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DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN DUNGENESS CRABS (*CANCER MAGISTER*) NEAR AN ALUMINUM SMELTER IN KITIMAT ARM, BRITISH COLUMBIA, CANADA

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Abstract—An aluminum smelter situated at the head of Kitimat Arm (BC, Canada) has discharged polycyclic aromatic hydrocarbons (PAHs) into the receiving waters since 1954. The purpose of the present study was to examine the distribution of PAHs contaminants in dungeness crabs (*Cancer magister*) collected in Kitimat Arm and Douglas Channel (BC, Canada) by determining the concentrations of PAHs in the hepatopancreas and muscle tissues of crabs by using gas chromatography–mass spectrometry. Crabs were collected at specific sites down the Arm from the smelter on four separate occasions over a three-year period. Hepatopancreas and muscle tissues of the crabs were analyzed for 10 of the 16 PAH priority pollutants recommended by the U.S. Environmental Protection Agency. Results of the studies showed that the crabs had detectable levels of PAHs in hepatopancreas and muscle tissues. The highest concentrations of PAHs in the tissues were found at a site near the aluminum smelter, the alleged point source of PAH discharge. The concentrations of PAH analytes were high in crabs collected close to the smelter and at lower levels in crabs collected throughout Douglas Channel. These results show that PAHs discharged by the smelter were bioavailable to the crabs. The concentration of each PAH analyte in the hepatopancreas was found to be strongly related to its water solubility. However, the PAH analyte concentrations in the hepatopancreas and muscle did not appear to correlate highly with each other.

Keywords—Dungeness crab *Cancer magister* Polycyclic aromatic hydrocarbons Biomonitoring Kitimat, British Columbia, Canada

INTRODUCTION

Kitimat Arm is part of a large group of fjords located on the coast of northern British Columbia, Canada. The head of Kitimat Arm is the site of an aluminum smelter, a methanol plant, and a pulp mill terminal (Fig. 1). The aluminum smelter is alleged to be the primary source of polycyclic aromatic hydrocarbon (PAH) input into the marine environment through loss of green coke during transport to the smelter and release of PAHs in airborne emissions, waterborne effluents, and surface runoff [1,2]. Most PAHs in the marine environment are associated with particulate materials such as black carbon (soot) and seem to be resistant to degradation and desorption [3]. Polycyclic aromatic hydrocarbons also may be associated with tars and oils in highly contaminated environments.

Concern about PAHs in the marine environment is largely related to their persistence in the environment and carcinogenic potential in fish and invertebrates. An unusually high prevalence of oral, dermal, and hepatic neoplasms have been observed in bottom-dwelling fish caught in PAH-contaminated waters and sediments [4]. The carcinogenic effects of PAHs seem to vary with the physicochemical properties of the PAH analyte; high molecular weight PAHs (HPAHs) such as benzo[*a*]pyrene (B*a*P) and benz[*a*]anthracene (B*a*A) are more potent carcinogens.

Polycyclic aromatic hydrocarbon contaminants in the sediments and biota of Kitimat Arm have been the subject of a number of studies [1,2,5–8]. Although these studies generally have demonstrated elevated levels of PAHs in the sediments, few studies have demonstrated significant levels of PAH contaminants in biota. Thus, although PAHs have been found in the tissues of crabs (W. Cretney, Institute of Ocean Studies, Sydney, BC, Canada, personal communication), other researchers have concluded that bioavailability of PAHs to the biota of Kitimat Arm is limited [7,8]. Because elevated levels of PAHs in sediments may cause adverse effects to sediment-dwelling organisms, investigation of the extent of PAH contamination in the dungeness crabs (*Cancer magister*) of Kitimat Arm is important. Crabs contaminated with PAHs also are a public health concern from the perspective of the human consumers.

Various species of crabs have been used to monitor PAH exposure around the world. Off the coast of North America, concentrations of PAHs have been determined in the blue crab (*Callinectes sapidus*) collected from Chesapeake Bay [9–11], in the rock crab (*Cancer irroratus*) in New York Bight and Long Island Sound [12,13], and in the snow crab (*Chionoecetes opilio*) and the spider crab (*Hyas coarctatus*) on the coast of Newfoundland [14]. Other species have been used to biomonitor PAHs elsewhere, such as the swim crab (*Polybius henslowi*) on the northern coast of France and Spain [15] and the mud crab (*Scylla serrata*) obtained from the Brisbane River estuary in Australia [16].

This paper describes the determination of 10 different PAH analytes in the hepatopancreas and muscle tissues of crabs collected from various sites within Kitimat Arm. The levels and isomer distribution patterns of the PAH analytes in crabs from different sampling sites were examined to gain a better

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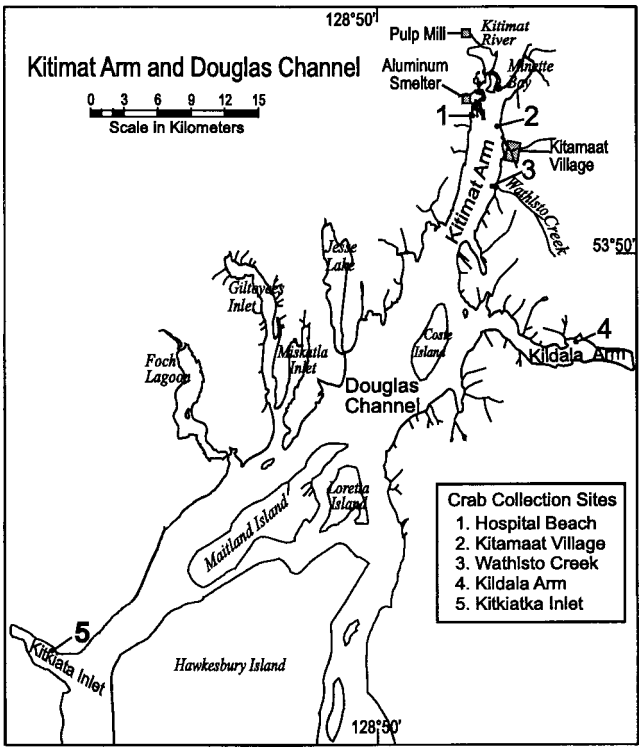

Fig. 1. Kitimat Arm and Douglas Channel (BC, Canada) showing locations of crab-collection sites.

understanding of the bioavailability and fate of PAHs in the crabs and to provide further evidence for the source(s) of PAH contamination in the area.

