Supporting Information

12 pgs, 4 figures, 4 tables

Predicting polycyclic aromatic hydrocarbon concentrations in resident aquatic organisms using passive samplers and partial least squares calibration

Norman D. Forsberg, Brian W. Smith, Greg J. Sower, Kim A. Anderson*

Environmental and Molecular Toxicology Department, Oregon State University, ALS 1007, Corvallis, OR, 97331, USA

*Corresponding Author:

E-mail: kim.anderson@orst.edu
Phone: 541-737-8501
Fax: 541-737-0497

MATERIAL AND METHOD DETAILS

Crayfish sample preparation and chemical analysis. The analytical method used to analyze PAHs in crayfish tissues was adapted from previous work (1). Briefly, 200 – 400 mg of tissue were transferred to a 15 mL BD Falcon tube, thawed, and spiked with 20 μL of a PAH surrogate standard mixture containing acenapthene-D10, pyrene-D10, and indeno[1,2,3-*cd*]pyrene-D12. Samples were subsequently spiked with 500 μL of H_2O, capped, and hand shaken for 1 min. A 10 mL aliquot of ethyl acetate, acetone, and iso-octane (2:2:1, v/v/v) was added to each tube and the resulting solution was shaken for 5 min. QuEChERS salts (650 mg) were added to the tube, mixed for five minutes by hand shaking, and tubes were then centrifuged at 3800 g for 5 min. Extracts (9mL) were transferred to 15 mL volumetric conical glass tubes, solvent exchanged to ~ 400 μL of *n*-hexane, and cleaned using *n*-hexane conditioned solid-phase extraction cartridges containing primary-secondary amines (Agilent Technologies, Santa Clara, CA). Analytes were

vacuum eluted with 7 mL of *n*-hexane at a flow rate of 2-3 drops/s, concentrated to a final volume of 100 µL, and spiked with 10 µL of recovery internal standard mixture composed of naphthalene-D8, acenaphthylene-D8, phenanthrene-D10, fluoranthene-D10, chrysene-D12, benzo[*a*]pyrene-D12, and benzo[*ghi*]perylene-D12 .

Crayfish samples were chemically analyzed using an Agilent 5975B GC-MS (Santa Clara, CA) equipped with electron impact ionization (70 eV) source and a DB-5MS capillary column (30 m length, 0.25 µm film thickness, 0.25 mm I.D., Agilent J&W). The GC injection port received a 1 µL injection and was operated at 300 °C in pulsed splitless mode. PAHs were chromatographically resolved using the following temperature program: initial oven temperature was 70 °C, 1 min hold, ramp to 300 °C at 10 °C/min, 4 min hold, ramp to 310 °C at 10 °C/min, 7 min hold for a total run time of 36 min. Seven point internal standard calibration curves ranged from 1 to 1000 ng/mL and were plotted as relative response ratios.

Quality control. PAHs were not detected in blank field or trip SPMDs, and RSDs between duplicate site averages were ≤ 15%. Analytical batches consisted of > 30% quality control samples including method blanks, instrument blanks, and continuing calibration verification standards. Crayfish method blanks contained trace level (< 5.8 ng/mL) background responses for NAP, FLO, and PHE, while all other target analytes were below reporting limits. PAH responses were at least three times greater than sample background, below detection in instrument blanks, and continuing calibration verification samples were within +/- 20% of expected values. Surrogate standard recoveries in crayfish samples were 62 ± 15, 75 ± 9, and 76 ± 16% of expected values for ACE-D10, PYR-D10, and IPY-D12 respectively, while average surrogate

recoveries in SPMD extracts ranged from 35% to 95% for NAP and BPL (2) . All results were surrogate recovery corrected.

