

Supplementary Information

Title: Passive samplers accurately predict PAH levels in resident crayfish

Authors: L. Blair Paulik¹, Brian W. Smith¹, Alan J. Bergmann¹, Greg J. Sower^{1,2}, Norman D. Forsberg¹, Justin G. Teeguarden³, Kim A. Anderson^{1*}

¹Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR 97331

²Ramboll ENVIRON US Corporation, 2111 East Highland Avenue, Suite 402, Phoenix, AZ 85016

³Pacific Northwest National Laboratory, 902 Battelle Boulevard, Richland, WA 99354

*Corresponding author:

kim.anderson@oregonstate.edu,

1007 Agriculture and Life Sciences Building,

Corvallis, Oregon, 97331

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Supplementary Information

Crayfish morphology

Crayfish collected in 2013 had average carapace lengths of 3.5 ± 0.57 cm, average body lengths of 10 ± 1.4 cm, and average body weights of 31 ± 13 g. Half of the crayfish were female and half were male. On average, viscera contributed $13 \pm 4\%$ to the total wet weight of each crayfish, while tails contributed $11 \pm 2\%$.

Crayfish tissue extraction

All tissues were extracted with a 2:2:1 solution of ethyl acetate, acetone and iso-octane, and dried using QuEChERS AOAC salts. Viscera samples were cleaned using flow-through solid-phase extraction (SPE) cartridges containing primary-secondary amines, as described in Forsberg 2014 (Forsberg et al., 2014), while tail samples were cleaned with AOAC Fatty Samples dispersive SPE tubes, as described in Forsberg 2011 (Forsberg et al., 2011).

Passive water sampler preparation

LDPE strips were cut from pre-sized polyethylene tubing that was approximately 2.7 cm wide. Each polyethylene strip was approximately 100 cm long and had a volume of 5.1 cm^3 . LDPE was dried under filtered vacuum in stainless steel kegs, from AEB Kegs in Delebio, Italy. TurboVap[®] evaporators were from Biotage, in Charlotte, NC.

2013 passive water sampler deployment dates

Samplers were deployed at river miles 18.5, 12E, 3.5W and 1NW from September 30th to October 17th, 2013, and at RM 11E from October 17th to November 7th, 2013.

2012 passive water sampler deployment information

In 2012, three sampler cages with 5 strips of LDPE each were individually deployed at RM 7E (McCormick & Baxter). They were in the water from Nov 30, 2012 to Jan 17, 2013. In this sampling campaign, p,p'-DDE-d4 was used as a PRC instead of pyrene-d10.

Chemical information

Single PAH standards were purchased from Sigma Aldrich, in St. Louis, MO, Chiron, in Trondheim, Norway, or Fluka (part of Sigma-Aldrich). PAH mixes were purchased from Accustandard, in New Haven, CT. Labeled compounds used as performance reference compounds (PRCs), laboratory surrogates, or instrument internal standards were obtained from either CDN Isotopes, in Pointe-Claire, Quebec, Canada, Cambridge Isotope Laboratories, in Tewksbury, MA, or Fisher Scientific in Pittsburgh, PA. All solvents were Optima-grade (from Fisher Scientific, Pittsburgh, PA) or equivalent, and all laboratory glassware and other tools were baked at 450°C for 12 hours and/or solvent-rinsed before use. Water used to clean LDPE was filtered through a D7389 purifier purchased from Barnstead International, in Dubuque, IA.

Chemical analysis

An Agilent Select PAH column was used to chromatographically separate PAHs. Each PAH was calibrated with a curve of at least five points, with correlations ≥ 0.99 . The temperature profile in the GC-MS/MS analytical method was as follows: 60°C for 1 minute, increasing 40°C per minute to reach 180°C , then increasing 3°C per minute to reach 230°C , then increasing 1.5°C per minute to reach 235°C , then increasing 15°C per minute to reach 280°C , staying at 280°C for 10 minutes, then increasing 6°C per minute to reach 298°C , and finally ramping up 16°C per minute to reach 350°C with a hold time of 4 minutes. The dimensions of the Agilent Select PAH column were: 30 m, 0.25 mm, $0.15 \mu\text{m}$. Continuing calibration standards were run nominally every 10 samples, and/or at the

end of the sample set. If a closing standard did not meet the criteria, samples were re-run after the standard was verified.

