and then with 10 ml 5% diethyl ether–hexane. Each elutant was concentrated to 0.1 ml under nitrogen stream and quantified by GC/MS. The analytical assurances were certified by standard reference material 2974 (organics in freeze-dried mussel, *Mytilus edulis*, tissue) of the National Institute of Standards and Technology.

Result and discussion

PCBs in mussels

The concentrations of total PCBs (ΣPCBs) detected in mussels at each sampling site were classified roughly into 3 groups, namely, a first group with ΣPCB concentration of about 5 ng/g or more (sites I-3A, I-4, and I-6), a second group with ΣPCB concentration of about 2 ng/g (sites I-3B and I-3C), and a third group with ΣPCB concentration of about 1 ng/g or below (sites I-1, I-2, I-5B and I-7) (Fig. 2).

At the site I-5, the concentration of ΣPCBs was much lower than at the other sampling sites, and low molecular weight congeners (with less than 4 chlorines) made up the highest percentage of total PCBs. At the other sampling sites, 70 – 87% of total PCBs were made up of higher molecular weight congeners with over 5 chlorines. The dominant group of PCB congeners had 6 chlorines, and these accounted for 33 to 55% of total PCBs. The proportions of congeners with 6 and 7 chlorines were relatively higher at sites I-2, I-3A and I-3B (63%, 70% and 64%, respectively) than at other sampling sites (40 - 57 %).

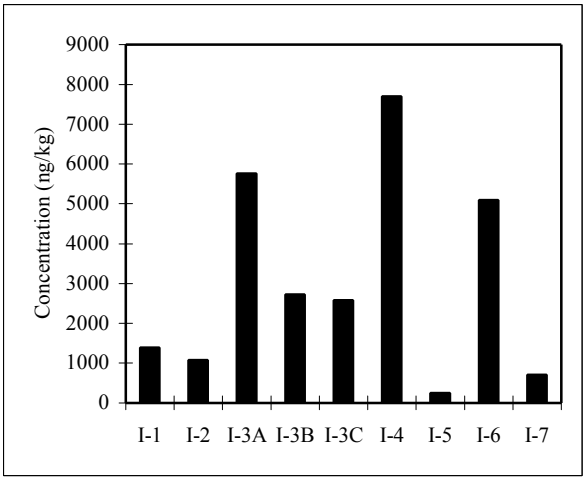

Fig. 2 Concentration of total PCBs in *M. trossulus.*

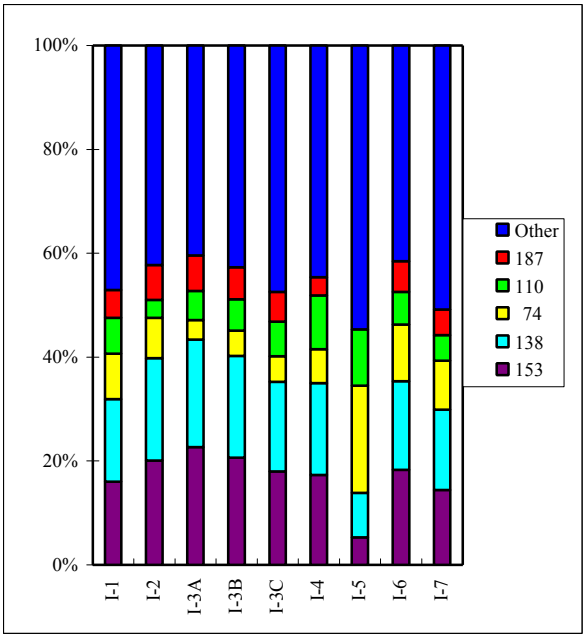

Fig. 3 Rate of PCB congeners in *M. Trossulus.*

For the individual PCB congeners in mussel, PCB 153 and 138 occurred at much higher concentrations than the other congeners. Furthermore, the concentrations of PCB 74, 110, and 187 were also relatively high. The ratios of these five congeners in ΣPCBs were over 50% at all sampling sites, except for site I-5B (45%) (Fig. 3).