MATERIALS AND METHODS

Study area

Crabs were collected from five different sites at or around Kitimat Arm: Hospital Beach (53°59.60′N, 128°41.58′W), Kitamaat Village (53°59.22′N, 128°39.25′W, 53°59.15′N, 128°39.30′W), Wathlsto Creek (53°57.09′N, 128°39.64′W), and one site each from Kildala Arm (53°50.52′N, 128°30.32′W) and Kitkiatka Inlet (53°38.1′N, 129°15.5′W) (Fig. 1). These sites were chosen based on their potential for collection of crabs and proximity to the smelter. The Hospital Beach site located on the western side of Kitimat Arm is the closest to the effluent outfall of the aluminum smelter. The Kitamaat Village and Wathlsto Creek sites located on the eastern side of Kitimat Arm are further away from the smelter. Kildala Arm and Kitkiatka Inlet were selected as clean reference sites.

Sample collection

Legal-size male dungeness crabs were caught at five different sites (Fig. 1) with round, stainless steel, commercial crab traps (Ladner Crab Traps, Ladner, BC, Canada) at a depth of 15 to 50 m on four separate dates: March 1994, May 1995, May 1996, and October 1996. Table 1 summarizes the sampling locations, dates and catch results of these expeditions. Kitamaat Village and Kitkiatka Inlet were the only sites visited in March 1994. The crabs (n = 187) were weighed (744 ± 10 g, mean ± standard error) and the carapace width between the tips of the 10th antennal spines (17.6 ± 0.1 cm, mean ± standard error) was measured. Crabs were dissected on site to remove muscle and hepatopancreas tissues. Tissues were frozen immediately at −20°C for subsequent analysis in our laboratory at Simon Fraser University (Burnaby, BC, Canada).

Table 1. Number of crabs collected at each sampling date and location in British Columbia, Canada

Sample date	Hospital Beach	Kitamaat Village	Wathlsto Creek	Kildala[a] Arm	Kitkiatka[a] Inlet
March 1994	—	20	—	—	15
May 1995	20	18	12	—	—
May 1996	26	3	6	15	—
October 1996	20	15	2	15	—
Total	66	56	20	30	15

[a] Reference sites.

Sample preparation

The tissue samples were homogenized individually with a model PT 10/35 Polytron homogenizer (Brinkman Company, Rexdale, ON, Canada). Excised muscle samples were solid, and required 0.9% saline (2:1 tissue:saline, w/w) for homogenization, whereas the hepatopancreas samples were soft and could be homogenized without added saline. An aliquot of the homogenate equivalent to 5 g of hepatopancreas or 15 g of muscle was weighed separately in a 50-ml conical centrifuge tube with a Teflon®-lined screw cap. Tissue samples were spiked separately with a solution containing the following deuterated internal PAH standards: acenaphthene-d_{10}, fluorene-d_{10}, anthracene-d_{10} (ANT-d_{10}), fluoranthene-d_{10} (FLR-d_{10}), pyrene-d_{10} (PYR-d_{10}), B*a*A-d_{12}, benzo[*b*]fluoranthene-d_{12}, benzo[*k*]fluoranthene-d_{12}, B*a*P-d_{12} (CDN Isotopes, Pointe Claire, PQ, Canada), phenanthrene-d_{10} (PHN-d_{10}), and chrysene-d_{12} (CRY-d_{12}) (Aldrich Chemical, Milwaukee, WI, USA). Acenaphthene-d_{10}, fluorene-d_{10}, and benzofluoranthenes-d_{12} were not included in the spiking solution used to analyze the PAH analytes in muscle tissues.

Each muscle homogenate was further split equally into two 50-ml conical centrifuge tubes before saponification. Both the hepatopancreas and muscle homogenates were saponified and extracted according to the procedure reported by the Axys Group [17] with recommendations from G. Brooks (Axys Group, Sydney, BC, Canada, personal communication) and modifications. Briefly, about 1 ml of 50% (w/v) potassium hydroxide and 7 ml of high-performance liquid chromatography–grade methanol were added to the homogenate. The mixture was refluxed at approximately 78°C for 1 h in a recirculating water bath. The samples were removed and vortexed after 20 and 40 min of refluxing. After a 1-h saponification, distilled, deionized H_2O (8 ml) was added to each sample. The procedure was repeated once. At the conclusion of a 2-h saponification, the samples were allowed to cool to room temperature before being shaken with 22 ml of pentane for 15 min. Each saponified muscle homogenate was extracted in two separate tubes because of volume restrictions. However, each hepatopancreas tissue sample could be extracted in a single tube. The pentane layer was removed from each tube. The extraction procedure was repeated twice with freshly added pentane. The extracts were combined into one tube per sample and reduced to about 6 ml under a gentle stream of N_2. One milliliter of isooctane was added to minimize PAH loss through vaporization before volume reduction. The extracts were washed with 2 ml of 0.1 M HCl to remove the contaminants with basic pH. The organic extract was removed and reduced to 1 ml before to silica gel column cleanup.

Table 2. Ion pairs for selective ion monitoring in gas chromatography mas spectrometry analysis

		Ion pairs			
		Deuterated standard		Analyte	
Analyte compound	Data acquisition start time (min)	Molecular ion	One half of molecular ion	Molecular ion	One half of molecular ion
Naphthalene[a]	9.00	136	68	128	64
Acenaphthylene[a]	13.00	160	80	152	76
Acenaphthene[a]	14.01	164	82	154	77
Fluorene[a]	15.30	176	88	166	83
Phenanthrene[a]	18.00	188	94	178	89
Anthracene[a]	18.80	188	94	178	89
Fluoranthene[b]	21.00	212	106	202	101
Pyrene[b]	22.00	212	106	202	101
Benz[*a*]anthracene[b]	24.60	240	120	228	114
Chrysene[b]	25.60	240	120	228	114
Benzofluoranthenes[b]	28.60	264	132	252	126
Benzo[*a*]pyrene[b]	28.80	264	132	252	126
Benzo(*ghi*)perylene[b]	34.50	288	144	276	138

[a] Low molecular weight polycyclic aromatic hydrocarbon.
[b] High molecular weight polycyclic aromatic hydrocarbon.