Differences in processing of crayfish collected in 2003

In the 2003 data set, the entire mass of each crayfish viscera was homogenized, extracted and analyzed. Additionally, the viscera tissue from each organism was homogenized, extracted, and analyzed separately. No compositing was done, and no tail tissues were retained for analysis. 2003 crayfish viscera were reanalyzed for 62 PAHs using an Agilent 6890N gas chromatograph coupled with an Agilent 5975B mass spectrometer.

An important site during the 2003 sampling campaign was RM 7E. This is the site of the former McCormick & Baxter creosote company. This site has been under investigation by the Oregon Department of Environmental Quality since 1990, and was added to the U.S. EPA's National Priority List in 1994, before the rest of the Portland Harbor Superfund. (EPA, 1996).

2012 PAH concentrations in water

Average \sum PAH measured in water (C_{free} calculated from water-deployed LDPE) at RM 7E (McCormick & Baxter) in 2012 was 56 ± 55 ng/mL. These data were used in the model to predict PAH levels in crayfish viscera at this site in 2012.

Water concentration calculations

Freely dissolved water concentrations (C_{free}) were determined through an empirical uptake model, as described below. Sampling rates were derived by measuring loss of performance reference compounds (PRCs) during deployment. PRCs allow for accurate assessment of *in situ* uptake rates for a wide range of analytes in variable environmental conditions (Bartkow et al., 2006; Huckins et al., 2002; Söderström and Bergqvist, 2004). The uptake calculations do not make any assumptions about the analyte being at equilibrium, so this model was used for water concentration calculations for all PAHs. PRCs shared similar physical and chemical properties with the target PAHs in this study and spanned a range of log K_{ow} values from 4.18 to 5.78 (Huckins et al., 2002). Water concentrations (C_w) of PAHs were determined using equations S1-S6, all presented in Huckins *et al* (Huckins et al., 2006):

Sampler-water partitioning coefficients (K_{sw}) were calculated for both PRCs and target PAHs using this quadratic equation:

$$\text{Eq S1. } \log K_{sw} = a_0 + (2.321 * \log K_{ow}) - (0.1618 * \log K_{ow}^2)$$

The a_0 term was determined by Huckins *et al* to be equal to -2.61 for PAHs and other similarly nonpolar compounds. To determine PRC sampling rates, a depuration rate (k_e) was needed. The following equation was used to calculate k_e , assuming first-order kinetics:

$$\text{Eq S2. } k_e = \frac{-\ln\left(\frac{PRC_t}{PRC_i}\right)}{t}$$

PRC_t is the amount of PRC remaining after a deployment period (t), and PRC_i is the initial amount spiked into the LDPE. Each PRC's sampling rate (R_{sPRC}) was calculated using:

$$\text{Eq S3. } R_{sPRC} = k_e * K_{sw} * V_s$$

V_s is the volume of the LDPE sampler. Sampling rates for target analytes (R_s) were determined using:

$$\text{Eq S4. } R_s = R_{sPRC} * \frac{\alpha_{analyte}}{\alpha_{PRC}}$$

The α terms are compound-specific adjustments made to account for differing chemistry between the PRC and the target analyte. This model is a best-fit polynomial, which gives α values for target analytes and PRCs, based on $\log K_{ow}$. These α terms were calculated using:

$$\text{Eq S5. } \log \alpha = (0.013 * \log K_{ow}^3) - (0.3173 * \log K_{ow}^2) + (2.244 * \log K_{ow})$$

Compound uptake during deployment was not assumed to be in any particular phase (kinetic, linear, or equilibrium), and no assumptions are necessary. C_w for target analytes were calculated using:

$$\text{Eq S6. } C_w = \frac{N_{analyte}}{V_s K_{sw} (1 - \exp(-\frac{R_s t}{V_s K_{sw}}))}$$

$N_{analyte}$ is the concentration of analyte measured in LDPE, and t is the length of the deployment in days.