RESULTS AND DISCUSSION DETAILS

PAH concentrations in the freely dissolved phase. The spatial variation of dissolved PAHs across the study area was investigated from 2002 to 2006 and coincided with crayfish collection reported in the present study (2). The fall 2003 subset of SPMD data was paired with crayfish collection and is presented here for direct comparison to crayfish. Similar to crayfish, median freely dissolved concentrations of ΣPAHs (350 ng/L) and ΣC-PAHs (132 ng/L) were significantly higher at sites within the Superfund compared to up-river sites (ΣPAH and ΣC-PAH = 100 and 18 ng/L, respectively). SPMDs from the McCormick and Baxter Superfund site at river mile 7eC were the only samples with significantly greater levels of individual PAHs compared to up-river samples, while median ΣC-PAH concentrations were higher down-stream at river mile 3.5 (260 ng/L compared to 19 ng/L at up-river sites). Though a number of significant site-specific differences were observed by Sower and Anderson (2), differences between sites in this study were not as stark, likely due in part to decreased statistical power resulting from fewer replicate measurements. Across all sites, crayfish ΣPAH concentrations were on average 280 (range from 21 to 930) times greater than freely dissolved ΣPAH concentrations.
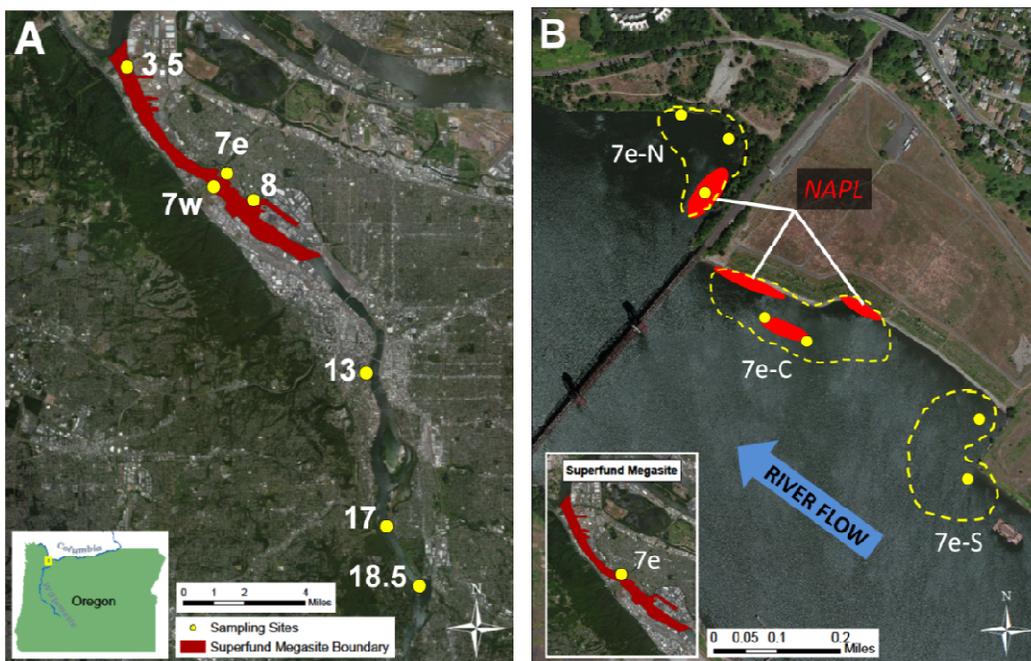
SI Figure 1. Approximate location (yellow dots) of SPMD and crayfish sampling sites in A) the lower 18.5 miles of the Willamette River and at B) river mile 7e during the fall 2003 sampling period. SPMDs and crayfish within yellow dashed circles in B) were paired. The portion of river highlighted red in A) represents the Superfund Mega-site boundary, while numbers indicate river mile. NAPL = non-aqueous phase liquid.
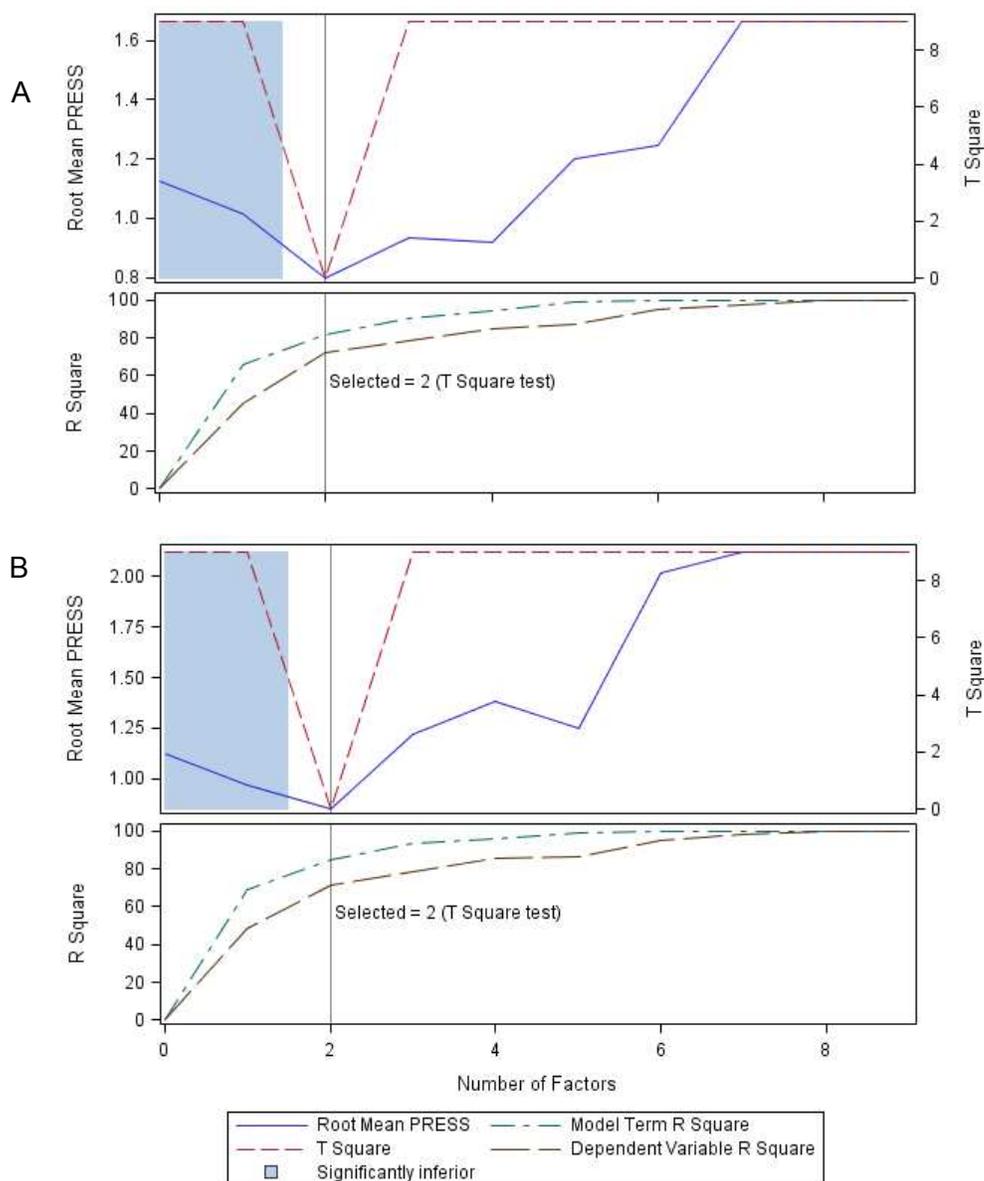
SI Figure 2. Results of leave-one-out-at-a-time cross-validation on site averages calculated by substituting A) 'zero' or B) method detection limits for measurements that were ≤ MDLs. Prior to PLS modeling, predictor variables were 4th root transformed then averaged by site, while response variables were averaged by site then 4th root transformed. Cross-validation revealed that a two-*factor* PLS model was optimal regardless of how values ≤ MDL were treated.
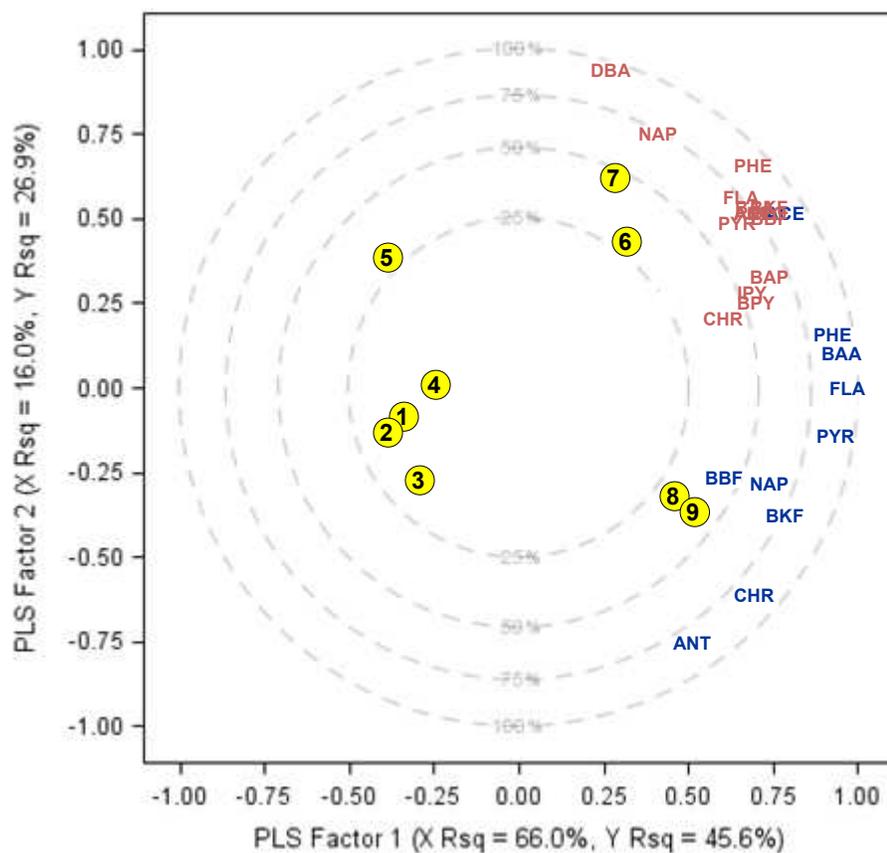
SI Figure 3. Partial least squares (PLS) correlation loading plot for the first two PLS *factors* modeled on 'zero' substituted data. Shown are sample scores labeled by site number, response variable loadings labeled with red letters, and predictor variable loadings