Sample cleanup was performed by liquid chromatography with a 205 × 14-mm glass column packed with 1 cm of sodium sulfate and 10 cm of 230- to 400-mesh Silica Gel 60 (Sigma-Aldrich Canada, Mississauga, ON, Canada). Column packing materials were mixed and degassed with pentane to form slurries before being added to the column. The packed column was rinsed with one bed volume of pentane before use. After adding the tissue extract, the column was rinsed with 12 ml of pentane and eluted with 15 ml of methylene chloride. The methylene chloride eluate was collected and evaporated down to near dryness with a gentle stream of N_2. A solution of methylene chloride (50 μl) containing the deuterated recovery standards (naphthalene-d_8, acenaphthylene-d_8 and benzo[*ghi*]perylene-d_{12}) was added, and the volume was adjusted to 100 μl before gas chromatography–mass spectrometry–selective ion monitoring (GC-MS-SIM) analysis.

Gas chromatography–mass spectrometry–selective ion monitoring analysis

Gas chromatography–mass spectrometry was used to determine the concentrations of the following U.S Environmental Protection Agency priority PAHs in the crab tissues: acenaphthene, fluorene, PHN, ANT, FLR, PYR, CRY, B*a*A, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, and B*a*P. An aliquot (1 μl) of the methylene chloride extract (see above) was injected directly into an Hewlett-Packard 5980 Series II gas chromatograph (Hewlett-Packard Canada, Mississauga, ON, Canada). The gas chromatograph was fitted with an HP 5971 mass selective detector and a 30-m × 0.25-mm (inner diameter) HP-5 MS capillary column (crosslinked 5% phenyl methyl silicone; 0.25-μm film thickness). The GC oven was temperature-programmed for an initial temperature of 70°C, held for 2 min, increased to 100°C in 1 min, held for 3 min, and then raised to 300°C in 20 min. The injector and mass selective detector temperature were set to 250°C and 280°C, respectively. Helium was the carrier gas and flowed at a rate of 40 to 45 ml/min. A splitless injection mode was used in the analysis.

The GC-MS system was operated in the electron impact mode and the electron energy was set at 70 eV. Initial mass calibration was performed according to the specification of Hewlett-Packard Canada. Two characteristic ions for each analyte were acquired when using the SIM mode. Data from GC-MS analyses were processed with the HP G1034C MS ChemStation (Ver C.03.00, Hewlett-Packard) on a 486 100-Mhz computer to integrate the ion pair peaks.

A total of four ions were monitored for each PAH analyte in the sample extract, one ion pair from the deuterated standard and the other from the analyte. The ion pair of each PAH analyte consisted of the molecular ion and a fragment ion at one half of the molecular weight. A summary of the ion pairs used in the SIM analysis is given in Table 2. Although all ion pairs were integrated and used in calculating recoveries, only the molecular ions were used in concentration determination.

A calibration curve for each PAH analyte was prepared by using serial dilutions of the U.S. Environmental Protection Agency 610 PAH standard mixture purchased from Supelco, a division of Sigma-Aldrich Canada. A total of six different standard concentrations were prepared, with each containing the recovery and the surrogate standards for the relative response factor calculation. The retention times and integrity of the surrogate standards were examined visually after each GC-MS-SIM analysis. A test mixture was also prepared by adding known amounts of PYR, B*a*A, and B*a*P to a blank hepatopancreas homogenate. This was used to check GC separation and MS sensitivity. The samples were prepared by other personnel in the laboratory and analyzed blindly by the primary author. A test mixture sample, a method blank, and a calibration standard were included with each set of 10 to 20 tissue extract samples.

The PAH analytes in the tissue extracts were identified by comparing with the retention times and mass ratios of the standard mixture. Polycyclic aromatic hydrocarbon concentrations were calculated from the relative response factor according to U.S. Environmental Protection Agency 600 Series Method 1624 (revision B) for isotope-dilution GC-MS [18; http://epa.gov/fedrgstr/index.html]. Background of the non-deuterated standards was subtracted from the calculated concentrations. Quantitation was performed by using a technique that self-corrected concentrations by recoveries of the deuterated standards. Mean (± standard error) recoveries of spiked deuterated PAH surrogate standards were as follows: acen-

apthene (63 ± 1%), PHN (88 ± 2%), ANT (87 ± 2%), FLR (90 ± 2%), PYR (96 ± 2%), B*a*A (92 ± 2%), CRY (89 ± 1%), benzofluoranthenes (86 ± 2%), and B*a*P (76 ± 1%), whereas the recoveries of blind spiked unlabeled PYR, B*a*A, and B*a*P in quality control samples were 81, 67, and 87%, respectively. No PAH analyte peaks were observed in the procedural blanks. Sample-specific estimated detection limits also were determined for each PAH analyte by using the U.S. Environmental Protection Agency Method 8290 [19; http://www.epa.gov/epaoswer/hazwaste/test/main.htm]. The estimated detection limits of the PAH analytes were found to range from 0.1 to 0.3 ng/g for the hepatopancreas and 0.01 to 0.09 ng/g for the muscle. These quality control results show that the GC-MS-SIM system met all the acceptance criteria of the U.S. Environmental Protection Agency method [18] and that the PAH analysis was precise and sensitive.

The benzo[*b*]fluoranthene and benzo[*k*]fluoranthene peaks coeluted under the chromatographic conditions. They were integrated together and reported as a single value for benzofluoranthenes. Despite efforts to minimize photolytic degradation, relative recoveries of the photolytically sensitive PAHs such as acenaphthene-d_{10} and benzofluoranthene-d_{12} were low. Therefore, acenaphthene analyte concentration was determined reliably in about one half of the hepatopancreas samples. Neither acenaphthene nor benzofluoranthenes were determined in the muscle because of rapid photolytic degradation of the deuterated surrogate standards. Also, fluorene was not determined in hepatopancreas and muscle tissues because of low recoveries.

Statistical analysis

Polycyclic aromatic hydrocarbon tissue concentration data were analyzed statistically by using version 6.12 SAS® for Windows (SAS Institute, Cary, NC, USA). Mixed-model analysis of variance (ANOVA) was used to resolve the difference in sample size for the PAH concentrations in the hepatopancreas data set. Hypotheses were tested at the $\alpha = 0.05$ significance level. Each PAH analyte was analyzed with respect to the date and location of sample collection. The data from Kitkiatka Inlet were not included in the statistical analysis because they were obtained on only one occasion. In addition, acenaphthene was not included in the statistical analysis because it could not be determined in all tissue samples because of degradation of the deuterated surrogate standard. Tissue PAH concentrations that were less than the estimated detection limits were included in the analysis to avoid left censoring of the data [20,21]. The data were log transformed before being analyzed because they were found to be log normally distributed. The PAH concentration correlation analysis between the hepatopancreas and muscle of crabs was performed on nontransformed data with Microsoft Excel 97 SR2 (Redmond, WA, USA).