Quantitative risk assessment calculations

Risk assessment was performed using equations S7-S9. The benzo[a]pyrene equivalent concentration (BaP_{eq}) was used in these calculations. This was determined using:

$$\text{Eq S7. } \sum BaP_{eq} = \sum (C_i * RPF_i)$$

C_i is the concentration of a given PAH in a crayfish sample, and RPF_i is the EPA's Relative Potency Factor of that PAH (EPA, 2010). Average daily dose (ADD) was calculated using:

$$\text{Eq S8. } ADD = \frac{\sum BaP_{eq} * CF * IR * EF * ED}{BW * AT}$$

ADD is the average daily dose, in mg/kg-day, C is the concentration in crayfish in ng/g, CF is a conversion factor, EF is the exposure frequency in days/year, ED is the exposure duration in years, BW is body weight in kg, and AT is the averaging time. The AT includes the lifetime in years multiplied by 365 days/year. In this work, $\sum BaP_{eq}$ for a given sample was used as the concentration in crayfish tissue. The IR was set at both 3.3 and 18 g/day, which are the average and 95th percentiles for crayfish consumption that were used in ATSDR's Portland Harbor Public Health Assessment to evaluate risks to local crayfish consumers (ATSDR, 2006). To mimic what was done in the ATSDR Public Health Assessment for Portland Harbor, average adult body weight was set at 70 kg, average lifetime (used in the AT) was set at 70 years, EF was set at 365 days/year and ED was set at 30 years (ATSDR, 2006).

$$\text{Eq S9. } ELCR = ADD * SF$$

ELCR is an estimate of excess lifetime cancer risk and SF is an oral slope factor. In this study, a SF of 7.3 mg/kg-d was used, based on the EPA's 2010 guidance (EPA, 2010).

There were 11 PAHs that were above the detection limits and had nonzero relative potency factors (RPFs) in crayfish (see **Table S4** for RPFs). Thus, these were the 11 PAHs used in the carcinogenic risk assessment. These PAHs were benzo[c]fluorene, benzo[b]fluoranthene, fluoranthene, benzo[a]pyrene, benzo[a]anthracene, chrysene, benzo[j]fluoranthene, indeno[1,2,3-c,d]pyrene, benzo[k]fluoranthene, anthanthrene, and benzo[g,h,i]perylene.

Assumptions in this risk assessment that may bias risk estimates high include assuming 1) exposure occurs every day for 30 years 2) 100% of crayfish eaten are from the stretch of river

discussed in this study, 3) ingestion rates are accurate, and do not change over time, 4) PAHs are 100% bioavailable via ingestion, 5) and the cancer slope factor from high-dose animal data are predictive of low-dose effects in the general population. However, these parameters were chosen to mimic assessment done in ATSDR's 2006 PHA [23]. Due to the dearth of data regarding the toxicity of the majority of PAHs, especially of alkylated PAHs, there is inherent uncertainty in PAH risk assessment. This risk assessment was conducted for adults, with no adjustments being made for the different exposures of children.

Heterogeneity in crayfish PAH levels – 2003 vs. 2013

The 2003 RM averages span two orders of magnitude, while the 2013 RM averages only differ by a factor of four (**Figure S6**). The comparatively small variability in PAH levels in crayfish seen in 2013 is partly explained by 2003 crayfish data representing averages of contaminants measured in individual organisms ($n=3$ at all sites except RM 7E, where $n=7$), while data for 2013 crayfish represent averages of 3 composites of tissue from 4 crayfish.

