Arch. Environ. Contam. Toxicol. 19, 10–16 (1990)

Archives of Environmental Contamination and Toxicology

Uptake of Aromatic and Chlorinated Hydrocarbons by Juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) in an Urban Estuary

B. B. McCain, D. C. Malins[1], M. M. Krahn, D. W. Brown, W. D. Gronlund, L. K. Moore, and S-L. Chan

Environmental Conservation Division, Northwest and Alaska Fisheries Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 2725 Montlake Boulevard East, Seattle, Washington 98112, USA

Abstract. A study was conducted to assess the potential for uptake of toxic chemicals by down-stream migrant salmon in an urban estuary. Juvenile chinook salmon (*Oncorhynchus tshawytscha*) were collected from the Duwamish Waterway (located in Seattle, Washington) and from the Nisqually River (a reference site). The mean concentrations of aromatic hydrocarbons and polychlorinated biphenyls (PCBs) in the stomach contents (food organisms) of salmon from the Duwamish Waterway were approximately 650 times and 4 times, respectively, higher than those in salmon from the Nisqually River. Similarly, the mean concentration of bile metabolites of aromatic compounds which fluoresce at benzo[*a*]pyrene wavelengths was 24 times higher in the urban salmon compared to the reference salmon, whereas the mean concentration of PCBs in liver of urban salmon was 3 times higher than that in reference salmon. The study clearly demonstrated that, during their residency in this urban estuary, juvenile chinook salmon bioaccumulate substantial levels of toxic chemicals. The possible effects of these chemical exposures on the health and survival of this species are not presently known.

Estuaries provide important habitats for juveniles of all five of the most important Pacific salmon species (Healy 1982). A number of urban estuaries in Puget Sound are used during the down-stream migration of wild and hatchery-reared juvenile salmonids, including the Duwamish Waterway in Seattle, the Puyallup Waterway in Tacoma, and the Snohomish River in Everett. Municipal and industrial wastes enter these urban estuaries from point and non-point sources and accumulate in bottom sediment and associated ecosystems (Dexter *et al.* 1985).

The Duwamish Waterway is perhaps the most chemically contaminated of these estuaries. Some of the contaminants measured in bottom sediments include aromatic hydrocarbons (AHs) and chlorinated hydrocarbons (CHs) (Malins *et al.* 1984; Hamilton *et al.* 1984). Malins *et al.* (1982) reported finding mean concentrations of summed AHs and polychlorinated biphenyls (PCBs) of 12.0 ± 11.0 and 0.43 ± 0.20 μg/g (± Standard Deviation, dry weight), respectively, in surface sediments (top 2 cm) at 5 sites sampled in this waterway. In contrast, sediment from the estuarine system near the Nisqually River, located in a rural region of Southern Puget Sound, had concentrations of AHs and PCBs which were at or below the limits of detection (<0.02 μg/g and 0.004 μg/g, dry weight, respectively) (McCain *et al.* 1988).

Bottom fish and benthic and epibenthic invertebrates in polluted waterways bioaccumulate substantial levels of CHs and AHs. For example, English sole (*Parophrys vetulus*) from the Duwamish Waterway have mean concentrations of 47.0 ± 25.0 μ/g (dry weight, n = 5) PCBs in liver tissue (Malins *et al.* 1984). Uptake of AHs by English sole has been demonstrated by measuring concentrations of AH metabolites in bile which fluoresce at the wavelength pair where benzo[*a*]pyrene (BaP) fluoresces (380/430 nm) (Krahn *et al.* 1984, 1986a, 1987). English sole (n = 58) from the Duwamish Waterway had a mean concentration of bile metabolites of 1.4 ± 2.2 μg/g (wet weight) (Krahn *et al.* 1986a), compared to 0.068 ± 0.043 μg/g (wet weight) for English sole from another reference site near the mouth of the Nisqually River (Malins *et al.* 1986). Amphipods (epibenthic crustacea) from the Duwamish Waterway had mean concentrations of AHs (1.3 ± 0.20 μg/g, dry weight) and PCBs (0.24 ± 0.010 μg/g, dry weight) which were substantially higher than those found in amphipods from near the Skagit River estuary (0.12 ± 0.04 μg/g, and 0.010 ± 0.0006 μg/g, respectively, dry weight), located in the rural region in Northern Puget Sound (Brown *et al.* 1985).