labeled with blue letters. PLS factors 1 and 2 explain 72.5% of the variation in the response variables and 82% of the variation in the predictor variables. The amount of individual variable variation explained corresponds to loading vector magnitudes which can be estimated using the dashed-line circles. Sampling sites are indicated by river mile: 1 – RM 18.5, 2 – RM 17, 3 – RM 13, 4 – RM 8, 5 – RM 7eS, 6 – RM 7eC, 7 – RM 7eN, 8 – RM 7w, 9 – RM 3.5.
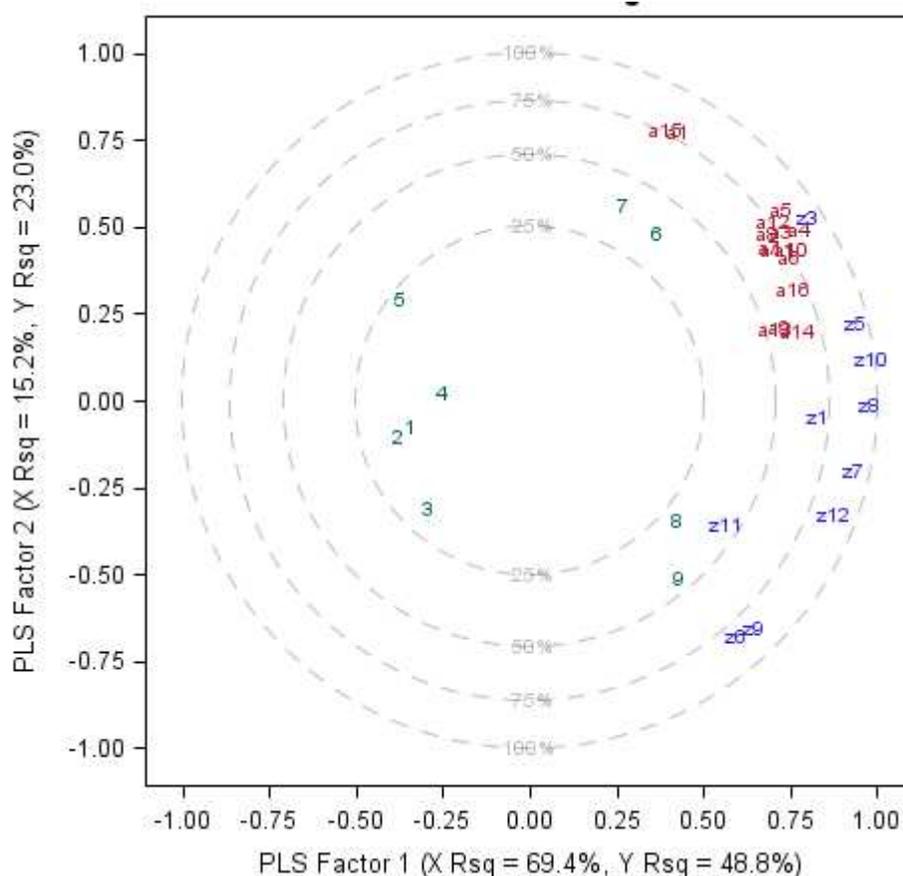
SI Figure 4. Partial least squares (PLS) correlation loading plot for the first two PLS factors modeled on 'detection limit' substituted data. Shown are sample scores labeled by site number, response variable loadings 'a' labeled with red letters (crayfish), and predictor variable loadings 'z' labeled with blue letters (freely dissolved). PLS *factors* 1 and 2 explain 71.8% of the variation in the response variables and 84.4% of the variation in the predictor variables. Sampling site are indicated by river mile: 1 – RM 18.5, 2 – RM 17, 3 – RM 13, 4 – RM 8, 5 – RM 7eS, 6 – RM 7eC, 7 – RM 7eN, 8 – RM 7w, 9 – RM 3.5. Numbers associated with loadings identify each PAH; NAP(1), ACE(3), FLO(4), PHE(5), ANT(6), PYR(7), FLA(8), CHR(9), BAA(10), BBF(11), BKF(12), IPY(13), BPY(14), DBA(15), BAP(16).