RESULTS

The PAH concentrations in the hepatopancreas of crabs collected from May 1995 to October 1996 are displayed in Table 3. Figure 2a displays the mean concentration of each PAH analyte in the hepatopancreas tissue of crabs from different sites. Among the PAH analytes in the hepatopancreas, acenaphthene had the highest concentration. A high level of PHN also was found in the hepatopancreas of crabs caught at different sites. A comparison of PAH analyte concentrations in different sampling sites by ANOVA showed that ANT and

Table 3. Concentration of polycyclic aromatic hydrocarbon (PAHs) in hepatopancreas of crabs collected in Douglas Channel (BC, Canada; means ± standard error)

Site	PAH concentration (ng/g wet wt)[a]								
	ACN	PHN	ANT	FLR	PYR	B*a*A	CRY	BF	B*a*P
March 1994									
Kitamaat Village	—	2.29 ± 0.30	0.92 ± 0.67	0.74 ± 0.12	0.71 ± 0.08	0.20 ± 0.02	0.53 ± 0.08	0.50 ± 0.03	0.33 ± 0.02
Kitkiatka Inlet	—	2.12 ± 0.19	0.12 ± 0.02	0.59 ± 0.06	0.51 ± 0.04	0.27 ± 0.04	0.53 ± 0.08	0.40 ± 0.04	0.43 ± 0.06
May 1995									
Hospital Beach	—	0.05 ± 0.01	2.32 ± 0.42	5.06 ± 0.81	1.36 ± 0.20	3.83 ± 1.25	3.67 ± 1.08	2.03 ± 0.29	0.98 ± 0.30
Kitamaat Village	—	0.02 ± 0.002	0.50 ± 0.12	0.63 ± 0.09	0.52 ± 0.05	0.31 ± 0.20	0.80 ± 0.23	0.40 ± 0.04	0.23 ± 0.05
Wathlsto Creek	—	0.02 ± 0.003	0.17 ± 0.06	0.33 ± 0.06	0.45 ± 0.10	0.09 ± 0.02	0.33 ± 0.04	0.28 ± 0.04	0.25 ± 0.04
May 1996									
Hospital Beach	29.1 ± 22.9	13.7 ± 2.28	1.48 ± 0.28	3.79 ± 1.11	1.38 ± 0.22	0.58 ± 0.22	1.38 ± 0.34	1.03 ± 0.11	0.76 ± 0.26
Kitamaat Village	0.28 ± 0.06	0.97 ± 0.13	0.11 ± 0.07	0.53 ± 0.04	0.64 ± 0.21	0.27 ± 0.05	0.51 ± 0.05	0.45 ± 0.05	0.003 ± 0.0005
Wathlsto Creek	0.34 ± 0.04	0.22 ± 0.16	0.23 ± 0.04	0.56 ± 0.10	0.62 ± 0.09	0.24 ± 0.03	0.43 ± 0.11	0.44 ± 0.06	0.003 ± 0.001
Kildala Arm	0.24 ± 0.03	1.16 ± 0.18	0.20 ± 0.10	0.51 ± 0.09	0.64 ± 0.06	0.22 ± 0.05	0.41 ± 0.35	0.59 ± 0.13	0.29 ± 0.18
October 1996									
Hospital Beach	0.36 ± 0.04	24.6 ± 12.4	7.14 ± 2.26	29.3 ± 21.2	15.7 ± 10.5	3.00 ± 2.59	20.5 ± 16.9	2.58 ± 0.63	0.82 ± 0.28
Kitamaat Village	0.42 ± 0.04	5.42 ± 1.26	7.77 ± 2.67	1.61 ± 0.30	1.00 ± 0.19	0.11 ± 0.02	0.99 ± 0.31	0.55 ± 0.03	0.20 ± 0.03
Wathlsto Creek	0.46 ± —	1.80 ± —	0.28 ± —	0.36 ± —	0.33 ± —	0.15 ± —	0.41 ± —	0.47 ± —	0.24 ± —
Kildala Arm	0.49 ± 0.02	1.44 ± 0.09	0.06 ± 0.01	0.21 ± 0.04	0.31 ± 0.04	0.11 ± 0.01	0.38 ± 0.05	0.35 ± 0.01	0.12 ± 0.02

[a] ACN = acenaphthalene; PHN = phenanthrene; ANT = anthracene; FLR = fluoranthene; PYR = pyrene; B*a*A = benz[*a*]anthracene; CRY = chrysene; BF = benzofluoranthene; B*a*P = benzo[*a*]pyrene.