Organochlorine pesticides in mussels

In this investigation, there was no common pattern in the distribution of organochlorine pesticides among the sampling sites (Fig. 4). At almost all sampling sites, the concentrations for α-, β-, and γ-HCHs were higher than those of other organochlorine pesticides. Levels of γ-HCH similar to the other HCHs were detected at all sampling sites, although the most common

isomers generally found in the environment are α-, β- and γ- (Walker. *et al*. 1999). The concentrations of α-HCH at sites I-1 and I-2 were relatively high (1.5 ng/g and 1.3 ng/g, respectively), although the concentrations of ΣPCBs at both sites were low. The distribution patterns and the concentrations of β- and γ-HCH were similar among all sampling sites. In particular, these concentrations were highest at site I-3C (3.2 ng/g for β-HCH and 3.4 ng/g for γ -HCH, respectively) among all sampling sites, although the concentrations of ΣPCBs were not so high at this site. On the other hand, although concentrations of ΣPCBs were highest at site I-4, concentrations of β- and γ -HCH were low (0.75 and 0.84 ng/g, respectively).

The highest concentrations of p,p'-DDT and its metabolites were detected at site I-4, except for DDD. The concentration of p,p'-DDD was remarkably high (7.7 ng/g). The distribution patterns for p,p'- DDT and metabolites were similar, if DDD at I-3C is excluded.

For heptachlor, it is remarkable that the concentrations were relatively high at sites I-1, I-5B, and I-7, where PCBs levels were low.

PAH in mussels

The maximum concentration of total PAHs (252 ng/g) was detected at site I-4 and the minimum was 53 ng/g at site I-5B. Fluoranthene was detected at highest concentrations at all sampling sites, and those concentrations were 5 - 81 ng/g. Furthermore, the concentrations of phenanthrene, chrysene, and pyrene were relatively high: more than 50% of total PAHs at all sites except for I-5B (Fig. 5).

The benzo[a]pyrene concentration was about 0.2 ng/g at all sampling sites. The concentrations of naphthalene, 1- and 2-methylnaphthalene, biphenyl, fluorene, and acenaphthene (group 1) were similar, and about 1–10 ng/g, at all sampling sites. On the other hand, the concentrations of phenanthrene, anthracene, fluoranthene, pyrene, chrysene, and 1,2-benzoanthracene (group 2) were different among the sites (Fig 6). However, the same compounds were found at all sites, and tended to co-occur with PCBs, although the concentrations of each PAH were quite different. These distributional similarities between PAHs of group 2 and PCBs could exist because PAHs were adsorbed through the food web. Broman *et al*. (1990) did not find biomagnification of PAHs from food in a natural Baltic Sea food chain. But for group 2 all octanol/water coefficients (in logarifmic scale, $\lg K_{OW}$) were above 4 (4.46 for phenanthrene, 4.45 for anthracene, 5.16 for fluoranthene, 4.88 for pyrene, 5.73 for chrysene, and 5.79 for 1,2-benzanthracene (Hansch *et al*. 1995). Spacie *et al*. (1982) estimated that