SI Table 1. Signal crayfish (*P. leniusculus*) sex and morphological characteristics grouped by site for the fall 2003 collection period.

Sampling site (river mile)	Sex		Pooled morphology (mean ± SEM)		
	Male	Female	Body length (cm)	Carapace length (cm)	Wet weight (g)
Up-river					
18.5	3	2	8.1 ± 0.9	3.9 ± 0.5	21.0 ± 10
17	5	3	9.2 ± 0.4	4.7 ± 0.2	28.4 ± 4.7
13	3	0	9.4 ± 0.4	4.5 ± 0.4	28.1 ±4.5
Superfund					
8	0	1	7.5	3.7	13.3
7e-S	3	4	9.5 ± 0.5	4.6 ± 0.2	20.6 ± 2.3
7e-C	1	4	8.5 ± 0.5	4.1 ± 0.2	13.9 ± 2.0
7e-N	11	14	9.3 ± 0.3	4.6 ± 0.2	22.9 ± 2.5
7w	2	1	9.8 ± 1.2	4.8 ± 0.6	27.4 ± 8.9
3	0	3	10.5 ± 0.3	5.2 ± 0.1	30.5 ± 2.2
All sites	28	32	9.2 ± 0.2	4.5 ± 0.1	23.1 ± 1.6

SI Table 2. Spatial distribution of PAHs (ng/g w.w.) in resident crayfish collected from within and outside of the Portland Harbor Superfund Mega-site, OR.

	River mile 18.5				River mile 17				River mile 13			
				n > MDL				n > MDL				n > MDL
compound	mean	SE	median	(n = 5)	mean	SE	median	(n = 8)	mean	SE	median	(n = 3)
NAP	23.6	9.0	14.0	5	3.6	1.1	4.9	5	7.8	4.9	6.5	2
ACY	<0.05			0	<0.05			0	<0.05			0
ACE	<0.25			0	<0.25			0	5.6	2.9	7.1	2
FLO	1.8	1.2	<0.17	2	<0.17			0	<0.17			0
PHE	3.6	1.5	3.4	4	0.6	0.4	<0.19	2	0.7	0.7	<0.19	1
ANT	0.4	0.3	<0.05	2	<0.05			0	<0.05			0
PYR	2.2	1.2	1.9	3	1.0	0.3	1.3	5	1.3	0.7	1.8	2
FLA	1.4	1.4	<0.25	1	0.4	0.3	<0.25	2	0.7	0.7	<0.25	1
CHR	11.8	4.1	7.2	5	1.9	1.1	<0.25	3	2.9	0.5	3.0	3
BAA	<0.15			0	<0.15			0	<0.15			0
BBF	<0.2			0	<0.2			0	<0.2			0
BKF	<0.24			0	<0.24			0	<0.24			0
BAP	<0.21			0	0.1	0.1	<0.21	1	0.3	0.3	<0.21	1
IPY	<0.19			0	<0.19			0	0.5	0.5	<0.19	1
BPY	<0.13			0	<0.13			0	0.6	0.6	<0.13	1
DBA	<1.25			0	<1.25			0	<1.25			0