Heterogeneity is to be expected among PAH levels in crayfish. Indeed, reduced variability is one of the main selling points for the use of PSDs to estimate organismal concentrations in lieu of collecting and analyzing the organisms themselves (Booij et al., 2006; Forsberg et al., 2014). While their home range is relatively small, crayfish are still mobile organisms. Additionally, many of the known point sources in the Superfund sites in this study include sediment contamination, which is notoriously heterogeneous. If crayfish are feeding on detritus near contaminated sediment, they are likely exposed to a wide range of contamination levels (Levengood and Schaeffer, 2011). Crayfish are opportunistic omnivores, so their range of diets could further exaggerate the variation in their internal contaminant levels. Forsberg *et al* discussed this phenomenon, reasoning that the substantial variation measured in crayfish collected near the McCormick & Baxter site was likely related to the crayfish being exposed to heterogeneous sediment contamination resulting from the creosoting operations that took place on the nearby shore (Forsberg et al., 2014). This is consistent with the data in the present study, in which crayfish collected at McCormick and Baxter in 2003 have by far the greatest variability, with one crayfish having Σ PAH levels 2 orders of magnitude greater than the rest, making the average for this site three times larger than the median (**Figure S6**). Fernandez and Gschwend made a similar point, suggesting that the atypically heterogeneous PAH levels in clams collected at one site were due to coal tar contamination in the sediment (Fernandez and Gschwend, 2015). Levengood *et al* observed large variability in PAH levels measured in crayfish at a site with elevated sediment contamination, and suggested that this was partly due to the spatial heterogeneity of sediment contamination (Levengood and Schaeffer, 2011).

PAH profiling – 2,6-dimethylnaphthalene discussion

Specifically, 2,6-DMN contributed 49 to 85% to Σ PAH in crayfish viscera and 55-95% to Σ PAH in crayfish tails, while it only contributed 1.9 to 5.7% to Σ PAH in water. In a previous study comparing co-deployed SPMDs and Asiatic clams, *Corbicula fluminea*, 2,6-DMN was one of only three PAHs measured in the clams, while 24 PAHs were measured in SPMDs (Moring and Rose, 1997). Thus, it is possible that shellfish preferentially accumulate 2,6-DMN relative to other PAHs. Additionally, Eisler reported that the BCF for dimethylnaphthalenes in crustaceans was two orders of magnitude higher than the BCF for naphthalene in clams, and one order of magnitude higher than the BCF for naphthalene in crustaceans (Eisler, 1987). This could begin to explain why 2,6-DMN was an order of magnitude higher in crayfish tissues than the rest of the PAHs in the present work, but more investigation is needed. For instance, Eisler's BCF does not explain why this heightened bioconcentration would occur for 2,6-DMN, and not with any of the six other dimethylnaphthalenes present in the analytical method.

The dominance of 2,6-DMN in 2013 crayfish tissue is at odds with the positive correlation between BAF and $\log K_{ow}$ that has been observed in mussels (Booij et al., 2006). This disparity could

be due in part to differential PAH uptake and/or metabolism in mussels and crayfish. This idea is supported by the significant negative correlation between BAF and $\log K_{ow}$ that has been observed in crayfish (Levengood and Schaeffer, 2011). PAH accumulation differ between bivalves and crustaceans, predominantly due to crustaceans' more mobile lifestyles and increased capacity for PAH metabolism (Neff, 1979). Studies have suggested that crayfish metabolize xenobiotics through oxidative metabolism with P450s (James and Boyle, 1998; Jewell and Winston, 1989; Jewell et al., 1997). Perhaps the increased oxidative metabolism of xenobiotics in crayfish relative to bivalves reduces crayfish' load of higher molecular weight PAHs, but enriches crayfish tissues in 2,6-DMN. Additionally, a negative correlation has been observed between PAH uptake rate (from water into *P. leniusculus*) and $\log K_{ow}$. This begins to explain the heightened uptake of PAHs with lower $\log K_{ows}$ in crayfish, in both viscera and tail tissue (Gossiaux and Landrum, 2005), However, it is still unclear why this effect was so dramatic with 2,6-DMN specifically,

It is worth noting that this phenomenon was much less dramatic in 2003 crayfish tissue, in which 2,6-DMN was not measured in all samples, and its contribution to \sum PAH ranged from 0 to 30%.

Linear range of the predictive model

The predictive model was built using individual PAH concentrations from the 2013 sampling campaign. Measured individual PAH concentrations used to build the model ranged from 0.01 to 5.7 ng/ml in water and from 0.11 to 18 ng/g in crayfish viscera.