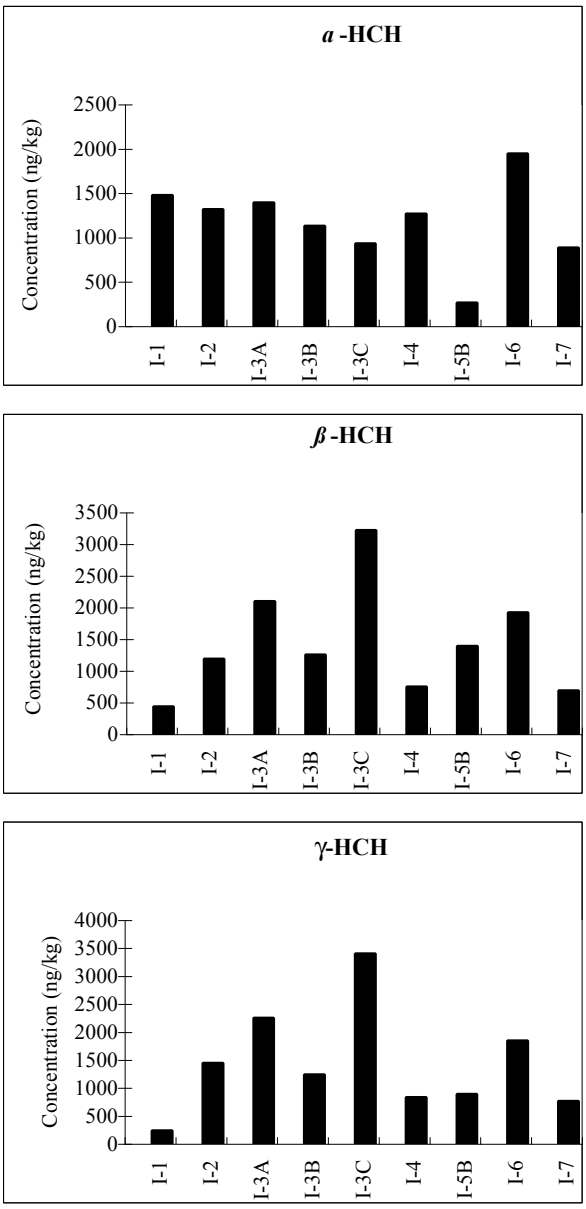

Fig. 4 Concentrations of organochlorine pesticides in *M. trossulus.*

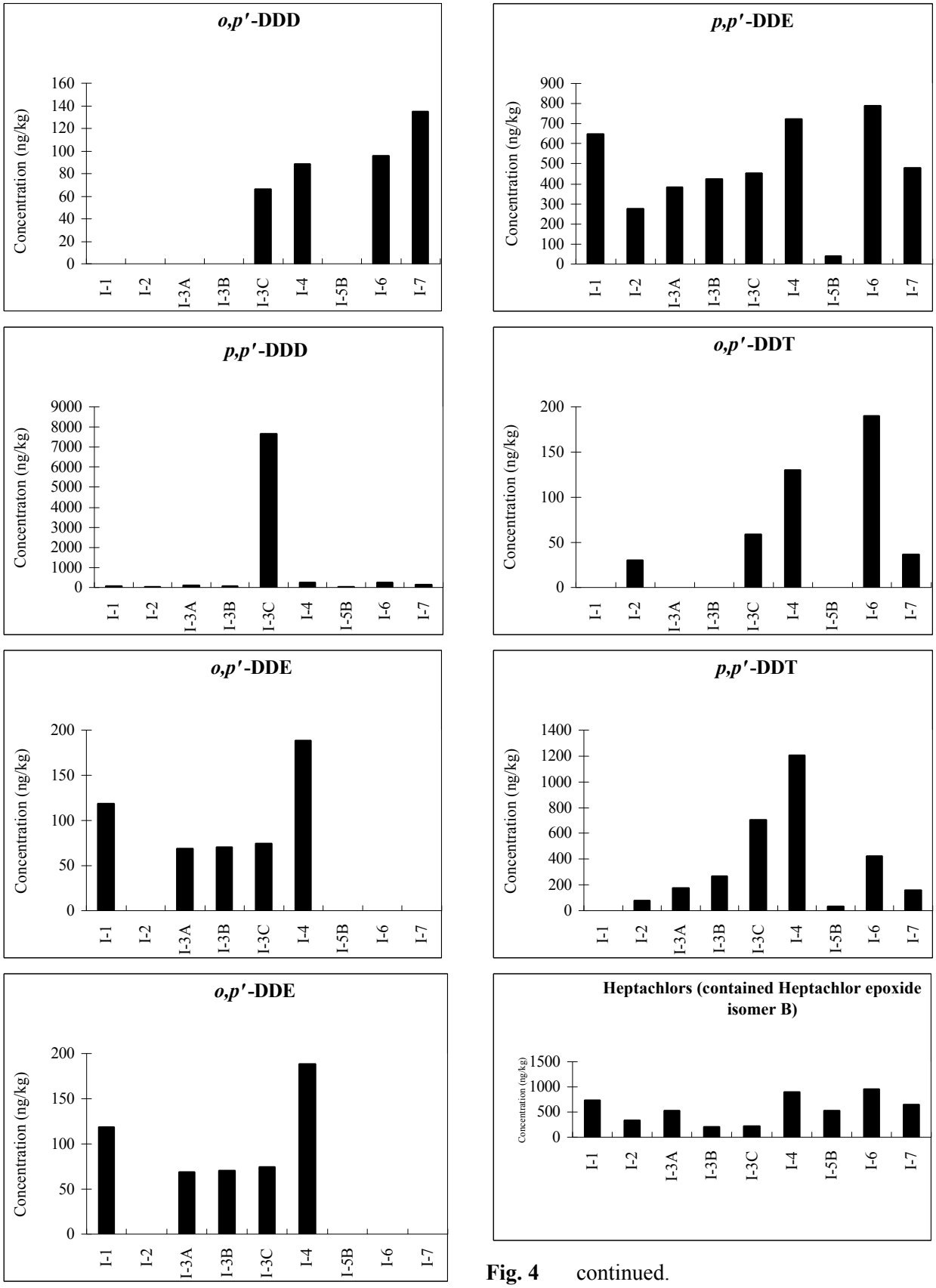

Fig. 4 continued.

the uptake of chemicals with $lgK_{ow} > 5$ through the gill membrane declines gradually. Therefore, a large amount of PAHs in group 2 mussels could be taken up through the food web, although lgK_{ow} for phenanthrene and anthracene is small.

Differences between species

Differences of contaminant concentrations were observed between mussel and other species. As an example, at site I-4, the concentrations of ΣPCBs in Pacific littleneck (*Protothaca staminea*), Nuttall's cockle (*Clinocardium nuttallii*), and Butter clam (*Saxidomus gigantea*) were higher than that in mussel. On the other hand, the concentrations of PAHs in these three species of bivalves were lower than that in mussel. There was no consistent pattern in organochlorine pesticide concentrations among the four species of bivalves.