Of all the species of Pacific salmon at the juvenile stage, chinook salmon (*Oncorhynchus tshawytscha*) are most dependent upon estuaries as a feeding ground (Thom 1987). Depending upon their age when they enter an estuary, juvenile chinook salmon may reside in estuaries for up to 6 months; younger fish tend to remain longest (Simenstad *et al.* 1982). The usual residence time for juveniles of this species in the Duwamish Waterway is about two months (early April to early June) (Meyer *et al.* 1981). Most of these juveniles are released into the Green River/Duwamish Wa-

[1] Present Address: Pacific Research Foundation, 720 Broadway, Seattle, WA 98104, USA

terway system from the hatchery at Soos Creek operated by the Washington State Department of Fisheries (Meyer *et al.* 1981). They feed on a variety of prey organisms, including epibenthic crustaceans, such as copepods, gammarid amphipods, mysids, cumaceans, and insects (Healy 1982; Simenstad *et al.* 1982). In the Duwamish Waterway, small (60 to 69 mm) juvenile chinook salmon tend to consume a higher proportion of gammarid amphipods than do larger juveniles (Meyer *et al.* 1981).

The extended residence time of juvenile chinook salmon and the presence of chemically contaminated amphipods, a primary juvenile salmonid food organism, in the polluted Duwamish Waterway suggested that these fish may be exposed to high concentrations of toxic chemicals. A study was initiated in the spring of 1986 to estimate the uptake of AHs and CHs by chinook salmon juveniles in the Duwamish Waterway. Similarly aged chinook salmon from the Nisqually River and from salmon hatcheries on the Green River/Duwamish Waterway system and on the Nisqually River were sampled in the same manner and at the same time for comparative purposes. We report here the results of this study.

Study Areas

The Duwamish Waterway constitutes the lower 8.4 km of the Green-Duwamish River system and is largely estuarine (Williams *et al.* 1975) (Figure 1). This waterway passes through the industrial section of Seattle and enters Elliott Bay. Portions of the waterway are routinely dredged. Fifteen small streams and over 20 storm drains flow into the waterway. Three species of Pacific salmon, including coho (*O. kisutch*), chum (*O. keta*) and chinook salmon use the waterway during downstream and upstream migrations (Williams *et al.* 1975). The Washington Department of Fisheries operates the Green River Salmon Hatchery located about 55 km upstream. This hatchery cultures and releases all three species.

The Nisqually River flows through predominantly rural areas, and enters Puget Sound about 80 km south of Seattle. Chinook salmon are cultured in the Kalama Creek Hatchery operated by the Nisqually Indian Tribe and released in the Nisqually River.

Methods

Fish Sampling

Juvenile chinook salmon were collected at four sites: the Duwamish Waterway, the Nisqually River, the Kalama Creek Hatchery of the Nisqually River and the Green River Hatchery on the Green-Duwamish River system (Figure 1). Fish at the estuarine sites were captured with a 30 m beach sein. Collections were made periodically between May 8 and June 16, 1986 (Tables 1 and 2). Immediately after capture, live fish were transported to the laboratory. Within 5 hr of capture, specimens were sacrificed and samples of liver, stomach contents (food organisms), and bile were collected for chemical analyses. Because hatchery fish were fed a standard pelletized commercial diet, their stomach contents were not collected. Due to the small size of the fish (mean lengths of 78 to 96 mm, Table 2), samples from each site were divided into 2 to 3 composites of 10 to 35 fish each. Different composites at each site were referred to by Roman numeral. Prior to chemical analysis, composites of stomach contents were examined with a dissecting microscope in order to estimate their taxonomic composition.

Chemical Analyses

Stomach content samples were analyzed for 17 AHs (Table 1) by capillary column gas chromatography (GC) with flame ionization detection or by mass spectrometry (MacLeod *et al.* 1985). PCBs (Table 1) were analyzed by capillary GC with electron-capture detection (ECD) (MacLeod *et al.* 1985). A 30 m × 0.25 mm I.D. DB-5 capillary column (J & W Scientific)[1] was used in a Hewlett-Packard Model[1] 5880 gas chromatograph. For AHs, 3 µl of the sample was injected splitless, and the split valve was opened after 18 sec. The oven temperature of 50°C was held for 1 min and then programmed at 4°C/min to 170°C, then at 1°C/min to 210°C, and finally at 4°C/min to 300°C. Helium was the carrier gas and the flow-rate was set to 30 cm/s at 300°C, split ratio of 20:1. The GC-mass spectrometry was performed with a Hewlett-Packard model 5840 GC interfaced with a Finnigan 3200 mass spectrometer (Finnigan MAT Corp., Sunnyvale, CA, USA) and a Finnigan IA/COS 2300 data system. The quality assurance/quality control procedures, as specified in MacLeod *et al.* (1985), included the use of internal standards (often called surrogate standards and calibration standards), method blanks, reference material, and analyses of replicate samples. An internal standard, 4,4′-dibromooctafluorobiphenyl, was added to each sample prior to analysis. The recovery of this standard was 77 ± 8.6% (n = 15). In addition, the recoveries of analytes added to a method blank analyzed concurrently with the other samples averaged 118 ± 6.6% (n = 9). Two concurrently analyzed method blanks had very low concentrations of analytes, demonstrating that the sample preparation procedures did not introduce unacceptable levels of contaminants.