Validation of predictive model with training set and test set

A training set (80% of the paired PAH data measured in crayfish viscera and in water in the 2013 sampling campaign) was used to build a model to validate the main predictive model. The equation of the line of best fit in the model built by this training set was: $[\text{PAH}]_{\text{crayfish}} = 0.89 \times [\text{PAH}]_{\text{water}} + 0.57$. This model is very similar to the main model, which was built using all of the data, and which is presented as Equation 1 in the main text. Additionally, the test set (the remaining 20% of the data) was used to compare PAH concentrations predicted by the training model, to what was actually measured in crayfish tissues (**Figure S5**). Root mean squared errors (RMSE) were computed to assess the models, by taking the square root of the average of the squared residuals. RMSE values were computed to compare how well the the full model and the training model predicted concentrations for the test set, relative to what was measured in crayfish. These root mean squared errors were very similar for the full model and the training model (0.222 and 0.226, respectively).

Risk assessment - EPA 2010 vs. EPA 16 priority pollutants

There are 23 PAHs with EPA 2010 RPFs included in the present analytical method, while only 11 of the EPA Priority Pollutants have RPFs. When all 23 of these PAHs are included, average $\sum \text{BaP}_{\text{eq}}$ doubles for crayfish collected at RM 7E and in the PHSM in 2003. For 2013 crayfish collected within the PHSM, average $\sum \text{BaP}_{\text{eq}}$ increases by 45% when the additional PAHs are included. This trend did not hold true for crayfish viscera collected upriver of the Superfunds in both 2003 and 2013, or for crayfish tails. $\sum \text{BaP}_{\text{eq}}$ was not affected by the additional PAHs in crayfish viscera collected upstream of the Superfunds (no change in 2003; 3.7% increase in 2013). Additionally, including the longer PAH list did not change $\sum \text{BaP}_{\text{eq}}$ for crayfish tails. This is explained by the fact that the only 2 PAHs contributing to $\sum \text{BaP}_{\text{eq}}$ (fluoranthene and benzo[ghi]perylene) in tails are both on the 16 PP list.

Figure S1. Map of sampling sites. This map depicts the Portland Harbor study area in 2003, 2012, and 2013, and all sampling sites referenced in the present work.