In this investigation, the concentrations of contaminants in Pacific oyster (*Crassostrea gigas*) were much higher than in other bivalves. For example, the concentration of total PCBs was 30 ng/g in Pacific oyster at site I-6, and that was about 6 times higher than in mussel (4.8 ng/g) from the same site. The concentration of total PAHs in oyster was also about two times higher than that in mussel at this site. Furthermore, all organochlorine pesticides were detected at higher levels in oysters than in mussels (Fig. 7). Results show that Pacific oyster could accumulate more contaminants than mussel.

Conclusions

In this study, the contaminants in mussels and other bivalves were investigated at Vancouver Harbour, Canada, during the PICES Practical Workshop. The highest concentrations of PCBs and PAHs were detected at site I-4 near Cates Park. The distributional patterns of organochlorine pesticide concentrations were individually different. The main congeners of PCBs were IUPAC No. 153, 138, 74, 110, and 187, those of PAHs were phenanthrene, chrysene, and pyrene, and those of organochlorine pesticides were α-, β-, and γ-HCHs.

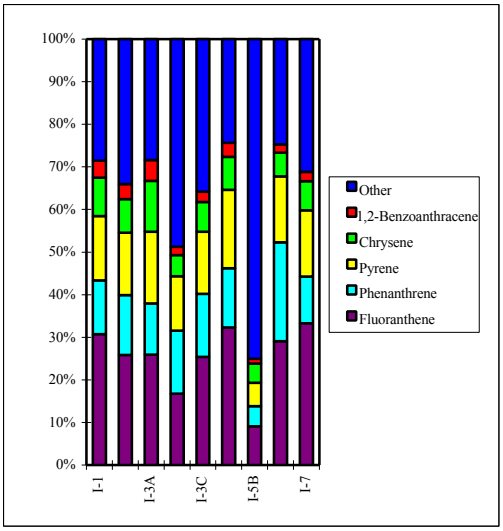

Fig. 5 Rate of PAHs in *M. trossulus* at Vancouver Harbour.