A high-pressure liquid chromatographic/fluorescence (HPLC) detection technique (Krahn *et al.* 1984, 1986a, 1987) was employed to measure metabolites of aromatic compounds in bile. This technique was used because analyses of AHs (*e.g.*, components of fossil fuels and their combustion products) in tissues are of limited value due to the extensive metabolism of these compounds, especially in the liver (Roubal *et al.* 1977; Varanasi and Gmur 1981). Quality assurance procedures included the use of a "bile pool" reference material, replicate analysis of calibration standards and of selected samples (Krahn *et al.* 1986b). The term "concentration of bile metabolites", when used in this manuscript, refers to the BaP equivalents calculated from HPLC chromatograms and is a relative rather than an absolute concentration (Krahn *et al.* 1986b). The same is true of naphthalene (NPH) equivalents. Samples of liver tissues were analyzed (MacLeod *et al.* 1985) for the more metabolically resistant CHs. Significant differences between concentrations of chemicals in Duwamish Waterway and reference salmon were evaluated by the Student's t-test (Zar 1974).

Results

Characteristics of Juvenile Chinook Salmon

Juvenile chinook salmon were released from the Green River and Kalama Creek Hatcheries during mid May, 1986. The greatest numbers of juveniles were captured in the re-

[1] Mention of trade names is for information only and does not constitute endorsement by the U.S. Department of Commerce.

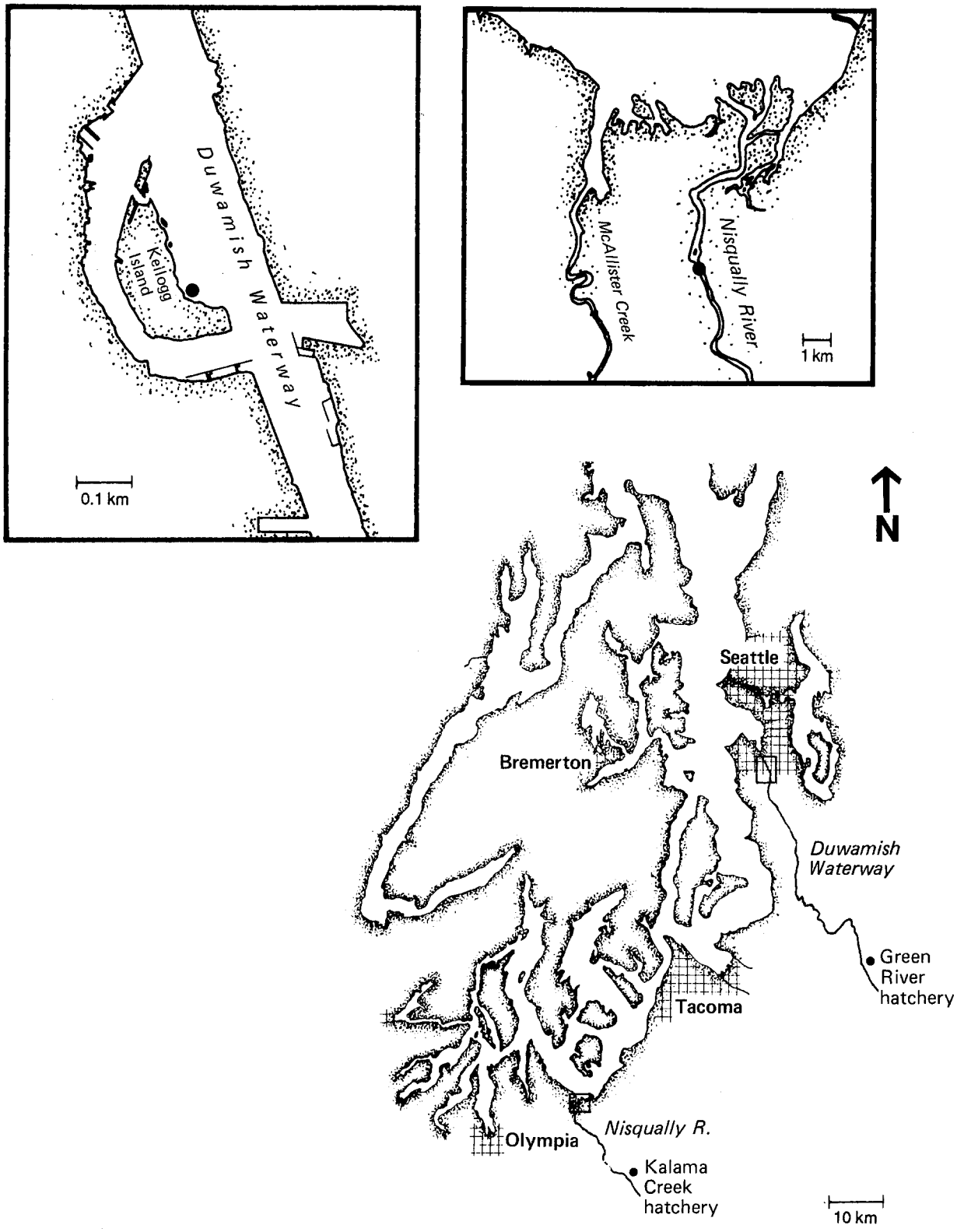

Fig. 1. Map of Puget Sound showing sampling sites (insets in the Duwamish Waterway and the Nisqually River)