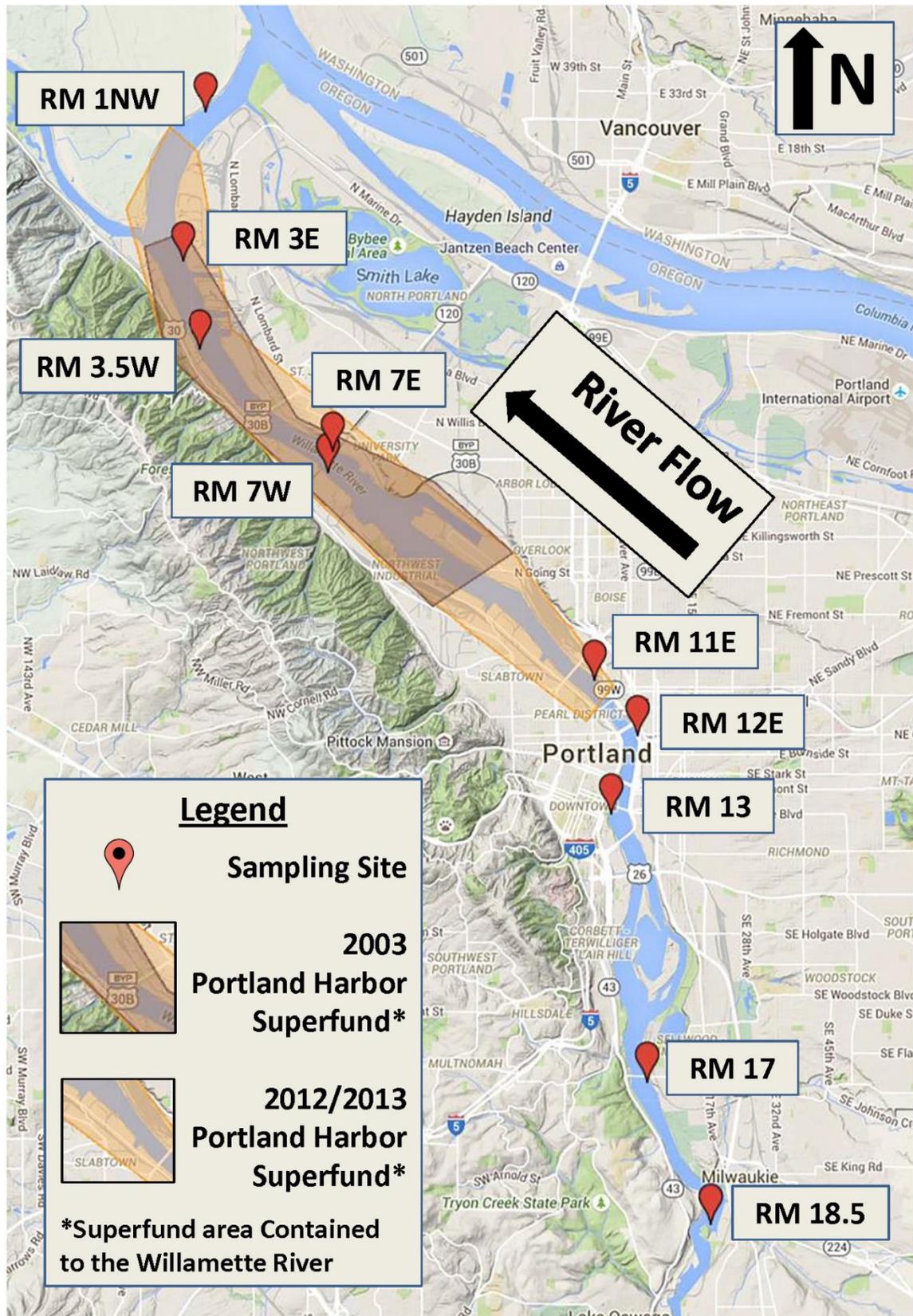


Table S1. GPS coordinates for sampling sites. Coordinates are given for locations of crayfish collection and LDPE deployment presented in this study from **A. 2013** and **B. 2003**. Deployment of LDPE at RM 7E in 2012 was at approximately the same location as crayfish collection at that site in 2003.

A. 2013

Approximate River Mile (used as sampling site identifier)	Latitude	Longitude
RM 18.5	45° 26' 14.88"N	122° 38' 49.05"W
RM 12E	45° 31' 34.87"N	122° 39' 57.88"W
RM 11E	45° 32' 11.50"N	122° 40' 37.74"W
RM 3.5W	45° 35' 51.59"N	122° 46' 51.70"W
RM 1NW	45° 38' 30.24"N	122° 46' 46.35"W

B. 2003

Approximate River Mile (used as sampling site identifier)	Latitude	Longitude
RM 17	45° 27' 47.67"N	122° 39' 49.72"W
RM 13	45° 30' 43.21"N	122° 40' 21.70"W
RM 7W	45° 34' 28.96"N	122° 44' 48.94"W
RM 7E	45° 34' 43.05"N	122° 44' 45.03"W
RM 3E	45° 36' 50.38"N	122° 47' 7.55"W

Figure S2. Conceptual diagram of in-lab sample processing. Diagram shows processing steps for **A. crayfish** and **B. LDPE** passive sampling devices.