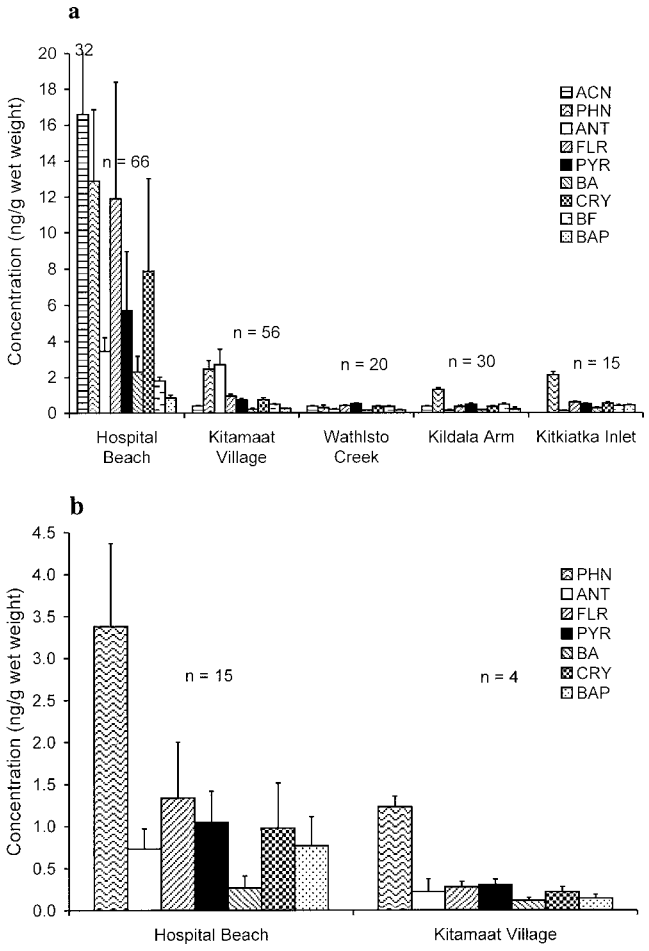

Fig. 2. (**a**) Mean polycyclic aromatic hydrocarbon (PAH) concentrations in the hepatopancreas and (**b**) PAH concentrations in the muscle of selected crabs at different sampling sites in Kitimat Arm Douglas Channel (BC, Canada) (1994–1996). ACN = acenaphthene, PHN = phenanthrene; ANT = anthracene; FLR = fluoranthene; PYR = pyrene; BA = benz[*a*]anthracene; CRY = chrysene; BF = benzofluoranthenes; BAP = benzo[*a*]pyrene. Error bars indicate standard error of the mean (SEM). Values at top of error bars indicate upper SEM value.

PYR tissue concentrations of Hospital Beach samples were significantly higher than those of Kildala Arm ($t = 3.34$, $p = 0.023$; $t = 2.7$, $p = 0.038$); and FLR and B*a*P tissue concentrations of crabs from Hospital Beach were significantly higher than those of Wathlsto creek ($t = 3.73$, $p = 0.0047$; $t = 2.21$, $p = 0.0286$) and Kildala arm ($t = 4.5$, $p = 0.005$; $t = 2.41$, $p = 0.0171$). In contrast, B*a*A, benzofluoranthenes, and CRY concentrations of Hospital Beach samples were not found to be significantly different to those of the other sites. The site-specific tissue concentration data were analyzed further by gradient analysis. Results of the ANOVA analysis showed that FLR ($p = 0.0034$) and B*a*P ($p = 0.0152$) concentrations significantly decreased in the hepatopancreas of crabs caught at an increasing distance down the Arm from Hospital Beach. The PAH concentrations in the hepatopancreas of the two reference sites, Kildala Arm and Kitkiatka Inlet, appeared similar. No significant differences in PAH concentrations were found at these sites when using the ANOVA test (Figs. 2a and 3), despite of the fact that these sites were far apart (Fig. 1). At both sites, PHN was found to be the predominant PAH in the hepatopancreas (Fig. 2a).

A number of crabs were also analyzed for PAH analytes in the muscle (Fig. 2b). These crabs were caught either at Hospital Beach or near Kitamaat Village. As with the hepatopancreas data, PAH concentrations in the muscle of crabs from Hospital Beach were high and variable, but were not statistically different from those of the crabs from Kitamaat Village (Fig. 2b).

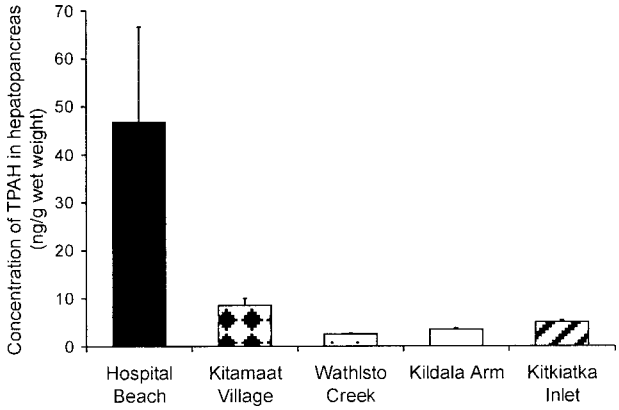

Fig. 3. Mean total polycyclic aromatic hydrocarbon (TPAH) concentrations in the hepatopancreas of crabs at different sampling sites in Douglas Channel (BC, Canada) (1994–1996). Error bars indicate standard error of the mean.

Total PAH (TPAH) concentrations in hepatopancreas samples were calculated as the sum of all PAH analytes determined. Figure 3 shows that mean TPAH concentrations in the crabs of Hospital Beach were the highest among all sites. The TPAH concentration at Kitamaat Village was significantly different from those of Wathlsto Creek and Kildala Arm, but not Kitkiatka Inlet ($F = 45.97$, $p < 0.0001$).

DISCUSSION

In the present study, PAH-contaminated crabs were found throughout the Kitimat and Kildala Arms and down the Douglas Channel as far as Kitkiatka Inlet. These results show that the PAH contaminants in Kitimat Arm are detectable in crab tissues and, therefore, bioavailable to the crabs. The highest tissue PAH concentrations are found in crabs caught near Hospital Beach, which is very close to the effluent discharge of the smelter's waste lagoons. Paine et al. [8] suggested that PAH bioavailability to the dungeness crabs is limited in Kitimat Arm because they found few PAH-contaminated crabs, and only found them in the Inner Harbour. A plausible explanation for the discrepancy in results between Paine et al. [8] and the present study may be related to the less sensitive analytical procedure employed by Paine et al. [8]. For example, the detection limits of PAH analytes in our study range from 0.1 to 0.3 μg/kg, whereas those of Paine et al. [8] ranged from 5 to 20 μg/kg. Because most of the PAH concentrations in the crab tissues were below 10 μg/kg (Figs. 2 and 3), detection of the PAH in the crab tissues by Paine et al. [8] would have been difficult if not impossible. However, one might conclude that PAH bioavailability is low in crabs because only a low level of PAHs is found in the tissues as compared to that in the sediments. However, the bioavailability of PAHs cannot be fully appreciated based on the concentrations of parent compounds in the tissues alone because crabs are able to metabolize PAHs [22–24]. For example, we have found parent PAH difficult to detect in groundfish such as the starry flounder [25] because fish are capable of rapidly metabolizing PAHs such as PYR, which is conjugated to form pyrene-1-glucu-

ronide, and excreted in the bile. Therefore, monitoring the metabolites of PAHs instead of the unchanged PAHs in the bile of fish often is more effective. Although invertebrates such as crabs do not metabolize PAHs as quickly as fish, a significant level of metabolic activity also takes place in the invertebrates. Indeed, we have successfully adapted a synchronous fluorescence spectrometry method [26] to screen for pyrene-1-glucoside and 1-hydroxypyrene in the hemolymph of crabs [8,22,27].