Table 1. Concentrations of chemicals (μg/g dry weight[a]) in composites of stomach organisms from juvenile chinook salmon captured from the Duwamish Waterway (on 6/12/86) and the Nisqually River (on 6/16/87)

Chemicals	Duwamish Waterway I	Duwamish Waterway II	Nisqually River I	Nisqually River II
Aromatic hydrocarbons[b]				
Naphthalene	2.4	1.7	0.09	0.09
2-Methylnapthalene	6.5	4.2	<0.07[c]	0.11
1-Methylnaphthalene	3.5	2.0	<0.07	<0.05
Biphenyl	1.7	1.2	<0.07	<0.05
2,6-Dimethylnaphthalene	<.1	<0.09	<0.07	<0.05
Acenaphthene	11.0	5.0	<0.07	<0.06
Fluorene	11.0	5.5	<0.07	<0.05
Phenanthrene	38.0	18.0	<0.07	<0.05
Anthracene	0.65	0.44	<0.07	<0.05
1-Methylphenanthrene	1.8	1.6	<0.07	<0.05
Fluoranthene	21.0	10.0	<0.07	<0.05
Pyrene	10.0	4.6	<0.07	<0.05
Benz[*a*]anthracene	4.0	1.3	<0.07	<0.06
Chyrsene	3.8	2.3	<0.08	<0.06
Benzo[*e*]pyrene	1.4	1.0	<0.08	<0.06
Benzo[*a*]pyrene	<0.13	1.0	<0.08	<0.06
Perylene	1.6	0.85	<0.08	<0.07
Total AHs	120.0	61.0	0.09	0.20
Mean (± S.D.)	91.0 ± 42.0[d]		0.14 ± 0.08	
Polychlorinated biphenyls[e]				
dichlorobiphenyls	<0.02	<0.02	<0.01	0.03
trichlorobiphenyls	0.07	0.07	0.02	0.02
tetrachlorobiphenyls	0.65	<0.01	0.18	0.16
pentachlorobiphenyls	1.2	1.2	0.26	0.31
hexachlorobiphenyls	0.9	1.0	0.20	0.32
heptachlorobiphenyls	0.36	0.34	0.05	0.06
octachlorobiphenyls	0.07	0.08	0.03	0.02
nonachlorobiphenyls	0.01	<0.01	<0.001	<0.001
Total PCBs	3.2	2.7	0.72	0.92
Mean (± S.D.)	3.0 ± 0.35[d]		0.82 ± 0.14	
Composite weight (g)	0.39	0.48	1.11	1.25
No. of samples per composite	14	23	26	31
Mean Length (mm)	84.4 ± 4.2	82.1 ± 4.6	83.2 ± 5.6	84.5 ± 9.4

[a] An estimated 20% of dry weight was used for the calculations based on determinations of previously analyzed stomach contents
[b] The concentrations of analytes were calculated, using hexamethylbenzene as the internal standard (*i.e.*, data are not corrected for recovery)
[c] "<" Indicates that the analyte was not detected in concentrations above the stated value
[d] Significantly ($p \leq 0.05$) higher than value for Nisqually River fish
[e] The concentrations of analytes were calculated, using 4,4″-dibromoctafluorobiphenyl as the internal standard, and the data are corrected for recovery

spective estuaries approximately three to five weeks later. The mean length of salmon just prior to release from the Green River Hatchery was 78.1 ± 4.3 mm (n = 31) compared to 82.7 ± 5.2 mm (n = 85) for salmon captured approximately five weeks later in the Duwamish Waterway. The salmon at the Kalama Creek Hatchery were somewhat larger [mean length 96.6 ± 5.2 mm (n = 40)] than those at the Green River Hatchery; however, the salmon captured in the Nisqually River five weeks later were similar in size to those collected in the Duwamish Waterway [mean length 83.9 ± 7.8 mm (n = 98)]. (Not all salmon collected at the Kalama Creek Hatchery or in the Nisqually River were used for chemical analyses.) The smaller mean length of the juveniles in the Nisqually River compared to that of the hatchery fish was possibly due to the longer residence time of smaller chinook juveniles in estuaries compared to the residence times of larger juveniles (Simenstad *et al.* 1982).

Stomach Contents

The food organisms found in the stomachs of juvenile salmon from the Duwamish Waterway consisted primarily of cumaceans, amphipods, and small fish.