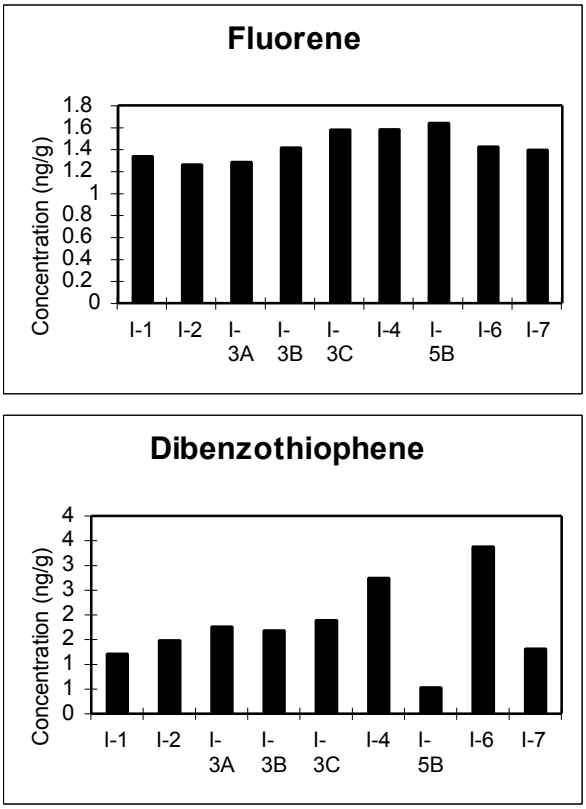

Fig. 6 Concentrations of individual PAHs in *M. trossulus*.