Examination of our results shows that tissue PAH concentrations generally are higher in crabs caught off Hospital Beach than those caught in other sites (Figs. 2 and 3). This is consistent with the findings of previous studies that sediment PAH concentrations are the highest when they are sampled close to the smelter but decline sharply in concentrations at sites further down the Arm [1,8]. A similar observation also was reported in the marine sediments near a Norwegian aluminum smelter in the Sunndalsfjord [28]. This Norwegian smelter also is set at the head of a long fjord remarkably similar in geography to Kitimat Arm [27].

We hypothesized that a PAH concentration gradient in crab tissues should exist in the Arm with the highest concentration near the head of the Arm at Hospital Beach, a lower concentration further down Kitimat Arm, and the lowest levels at Kildala Arm and Kitkiatka Inlet. However, Figures 2a and b and 3 show that generally, PAH concentrations are highest at Hospital Beach, the site closest to the smelter, and occur at a much lower level at other sites throughout Douglas Channel (ANOVA, Tukey–Kramer, $\alpha = 0.05$, $p < 0.0001$). We have been unable to demonstrate a significant concentration gradient of PAH concentrations in crab tissues, although a significant tissue concentration difference was found between Hospital Beach and the reference sites. The total concentration of all PAH analytes (TPAHs) determined in the tissues at all sites over time reflects the same trend (Fig. 3), although a significant difference was found between the TPAH concentration in crab hepatopancreas from Kitamaat Village compared to Wathlsto Creek and Kildala Arm (ANOVA, Tukey–Kramer, $\alpha = 0.05$, $p < 0.0001$). Our inability to demonstrate a spatial difference in concentration for these PAH analytes could be explained by the fact that the PAH emissions from the smelter are evenly distributed by the atmosphere over a very large area, and that cycling of PAHs may occur throughout Douglas Channel. Quite possibly, the background level of PAHs is comparable or perhaps greater than those attributed to the smelter's current emissions.

Kildala Arm and Kitkiatka Inlet were chosen to represent clean reference sites. However, PAH levels in the hepatopancreas of crabs collected from these sites were similar to those collected from Wathlsto Creek in Kitimat Arm (Fig. 1). Aerial transport of PAHs from the smelter, cycling of contaminants throughout Douglas Channel, as well as local logging operations and boat traffic may account for the PAHs found in the crabs from these clean sites.

The PAH concentrations in crab tissues were quite variable. This is especially evident in the crabs caught in Hospital Beach (Figs. 2 and 3). The variability in tissue PAH concentration appears to parallel that of the sediments and may have resulted from the randomness of soot, coal dust, and tar ball deposition or pitch spillage near the smelter [1,2].

Our results show that low molecular weight PAHs (LPAHs), including naphthalene, acenaphthene, fluorene, PHN, and ANT [1], often were found at higher concentrations than HPAHs

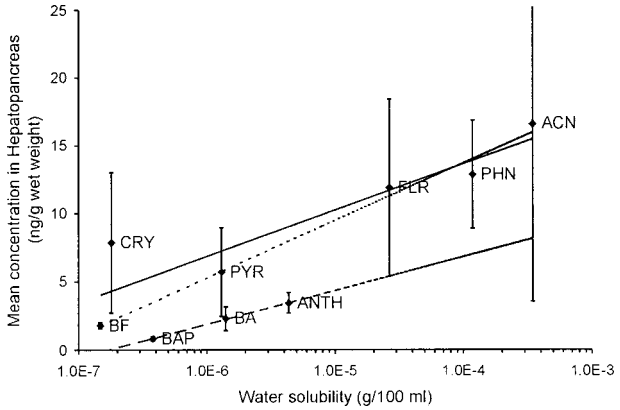

Fig. 4. Mean concentration of analyte polycyclic aromatic hydrocarbons (PAHs) in the hepatopancreas of crabs collected from Hospital Beach (BC, Canada) (1994–1996) versus water solubility. ANT = anthracene; BA = benz[*a*]anthracene; BAP = benzo[*a*]pyrene; BF = benzofluoranthene; CRY = chrysene; FLR = fluoranthene; PHN = phenanthrene; PYR = pyrene. Regression lines: solid line, $r^2 = 0.845$; short dashes, $r^2 = 0.985$; long dashes, $r^2 = 0.999$. Error bars indicate standard error of the mean.

(FLR, PYR, CRY, B*a*A, benzofluoranthenes, and B*a*P) in crab tissues. For example, LPAHs, such as acenaphthene and PHN, are found at high concentrations in the hepatopancreas of crabs (Fig. 2a and b). This is consistent with the finding that a relatively high level of LPAHs was found in the crab tissues in an earlier study conducted in Kitimat Arm [8]. Acenaphthene or PHN often were the predominant PAHs determined in crab tissues from Kitimat Arm. Phenanthrene also was determined at high concentrations in crabs, particularly at Hospital Beach, and was also the most prominent PAH determined in the tissues of two crab species, the snow crab (*Chionoecetes opilio*) and the spider crab (*Hyas coarctatus*), collected from Conception Bay, Newfoundland [14]. Moreover, high concentrations of LPAHs, such as PHN and ANT, and HPAHs, such as FLR and PYR, were determined in the tissues of *Polybius henslowi*, an edible crab species caught on the coast of France and Spain in the North Atlantic [15]. This study also found that both LPAHs and HPAHs accumulated in the tissues of crabs, but HPAHs such as B*a*A, benzofluoranthenes, and B*a*P did not accumulate to the same degree as the LPAHs and other HPAHs such as FLR, PYR, and CRY.

In contrast, higher levels of HPAHs than LPAHs were reported in the sediments collected throughout Kitimat Arm [1]. Because LPAHs are more water soluble and therefore are less tightly bound to the organic materials of sediments than HPAHs, crabs may be exposed to higher concentrations of LPAHs than HPAHs in water. This hypothesis is further supported by the finding that PHN is found at a high concentration in the sediment and was the only LPAH detected in the pore waters [7]. Baumard et al. [15] reported a high ratio of PHN to ANT in crab tissues, and stated that PHN is 20 times more water soluble than ANT and, therefore, is more available in the water column for uptake by crabs. Hellou et al. [14] stated that composition of diet, bioavailability of contaminants, differences in PAH distribution and location, and metabolism are factors that are involved in the differential accumulation of HPAHs and LPAHs by crabs. Our results are consistent with the observations of these studies.