	River mile 8				River mile 7eS				River mile 7eC			
				n > MDL				n > MDL				n > MDL
compound	mean	SE	median	(n = 1)	mean	SE	median	(n = 7)	mean	SE	median	(n = 5)
NAP	29.0		29.0	1	19.0	6.2	12.9	7	65.9	8.2	64.9	5
ACY	<0.05			0	<0.05			0	<0.05			0
ACE	<0.25			0	17.7	11.2	5.9	6	57.8	13.7	58.9	5
FLO	<0.17			0	6.8	2.8	5.3	6	47.3	10.9	46.8	5
PHE	5.0		5.0	1	21.8	10.2	6.9	7	60.7	10.3	61.8	5
ANT	3.4		3.4	1	8.3	3.6	4.4	7	18.9	3.5	21.1	5
PYR	<0.25			0	21.0	8.2	9.6	7	24.3	2.7	27.4	5
FLA	<0.25			0	25.1	10.5	10.9	7	32.2	4.2	35.8	5
CHR	<0.25			0	9.6	3.9	7.4	6	18.9	4.4	19.7	5
BAA	<0.15			0	8.9	2.5	7.1	7	12.3	1.4	13.8	5
BBF	<0.2			0	7.3	2.0	5.4	7	5.9	1.2	4.6	5
BKF	<0.24			0	2.4	0.7	1.8	6	2.8	0.4	2.5	5
BAP	<0.21			0	2.4	0.9	1.5	5	2.2	0.5	1.7	5
IPY	<0.19			0	2.6	1.0	1.7	5	1.1	0.7	<0.19	2
BPY	<0.13			0	1.8	0.9	<0.13	3	1.6	1.2	<0.13	2
DBA	<1.25			0	0.3	0.3	<1.25	1	0.7	0.7	<1.25	1

	River mile 7eN				River mile 7w				River mile 3e			
				n > MDL				n > MDL				n > MDL
compound	mean	SE	median	(n = 25)	mean	SE	median	(n = 3)	mean	SE	median	(n = 3)
NAP	91.7	28.0	49.5	25	27.4	16.3	12.6	3	6.7	1.2	6.1	3
ACY	4.9	2.9	<0.05	4	<0.05			0	5.4	5.4	<0.05	1
ACE	119	34.4	50.2	22	18.1	9.8	12.2	3	20.8	13.8	8.4	3
FLO	98.2	30.0	39.4	24	9.5	4.1	6.8	3	27.6	20.5	10.3	3
PHE	179	96.8	57.9	24	16.1	7.8	10.3	3	47.0	21.5	52.1	3
ANT	42.0	14.2	17.6	25	5.0	2.7	2.5	3	30.8	27.8	3.7	3
PYR	77.2	42.7	22.8	25	13.4	3.1	12.5	3	27.1	7.3	24.8	3
FLA	115	66.3	33.1	25	12.6	2.9	11.1	3	33.2	11.9	32.2	3
CHR	34.9	21.1	10.0	23	22.7	11.8	15.7	3	21.4	8.0	16.5	3
BAA	35.9	22.0	7.8	23	8.1	2.1	7.6	3	12.8	7.1	6.6	3
BBF	29.1	20.0	4.6	17	6.2	2.3	7.3	3	7.8	3.7	5.7	3
BKF	8.6	5.7	1.2	13	1.8	1.0	2.1	2	2.3	1.5	1.8	2
BAP	7.9	5.1	1.4	14	3.9	1.2	4.7	3	3.1	1.4	2.5	3
IPY	6.4	3.8	<0.19	12	3.5	1.1	3.7	3	3.4	1.2	2.8	3
BPY	3.9	2.8	<0.13	8	3.4	1.7	4.6	2	2.3	1.1	3.3	2
DBA	1.2	1.2	<1.25	1	<1.25			0	<1.25			0