Of the 7 individual AHs detected in stomach contents from Duwamish Waterway fish, only the low molecular weight AHs (*e.g.*, naphthalene and 2-methylnaphthalene) were found in detectable concentrations in stomach contents from the Nisqually River fish (Table 1). The PCB concentrations in stomach contents of Duwamish Waterway and Nis-

Table 2. PCB concentrations (ng/g, dry wt[a]) in composites of liver from Chinook salmon

Chemicals	Green R. Hatchery	Duwamish Waterway I	Duwamish Waterway II	Duwamish Waterway III	Kalama Creek Hatchery I	Kalama Creek Hatchery II	Nisqually River I	Nisqually River II
Dichlorobiphenyls	<0.02	<0.03	<0.02	0.43	<0.03	<0.005	<0.01	<0.005
Trichlorobiphenyls	0.02	0.16	0.10	0.05	0.03	0.02	0.02	0.03
Tetrachlorobiphenyls	0.04	0.42	0.27	0.39	0.07	0.20	0.10	0.20
Pentachlorobiphenyls	0.13	1.2	0.74	0.76	0.19	0.28	0.35	0.35
Hexachlorobiphenyls	0.09	0.93	0.62	0.86	0.11	0.34	0.22	0.36
Heptachlorobiphenyls	0.02	0.30	0.22	0.20	0.02	0.09	0.07	0.07
Octachlorobiphenyls	0.004	0.06	0.040	0.007	0.006	0.02	0.2	0.02
Nonachlorobiphenyls	0.007	<0.01	<0.006	0.005	<0.01	<0.005	0.006	0.003
Total PCBs	0.29	3.1	2.0	2.8	0.42	0.95	0.78	1.0
Mean (±S.D.)		2.6 ± 0.56[b]			0.68 ± 0.37		0.90 ± 0.18	
Composite weight (g)	1.52	1.12	1.36	1.21	1.02	0.76	2.00	1.87
No. of livers per composite	31	30	30	25	10	10	35	31
Mean length (mm)	78.3 ± 4.3	81.7 ± 5.2	82.6 ± 5.5	82.5 ± 5.4	94.9 ± 6.5	95.9 ± 4.8	83.6 ± 8.0	84.5 ± 9.4
Date of collection	5/9/86	6/5/86	6/12/86	6/12/86	5/8/86	5/8/86	6/16/86	6/16/86

[a] Percent dry weight was estimated to be 18.6%

[b] Significantly ($p \leq 0.05$) higher than value for Nisqually River fish

qually River salmon were not as markedly different as the concentrations of AHs. Nevertheless, the concentration of individual PCBs in stomach contents from Duwamish Waterway salmon was significantly ($p \leq 0.05$) higher than that for Nisqually River fish.

Liver

Because of the aforementioned ability of salmon to rapidly metabolize aromatic compounds, chemical analyses of composites of liver tissue were conducted only for PCBs which are metabolically resistant. The same individual PCB compounds were found in livers of salmon from the hatcheries and the estuaries (Table 2). The concentrations of PCBs in livers of fish from the Kalama Creek Hatchery and the Nisqually River Estuary were not significantly different ($p \leq 0.05$). The concentration of PCBs in livers of Duwamish Waterway salmon, however, was significantly different ($p \leq 0.05$) (higher) than that of salmon from the Green River Hatchery, the Kalama Creek Hatchery and the Nisqually River estuary.

Bile

Analyses of bile indicated large differences in concentrations of metabolites of aromatic compounds in salmon from the Duwamish Waterway compared to fish from the Nisqually River estuary, regardless of whether the values were obtained at BaP (380/430 nm) or NPH (290/335 nm) fluorescence wavelengths (Table 3). The values obtained at BaP and NPH fluorescence wavelengths primarily represent metabolites of polynuclear AHs, characteristic of fossil fuel combustion products, and metabolites of diaromatic hydrocarbons present in the kerosene/gasoline fraction of petroleum, respectively (Krahn *et al.* 1987). The concentration of metabolites fluorescing at BaP wavelengths in the bile of salmon from the Duwamish Waterway was significantly different ($p \leq 0.05$) (higher) than those obtained from fish in the Nisqually River and the Kalama Creek Hatchery. Due to the small size of the 30 fish from the Green River Hatchery, an adequate sample of bile could not be obtained for analysis. The mean concentration of metabolites measured at NPH fluorescence wavelengths in bile of Duwamish Waterway fish was also significantly ($p \leq 0.05$) different (higher) from that in Nisqually River fish. Fish from the Kalama Creek Hatchery had a mean concentration of these metabolites which was significantly different ($p \leq 0.05$) (lower) than those in bile of salmon from both estuaries.

Discussion

The results of this investigation clearly demonstrated that juvenile chinook salmon captured in the Duwamish Waterway had been exposed to substantially higher concentrations of toxic chemicals than had juvenile chinook salmon from the Nisqually River estuary. The most pronounced differences were observed for concentrations of AHs in stomach contents and of metabolites of AHs in bile. This result is consistent with the high concentrations of AHs found in the Duwamish Waterway (Malins *et al.* 1984; Hamilton *et al.* 1984).