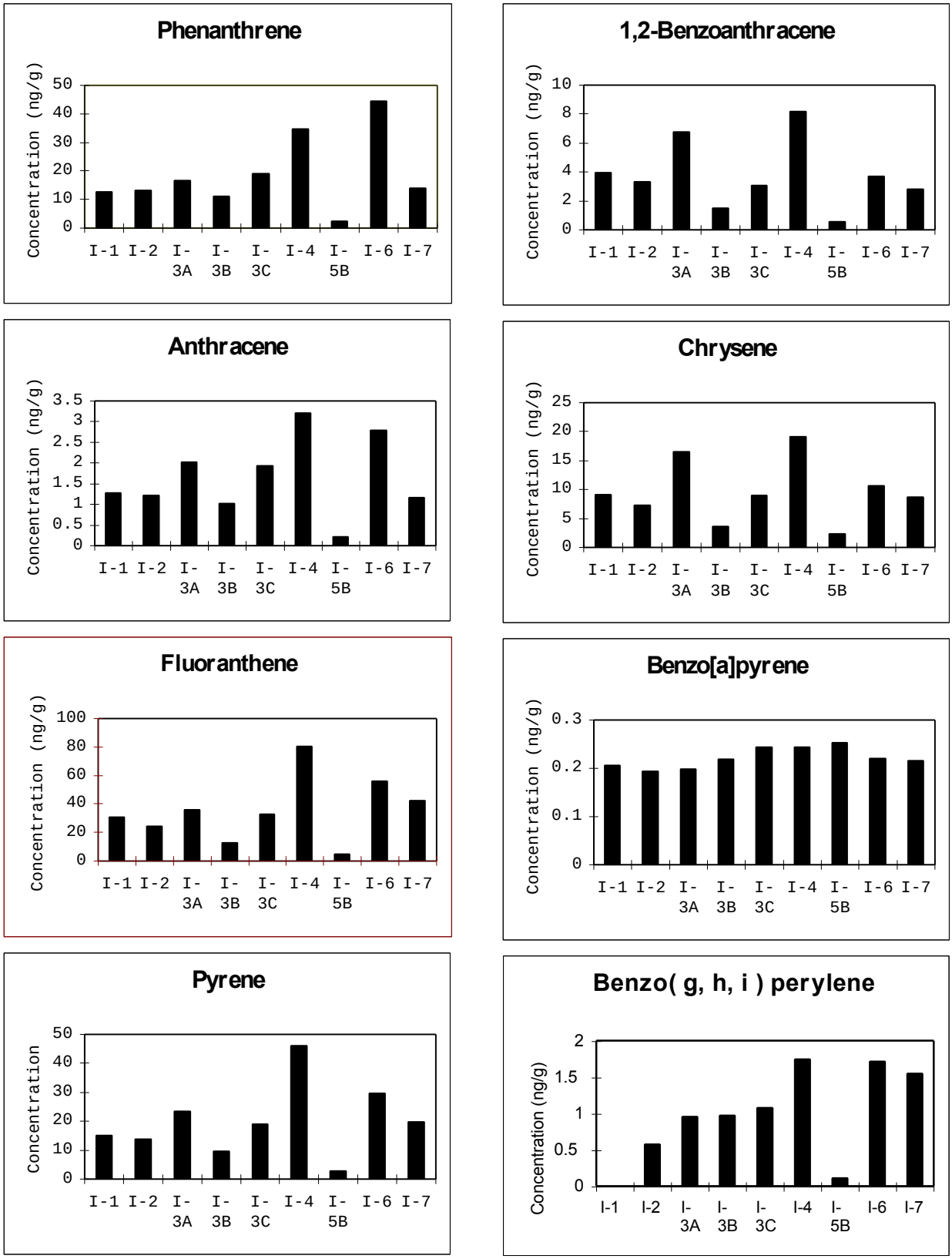

Fig. 6 continued.

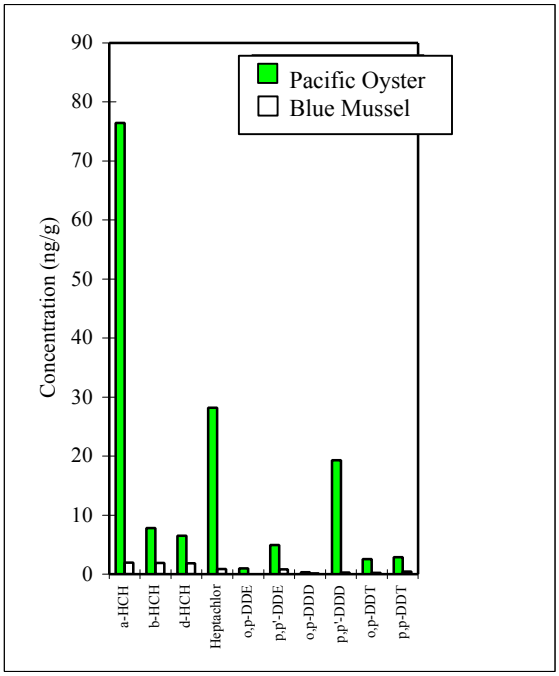

Fig. 7 Concentrations of organochlorine pesticides in the Pacific Oyster (*C. gigas*) and Blue Mussel (*M. trossulus*) at site I-6.

The intakes of phenanthrene, anthracene, fluoranthene, pyrene, chrysene, and 1,2-benzo-anthracene could be through the food web. Since the patterns of distribution among sampling sites were similar with that of ΣPCBs, most of these contaminants were thought to be absorbed from food.

In general, since most of the PCBs and organochlorine pesticides in aquatic animals are accumulated through the food web, these concentrations could be variable seasonally with the dietary quantity (Hühnerfuss *et al*. 1995). Therefore, it is necessary to conduct a few investigations at the same sites for a year to understand these pollution levels.

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