SI Table 3. Spatial distribution of freely dissolved PAHs (ng/L) collected by SPMDs from within and outside of the Portland Harbor Superfund mega-site[a]

	River mile 18.5			n > MDL	River mile 17			n > MDL	River mile 13			n > MDL
compound	mean	SE	median	(n = 2)	mean	SE	median	(n = 2)	mean	SE	median	(n = 1)
NAP	5.1	5.1	5.1	1	4.3	4.3	4.3	1	<1.4			0
ACY	11.7	11.7	11.7	1	13.5	13.5	13.5	1	27.3		27.3	1
ACE	<0.14			0	<0.14			0	<0.14			0
FLO	NQ[b]				NQ				NQ			
PHE	20.9	4.3	20.9	2	18.2	6.4	18.2	2	28.8		28.8	1
ANT	3.4	3.4	3.4	1	3.9	3.9	3.9	1	8.3		8.3	1
PYR	16.9	0.9	16.9	2	13.4	0.8	13.4	2	20.4		20.4	1
FLA	17.4	1.3	17.4	2	14.8	1.8	14.8	2	22.6		22.6	1
CHR	<0.94			0	<0.94			0	<0.94			0
BAA	<0.36			0	<0.36			0	<0.36			0
BBF	<0.83			0	<0.83			0	<0.83			0
BKF	1.3	1.3	1.3	1	1.0	1.0	1.0	1	2.8		2.8	1
BAP	NQ				NQ				NQ			
IPY	<15.2			0	<15.2			0	<15.2			0
BPY	<14.9			0	<14.9			0	<14.9			0
DBA	<7.0			0	<7.0			0	<7.0			0

	River mile 8			n > MDL	River mile 7eS			n > MDL	River mile 7eC			n > MDL
compound	mean	SE	median	(n = 2)	mean	SE	median	(n = 1)	mean	SE	median	(n = 10)
NAP	2.2	2.2	2.2	1	<1.4			0	27.2	11.7	<1.4	4
ACY	7.1	7.1	7.1	1	<2.1			0	16.6	7.9	2.4	5
ACE	<0.14			0	<0.14			0	107	37	62.2	9
FLO	NQ				NQ				NQ			
PHE	24.5	5.5	24.5	2	23.9		23.9	1	133	24	116	9
ANT	3.8	3.8	3.8	1	<0.39			0	10.8	6.4	2.3	6
PYR	33.7	10.0	33.7	2	24.3		24.3	1	107	29	109	8
FLA	30.5	3.0	30.5	2	26.7		26.7	1	160	44	144	8
CHR	<0.94			0	<0.94			0	<0.94			0
BAA	3.2	3.2	3.2	1	<0.36			0	31.6	10.0	28.2	9
BBF	<0.83			0	<0.83			0	1.6	1.6	<0.83	1
BKF	1.3	1.3	1.3	1	<0.15			0	6.7	2.4	6.0	7
BAP	NQ				NQ				NQ			
IPY	<15.2			0	<15.2			0	<15.2			0
BPY	<14.9			0	<14.9			0	<14.9			0
DBA	<7.0			0	<7.0			0	<7.0			0

	River mile 7eN			n > MDL	River mile 7w			n > MDL	River mile 3e			n > MDL
compound	mean	SE	median	(n = 4)	mean	SE	median	(n = 2)	mean	SE	median	(n = 3)
NAP	21.9	21.9	<1.4	1	20.9	11	20.9	2	8.6	5.2	7.8	2
ACY	12.2	7.3	8.1	3	7.1	7.1	7.1	1	5.7	5.7	<2.1	1
ACE	30.5	8.7	33.0	4	19.7	19.7	19.7	1	4.0	2.2	4.4	2
FLO	NQ				NQ				NQ			
PHE	93.4	8.4	88.1	4	109	37	109	2	60.2	3.9	57.4	3
ANT	7.8	7.8	<0.39	1	21.8	3.0	21.8	2	10.6	3.2	7.4	3
PYR	73.6	2.3	74.2	4	151	85	151	2	204	65	268	3
FLA	94.2	6.6	92.0	4	153	68	153	2	142	27	167	3
CHR	<0.94			0	17.9	17.9	17.9	1	32.4	16.3	45.9	2
BAA	21.2	1.0	21.0	4	21.5	13.3	21.5	2	27.6	9.1	35.5	3
BBF	<0.83			0	<0.83			0	4.7	4.7	<0.83	1
BKF	4.7	1.5	6.0	4	5.8	1.5	5.8	2	7.2	1.4	8.6	3
BAP	NQ				NQ				NQ			
IPY	<15.2			0	<15.2			0	<15.2			0
BPY	<14.9			0	<14.9			0	<14.9			0
DBA	<7.0			0	<7.0			0	<7.0			0