The differences between the PCB concentrations in samples of stomach contents and liver of salmon from the Duwamish Waterway and the Nisqually River estuary are not as great as would be predicted on the basis of the concentrations of PCBs in sediments from these two areas. One possible explanation is that a high proportion of the juvenile chinook salmon captured in the Nisqually River were from the Kalama Creek Hatchery and these fish may have been exposed to measurable levels of PCBs prior to release into the river. The fact that the mean PCB concentrations in liver samples of salmon from the Kalama Creek Hatchery (0.68 ± 0.37 μg/g, dry weight) and the Nisqually River estuary (0.90 ± 0.118 μg/g, dry weight) were so similar supports this explanation (Table 2). Uptake of PCBs by salmonid juveniles

Table 3. Total concentration (μg/g, wet wt) of metabolites of aromatic compounds in fish bile composites (n = 25) measured at the fluorescence wavelength pairs 380/430 nm and 290/335 nm [where benzo[*a*]pyrene (BaP) and naphthalene (NPH) metabolites fluoresce, respectively]

Sampling site	Collection date	Number of fish per composite	Mean length (mm)	Concentrations in composites BaP wavelengths (380/430 nm)	NPH wavelengths (290/335)
Duwamish Waterway	6/5	22	81.0 ± 6.4	0.99	490.0
		27	82.8 ± 5.4	1.9	550.0
		18	82.8 ± 5.2	0.89	410.0
			Mean ± S.D.	1.30 ± 0.56	480.0 ± 70.0
	6/12	17	83.2 ± 6.3	1.3	300.0
		19	83.9 ± 4.7	1.7	610.0
		17	83.2 + 5.4	0.95	420.0
			Mean ± S.D.	1.3 ± 0.38	440.0 ± 160.0
			Overall Mean ± S.D.	1.3 ± 0.430[a]	460.0 ± 110.0[a]
Nisqually River	6/16	21	82.5 ± 10.1	0.07	35.0
		21	84.5 ± 6.0	0.05	69.0
		20	85.8 ± 8.2	0.04	73.0
			Mean ± S.D.	0.05 ± 0.01	59.0 ± 21.0
Kalama Creek	5/8	10	95.9 ± 4.8	0.23	20.0
		10	94.9 ± 6.5	0.06	9.0
		10	97.2 ± 4.8	0.17	13.0
			Mean ± S.D.	0.15 ± 0.09	14.0 ± 5.6

[a] Significantly ($p \leq 0.05$) higher than Nisqually River and Kalama Creek Hatchery fish

fed commercial diets in hatcheries has been previously documented (Gruger *et al.* 1976). Concentrations of PCBs in a commercial feed commonly used at the Green River Hatchery have been reported to range from below limits of detection (10 ng/g) to 300 ng/g, dry weight (Mac *et al.* 1979). Therefore, it is likely that salmon in the Kalama Creek River Hatchery were fed a diet containing low levels of PCBs.

The period of exposure in the estuaries for the salmon sampled in this study very likely ranged from one to six weeks. Highest numbers of juvenile chinook salmon captured by beach seine were generally preceded one to three weeks by releases of large numbers of hatchery-reared chinook salmon. In the Green River/Duwamish Waterway system, Weitkamp and Campbell (1980) estimated that the transit time from hatchery to estuary for chinook salmon was 15–19 days. In the present study, the catch per unit effort (CPUE) in the Duwamish Waterway was 0.2 during the first week in May. During the second week of May, a major release of chinook salmon occurred at the Green River Hatchery, and by the end of May the CPUE in the Waterway had risen to 18.3. Similar values were obtained until the middle of June and by the end of June the CPUE values were again less than 0.2.

Reports in the literature suggest that juvenile salmon exposed to individual AHs or PCBs in the water column or their diet could take up substantial levels of these compounds within a few days to a few weeks, depending upon the level of exposure. In feeding studies, Roubal *et al.* (1977) demonstrated that when coho salmon were fed pelletized feed impregnated with ^{14}C-labeled anthracene or naphthalene, radioactivity was measured in the liver, muscle and brain within 24 hours. Roubal *et al.* (1977) also exposed juvenile coho salmon to the sea-water-soluble fraction of Prudhoe Bay crude oil in flow-through water. A variety of individual AHs were present in the water, including naphthalene (0.003 ± 0.002 μg/g). Salmon exposed for three weeks had significant concentrations of AHs in muscle tissue; for example, the concentration of naphthalene was 0.14 ± 0.07 μg/g (dry weight). Broyles and Noveck (1979) determined the rate of uptake of a PCB isomer, tetrachlorobiphenyl, by juvenile chinook salmon. The salmon were exposed to freshwater containing 5 ppb [^{14}C]hexachlorobiphenyl in a static system for 17 days. Significant uptake into total body tissues was measured after 4 days of exposure, and by 17 days the total body concentration was 3.6 μg/g. The rate of uptake of two hexachlorobiphenyl isomers and a tetrachlorobiphenyl isomer by juvenile coho salmon through the diet was investigated by Gruger *et al.* (1976). Fish were fed a pelletized diet containing approximately 3.3 μg/g of each compound three times a week for three months. After 24 days of feeding, the total body concentrations ranged from 0.49 ± 0.16 to 0.73 ± 0.18 μg/g (wet weight) compared to 0.050 to 0.090 μg/g (wet weight) for fish fed control diet.