Figure 4 shows the relationship between the water solubility of PAH analytes and their concentrations in the hepatopancreas of crabs collected near Hospital Beach. Generally, the higher

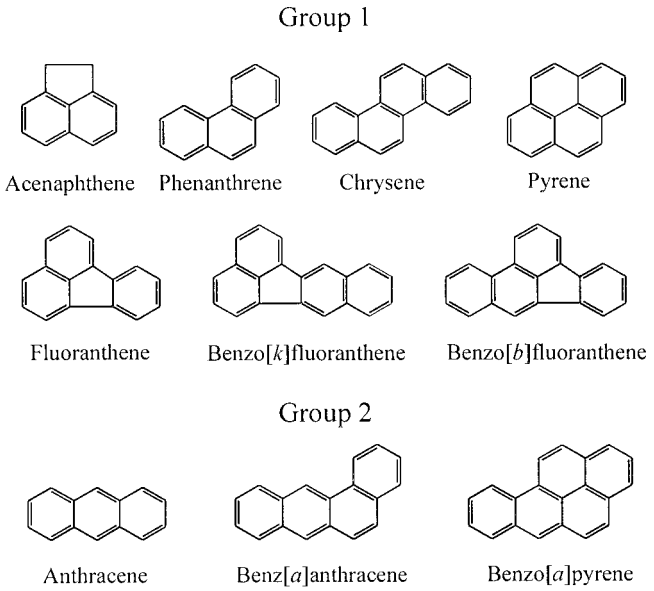

Fig. 5. Chemical structures of polycyclic aromatic hydrocarbon (PAH) analytes. Group 1 includes PAHs based on a two-ring subunit (naphthalene) and group 2 includes PAHs based on a three-ring structural subunit (anthracene).

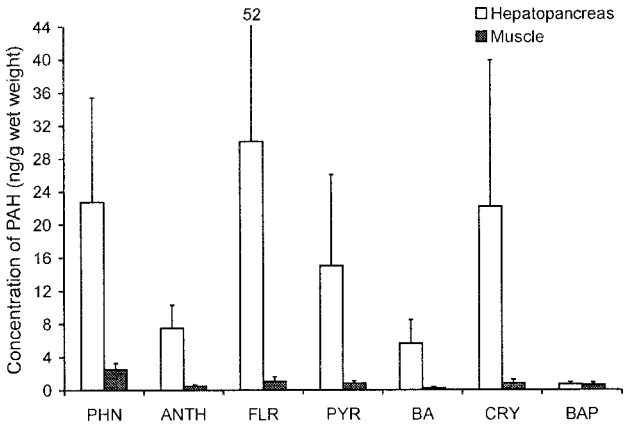

Fig. 6. Mean concentrations of polycyclic aromatic hydrocarbons (PAHs) determined in hepatopancreas and muscle tissues of selected crabs. PHN = phenanthrene; ANTH = anthracene; FLR = fluoranthene; PYR = pyrene; BA = benz[*a*]anthracene; CRY = chrysene; BAP = benzo[*a*]pyrene. Error bars indicate standard error of the mean (SEM). Values at top of error bars indicate upper SEM value.

the water solubility of the PAH, the higher the concentration in the hepatopancreas (Fig. 4). Apparently, PAHs can be classified into two groups based on their structure and the log-linear relationship between water solubility and hepatopancreas concentrations (Fig. 5). Group 1 is comprised of PAHs that contain a two-ringed naphthalene structural subunit, acenaphthene, PHN, CRY, PYR, FLR, and benzofluroanthenes; whereas group 2 compounds, ANT, B*a*A and B*a*P, are based on a three-ringed ANT structural subunit. Chrysene may be somewhat of an exception, because it seems to be concentrated in the hepatopancreas at a higher concentration relative to other PAHs in group 1 with equal or greater water solubility. Indeed, if regression lines are fit through the concentration versus water solubility data, the correlation (r^2) between the group 1 compounds ($r^2 = 0.845$) improves if CRY is not included ($r^2 = 0.986$; Fig. 4). A similarly strong correlation is found between the group 2 compounds ($r^2 = 0.999$). These observations indicate that the water solubility of PAHs seems to be an important factor in differential exposure and bioconcentration of PAHs by dungeness crabs. Although the relationships between water solubility, chemical stucture, and tissue concentration are interesting and may be useful for prediction of relative tissue concentrations, they are very simplistic. In fact, the concentration of PAHs in the tissues of crabs also is influenced by the relative absorption, distribution, metabolism, and excretion rates of each PAH in the crab. For example, in our laboratory, we have found that PYR and B*a*P are metabolized and excreted at different rates by the dungeness crab.

Because crabs are closely in contact with sediments, assuming that concentrations of PAHs in crabs generally reflect the sediments in Kitimat Arm is reasonable. Moreover, because the hepatopancreas of male crabs contains a larger amount of lipid than the muscle (6.6% and 0.9%, respectively) [29], the hepatopancreas is expected to accumulate a higher level of PAHs on a wet weight basis. As shown in Figure 6, mean PAH analyte concentrations are greater in the hepatopancreas than in the muscle of selected crabs. The muscle tissue analysis was performed on 19 crabs that had particularly high concentrations of PAHs in the hepatopancreas. The muscle tissue of these crabs was selected for analysis because crabs that had high levels of PAHs in the hepatopancreas would be expected to have detectable levels of PAHs in the muscle. The hepatopancreas:muscle concentration ratios (Table 4) also reflect the higher concentration of PAH analytes in the hepatopancreas. Hellou et al. [14] also reported higher mean concentrations of PAHs in the hepatopancreas compared to the muscle of crabs. In another study conducted with blue crabs from the Elizabeth River and Chesapeake Bay, PAH burdens in the

Table 4. Concentration ratios and correlation coefficients for concentrations of phenanthrene (PHN), anthracene (ANT), fluoranthene (FLR), pyrene (PYR), benz[*a*]anthracene (B*a*A), chrysene (CRY), and benzo[*a*]pyrene (B*a*P) determined in hepatopancreas and muscle samples from crabs collected at Hospital Beach (BC, Canada)

Parameter	PHN	ANT	FLR	PYR	B*a*A	CRY	B*a*P
Mean ratio of hepatopancreas/muscle concentrations in selected crabs	5	16	18	10	35	20	2
Hepatopancreas:muscle correlation coefficient	0.09	0.63	0.31	0.34	0.07	−0.40	0.62
Site-specific concentration differences	+	+	+	+	0	0	+
Molecular weight	178	178	202	202	228	228	252
Water solubility (g/100 ml)	1.18×10^{-4}	4.34×10^{-6}	2.65×10^{-5}	1.3×10^{-6}	1.4×10^{-6}	1.8×10^{-7}	3.8×10^{-7}
Log octanol–water partition coefficient (log K_{OW})[a]	4.5	4.45	5.2	5.2	5.6	5.9	6.3

[a] Mackay et al. [31].

hepatopancreas were found to be generally two- to fourfold greater than muscle burdens from the same crabs [9].