[a]Data were adapted from Sower and Anderson (2008), [b]'NQ' = not quantified due to chromatographic interferences.

SI Table 4. Summary of significant site-specific differences in median crayfish (ng/g w.w.) and freely dissolved water concentrations (ng/L) for the fall 2003 Portland Harbor, OR study

PAH	Superfund vs up-river[a]		Individual within Superfund sites vs control sites[b]			
	Crayfish	H_2O_{PSD}	Crayfish	River miles with P<0.05	H_2O_{PSD}	River miles with P<0.05
NAP	<0.001*[c]	0.6	<0.001*	7eN, 7eC	0.8	
ACY	0.2	0.4	0.3		0.8	
ACE	<0.001*	0.01*	<0.001*	7eN, 7eC	0.01*	7eC
FLO	<0.001*	NQ[d]	<0.001*	7eN, 7eC	NQ	
PHE	<0.001*	0.007*	<0.001*	7eN, 7eC	0.02*	7eC
ANT	<0.001*	0.9	<0.001*	7eN, 7eC	0.4	
PYR	<0.001*	0.005*	<0.001*	3.5, 7eN, 7eC, 7eS	0.06	
BPY	0.01*	BDL[e]	0.1		BDL	
ΣNC-PAH[f]	<0.001*	0.011*	<0.001*	7eN, 7eC	0.037*	7eC
FLA	<0.001*	0.005*	<0.001*	3.5, 7eN, 7eC, 7eS	0.08	
CHR	0.002*	0.4	0.01	None[g]	0.02*	None
BAA	<0.001*	0.003*	<0.001*	7eN, 7eC	0.02*	None
BBF	<0.001*	0.5	0.01*	None	0.7	
BKF	<0.001*	0.07	0.003*	7eN, 7eC	0.2	
BAP	<0.001	NQ	0.003*	7w	NQ	
IPY	<0.001*	BDL	0.008	None	BDL	
DBA	0.3	BDL	0.6		BDL	
ΣC-PAH[h]	<0.001*	0.002*	<0.001*	3.5, 7eN, 7eC	0.02*	3.5
ΣPAH[i]	<0.001*	0.007*	<0.001*	7eN, 7eC	0.03*	None

[a]Mann-Whitney rank sum tests between sampling sites, [b]Kruskal-Wallis one way ANOVA on ranks using Dunn's method for multiple comparisons between individual sites within the Superfund mega-site and up-river 'control' sites (river miles 13, 17, and 18.5), [c]Asterisk (*) indicates significance at α = 0.05, [d]Not quantified due to chromatographic interference, [e]Measurement was below method reporting limits, [f]ΣNC-PAH = the summed concentration of non-carcinogenic PAHs excluding FLO, [g]Indicates that no statistically significant differences were found during multiple-comparisons to controls, [h]ΣC-PAH = the summed concentration of carcinogenic PAHs excluding BAP, [i]ΣPAH = the summed concentration of PAHs exluding FLO and BAP.

REFERENCES

(1) Forsberg, N. D.; Wilson, G. R.; Anderson, K. A. Determination of parent and substituted polycyclic aromatic hydrocarbons in high-fat salmon using a modified QuEChERS extraction, dispersive SPE and GC–MS. *J. Agric. Food Chem.* **2011**, *59* (15), 8108-8116.

(2) Sower, G. J.; Anderson, K. A. Spatial and temporal variation of freely dissolved polycyclic aromatic hydrocarbons in an urban river undergoing Superfund remediation. *Environ. Sci. Technol.* **2008**, *42* (24), 9065-9071.