We presently have little information concerning the sources of the PCBs and AHs bioaccumulated by salmon in the respective estuaries. Although the high concentrations of PCBs and AHs in samples of stomach contents from Duwamish Waterway fish suggest that diet represents an important route of exposure, uptake from water as another important route cannot be ruled out. At the present time we do not know the relative amount of the contaminants in the stomach contents which may be excreted. We did not assess the importance of the water column route, because so little information is available on episodic increases in the concentrations of AHs and PCBs in this waterway.

No evidence has been found that the bioaccumulation of toxic chemicals (*i.e.*, AHs and PCBs) by juvenile chinook salmon in the Duwamish Waterway is related to adverse biological effects. Investigations (Larry Peck, Washington State Dept. of Fisheries, Personal Communication) of chinook salmon returning to the Green River Hatchery have not demonstrated consistent declines in the numbers of re-

turning adults compared to salmon runs in other Puget Sound rivers. However, a number of pathological conditions (*i.e.* hepatic lesions and fin erosion) have been reported in three species of bottom-dwelling fish from this waterway (Wellings *et al.* 1976; McCain *et al.* 1982; Malins *et al.* 1984). For example the prevalences of hepatic neoplasms in English sole from the Duwamish Waterway are among the highest (17%) found in Puget Sound (Malins *et al.* 1984). These lesions are considered to be of the chronic variety requiring months to years of exposure to produce identifiable pathological changes (Rhodes *et al.* 1987), whereas possible effects in juvenile salmon would have to be produced after exposures of one to several weeks.

Acknowledgments: We thank D. D. Weber, M. S. Myers, O. P. Olson, L. L. Johnson, and L. D. Rhodes for their help in sample collections; and W. D. MacLeod Jr., D. G. Burrows, K. L. Tilbury, S. M. Pierce, R. W. Pearce, C. A. Wigren, J. D. Drury and R. G. Bogart for chemical analyses of stomach content and liver samples. We also express our appreciation to S. Perry for typing; to J. Peacock for figures; and to J. B. Pritchard, U. Varanasi, E. Casillas and A. McElroy for manuscript review.

References

Brown DW, Chan S-L, Friedman AJ, Grams KL, Burrows DG, MacLeod WD Jr. (1985) Bioaccumulation study: Organic compounds in sediment and biota from Grays Harbor and reference area. Final Report to the U.S. Army Corps of Engineers, Seattle, WA, 83 pp

Broyles RH, Noveck MI (1979) Uptake and distribution of 2,4,5,2′,4′,5′-hexachlorobiphenyl in fry of lake trout and chinook salmon and its effects on vitality. Toxicol Applied Pharmacol 50:299–308

Dexter RN, Goldstein LS, Chapman PM, Quinlan EA (1985) Temporal trends in selected environmental parameters monitored in Puget Sound. NOAA Tech Memo NOS OMA 19, 166 pp

Gruger EH, Jr, Hornby T, Karrick NL (1976) Sublethal effects of structurally related tetrachloro-, pentachloro-, and hexachlorobiphenyl on juvenile coho salmon. Environ Sci Technol 10:1033–1037

Hamilton SE, Bates TS, Cline JD (1984) Sources and transport of hydrocarbons in the Green-Duwamish River, Washington. Environ Sci Technol 18:72–79

Healy MC (1982) Juvenile Pacific salmon in estuaries: The life support system. In: Kennedy VS (ed) Estuarine comparisons. Academic Press, NY, p 315

Krahn MM, Myers MS, Burrows DG, Malins DC (1984) Determination of metabolites of xenobiotics in bile of fish from polluted waterways. Xenobiotica 14:633–646

Krahn MM, Rhodes LD, Myers MS, Moore LK, MacLeod, WD Jr, Malins DC (1986a) Associations between metabolites of aromatic compounds in bile and the occurrence of hepatic lesions in English sole (*Parophrys vetulus*) from Puget Sound, Washington. Arch Environ Contam Toxicol 15:61–67

Krahn MM, Moore LK, MacLeod WD Jr (1986b) Standard Analytical Procedures of the NOAA National Analytical Facility, 1986: Metabolites of aromatic compounds in fish bile. NOAA Tech memo NMFS F/NWC-102,25 pp