Although the PAH levels seem to be clearly higher in the hepatopancreas, only the mean concentration of ANT in the muscle of crabs was found to be significantly different from that of the hepatopancreas when using Student's *t* test and one-way ANOVA ($t = 2.477$, $p = 0.0181$). The statistical tests probably were unable to resolve the difference in PAH analyte concentrations between these tissues because of a large variability in the hepatopancreas data set and a small sample size of 19 crabs, which had PAHs determined in both hepatopancreas and muscle tissues. Similarly, the hepatopancreas:muscle concentration ratios of PAHs at Hospital Beach likely were distorted by one or two extreme outlying values of the data set.

Statistical analysis was performed to examine the correlation between the concentration of individual PAHs in the hepatopancreas and that of the muscle taken from the same crabs. In general, a poor correlation was found between PAH concentrations in the hepatopancreas and muscle tissues. This may be because the hepatopancreas data are confounded by the outlying values and a relatively small sample size of muscle values. If the extreme outlying values in the data set are excluded, reasonably good correlations between the hepatopancreas and muscle tissue concentrations can be found for the following PAH analytes (correlation coefficients, *r*): ANT (0.63) and B*a*P (0.62), and PYR (0.34) and FLR (0.31) (Table 3). However, PHN (0.09) and B*a*A (0.07) display a low PAH concentration correlation between the hepatopancreas and muscle tissues and CRY shows a negative correlation (−0.40).

Interestingly, ANT, B*a*P, FLR, PYR, and PHN, which display a concentration gradient in the sample sites, also show a positive correlation of PAH concentrations between hepatopancreas and muscle tissues. In contrast, B*a*A and CRY, which do not display a concentration gradient in the sample sites, show a very low correlation of PAH concentrations between hepatopancreas and muscle tissues. These results indicate that the PAH analytes in Kitimat Arm vary markedly in their bioavailabilities, which cannot be determined solely by their molecular weights and K_{OW} values (Table 3). Thus, the accumulation of PAH analytes by crab tissues may be determined by a number of factors, including PAH distribution in sediments, pore water and prey PAH concentrations, water solubility and binding of PAH analytes to organic sediment particles, and the toxicokinetics of individual PAH analytes in the crab.

Although tissue lipid concentrations may influence the relative concentrations of PAHs in the muscle and hepatopancreas, lipid analysis was not performed on the tissues in this study. In retrospect, lipid analysis may have greatly enhanced this study by allowing for the normalization of contaminant concentrations based on lipid content, because dungeness crabs may exhibit significant seasonal and annual variations in lipid levels. Unfortunately, the influence of lipid tissue content on the apparent temporal trends in PAH concentrations cannot be examined for this data set.

The concentrations of PAHs determined in the hepatopancreas and muscle of the crabs collected in Kitimat Arm are much lower that those of blue crabs collected from the Elizabeth River, an industrial area with one of the highest recorded PAH loads recorded in the world [9]. Concentrations of total resolvable PAHs in the hepatopancreas of the blue crabs were between 2,900 to 11,000 μg/kg, whereas concentrations of TPAHs determined in dungeness crabs collected from sites in Kitimat Arm ranged from 0.7 to 308 μg/kg. Similar TPAH concentrations (<5–1,100 μg/kg) previously were determined in blue crabs collected in South Chesapeake Bay [10]. Concentrations of TPAHs between 55.5 and 123.1 μg/kg have been determined in tissues of mud crabs collected in the Brisbane River estuary on the coast of Australia [16]. Note that the TPAH concentrations reported in the present study are based solely on the 10 PAH analytes determined.

The dungeness crab seems to be an effective indicator species for monitoring PAH exposure to biota in Kitimat Arm for the following reasons. Crabs can be caught readily at many sites in the Arm. Detectable levels of PAHs are present in the muscle and the hepatopancreas. Crabs are an important benthic species of Kitimat Arm, and do not move about from one area to another as quickly as many fish species. Finally, crabs do not metabolize and excrete PAHs from their tissues as rapidly as fish [30]. Therefore, PAH levels in crabs may reflect differences in the relative amount and bioavailability of PAHs at different sites, particularly between Hospital Beach, and other sites downstream in Douglas Channel.

The study area focused mainly on sites along the eastern side of Kitimat Arm, such as Kitamaat Village and Wathlsto Creek (see Fig. 1). These sites have been chosen based on the potential of catching crabs, distance from the smelter, and proximity to human settlement. Future studies should determine the concentrations of PAHs in crabs on the western side of Kitimat Arm. As suggested by Simpson et al. [1], waterborne PAH-laden particles and atmospheric emissions tend to be deposited along the western side by prevailing currents and winds. In addition, the results of this study will be used to estimate the risk of cancer in a local population due to the consumption of crabs collected in Kitimat Arm.

CONCLUSIONS

In summary, this study has demonstrated that PAHs in Kitimat Arm and Douglas Channel are bioavailable to dungeness crabs. Concentrations of PAHs in the tissues were the highest and the most variable in crabs collected from the site close to the smelter and dropped off to lower levels along the eastern side of the arm and reference sites. The PAHs were detectable in the tissues of crabs and therefore were bioavailable. Water solubility and chemical structure seem to be important factors that affect PAH exposure and bioconcentration by crabs. Hepatopancreas PAH concentrations were higher and more variable than those in the muscle tissues. Overall, this study has shown that dungeness crabs can be used to monitor tissue concentrations of parent PAHs in biota in estuarine and marine environments such as Kitimat Arm.

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