Krahn MM, Burrows DG, MacLeod WD Jr, Malins DC (1987) Determination of individual metabolites of aromatic compounds in hydrolyzed bile of English sole (*Parophrys vetulus*) from polluted sites in Puget Sound, Washington. Arch Environ Contam Toxicol 16:511–522

Lee CO, Strand JA, Nevissi AE (1988) Modes of toluene uptake in adult chinook salmon. In: Proceedings of the First Annual Meeting on Puget Sound Research. Puget Sound Water Quality Authority, Seattle, WA

Mac MJ, Nicholson LW, McCauley CA (1979) PCBs and DDE in commercial fish feeds. Prog Fish-Culturist 41:210–211

MacLeod WD Jr, Brown DW, Friedman AJ, Burrows DG, Maynes O., Pearce R, Wigren CA, Bogar RG (1985) Standard analytical procedures of the NOAA National Analytical Facility, 1985–6: Extractable Toxic Organic Compounds, Second Edition NOAA Tech Memo NMFS F/NWC-92, 121 p

Malins DC, McCain BB, Brown DW, Sparks AK, Hodgins HO, Chan S-L (1982) Chemical contaminants and abnormalities in fish and invertebrates from Puget Sound. NOAA Tech Memo OMPA-19, 168 pp

Malins DC, McCain BB, Brown DW, Chan S-L, Myers MS, Landahl JT, Prohaska PG, Friedman AJ, Rhodes LD, Burrows DG, Gronlund WD, Hodgins HO (1984) Chemical pollutants in sediments and diseases in bottom-dwelling fish in Puget Sound, Washington. Environ Sci Technol 18:705–713

Malins DC, Chan S-L, MacLeod WD Jr, McCain BB, Clark RC Jr, Brown DW, Myers MS, Krahn MM (1986) Results of the 1984–85 National Benthic Surveillance Project: West Coast Oceans '86 Conference Record; The Institute of Electrical and Electronics Engineers, Piscataway, NJ, pp 566–671

McCain BB, Myers MS, Varanasi U, Brown DW, Rhodes LD, Gronlund WD, Elliott DG, Palsson WA, Hodgins HO, and Malins DC (1982) Pathology of two species of flatfish from urban estuaries in Puget Sound. DOC (EPA) Interagency Energy/Environment, Research and Development Program Report, EPA-600/7-82-001, 100 pp

McCain BB, Brown DW, Krahn MM, Myers MS, Clark RC Jr, Chan S-L, Malins DC (1988) Marine pollution problems, North American West Coast. Aquatic Toxicology 11:143–162

Meyer JH, Pearce TA, Patlan SB (1981) Distribution and food habits of juvenile salmonids in the Duwamish Estuary, Washington, 1980. Report to the U.S. Army Corps of Engineers, Seattle, WA, 42 pp

Rhodes LD, Myers MS, Gronlund WD, McCain BB (1987) Epizootic characteristics of hepatic and renal lesions in English sole (*Parophrys vetulus*) from Puget Sound. J Fish Biol 31:395–407

Roubal WT, Collier TK, Malins DC (1977) Accumulation and metabolism of carbon-14 labeled benzene, naphthalene, and anthracene by young coho salmon (*Oncorhynchus kisutch*). Arch Environ Contam Toxicol 5:513–529

Simenstad CA, Fresh KL, Salo EO (1982) The role of Puget Sound and Washington coastal estuaries in the life history of Pacific salmon: An unappreciated function. In: Kennedy VS (ed) Estuarine comparisons. Academic Press, NY, p 343

Thom RM (1987) The biological significance of Pacific Northwest Estuaries. Northwest Environ J 3:21–42

Varanasi U, Gmur DG (1981) Hydrocarbons and metabolites in English sole (*Parophrys vetulus*) exposed simultaneously to [^{3}H]benzo[*a*]pyrene and [^{14}C]naphthalene in oil-contaminated sediment. Aquat Toxicol 1:49–67

Weitkamp DE, Campbell RF (1980) Port of Seattle Terminal 107 fisheries study. Report to the Port of Seattle prepared by Parametrix, Inc, Belleuve, WA, 53 pp

Wellings SR, Alpers CE, McCain BB, and Miller BS (1976) Fin erosion disease of starry flounder (*Platichthys stellatus*) and English sole (*Parophrys vetulus*) in the estuary of the Duwamish River, Seattle, Washington. J Fish Res Board Can 33:2577–2586

Williams RC, Laramie RW, Ames JJ (1975) A catalog of Washington streams and salmon utilization. Volume I, Puget Sound Region. Wash Dept Fish, Olympia, WA

Zar JH (1974) Biostatistical analysis. Prentice-Hall, Englewood Cliffs, NJ, 620 pp

Manuscript received May 10, 1988 and in revised form September 21, 1988.