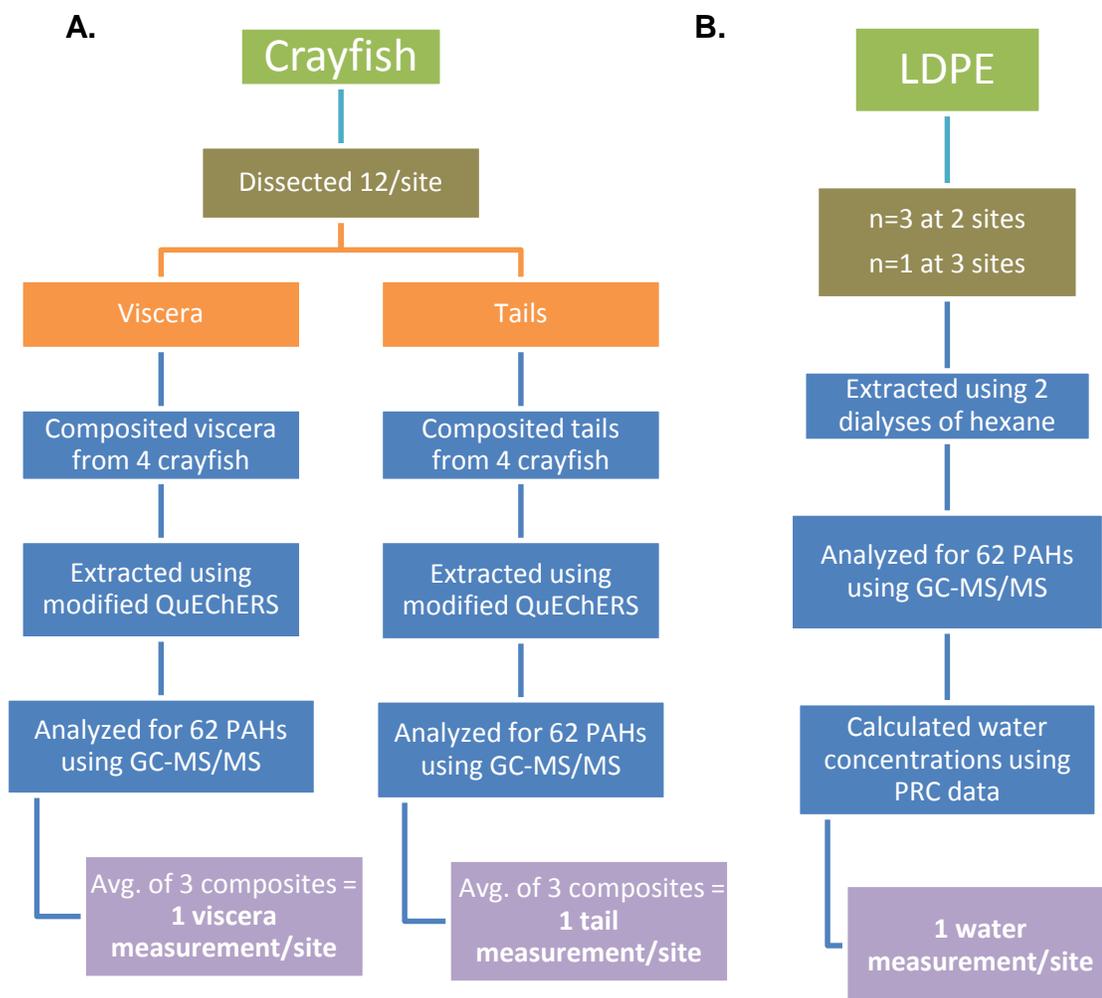


Table S2. List of QC and target PAHs in GC-MS/MS method. Performance reference compounds (PRCs), internal standard (IS), surrogates, and target polycyclic aromatic hydrocarbons (PAH) are given for the GC-MS Triple Quad method used for PAH analysis in this study, with instrument limits of detection (LOD) and limits of quantitation (LOQ).

PAH	Target, IS, PRC, or Surrogate?	CAS #	LOD (ng/mL)	LOQ (ng/mL)
Fluorene-d10	PRC	81103-79-9	0.33	1.0
Pyrene-d10	PRC	1718-52-1	0.42	2.1
Benzo[b]fluoranthene-d12	PRC	205-99-2	1.7	5.0
Perylene-d12	IS	1520-96-3	1.7	
Naphthalene-d8	Surrogate	1146-65-2	0.33	1.0
Acenaphthylene-d8	Surrogate	93951-97-4	0.33	1.0
Phenanthrene-d10	Surrogate	1517-22-2	1.7	5.0
Fluoranthene-d10	Surrogate	93951-69-0	1.7	5.0
Chrysene-d12	Surrogate	1719-03-5	1.7	5.0
Benzo[a]pyrene-d12	Surrogate	63466-71-7	1.7	5.0
Benzo[ghi]perylene-d12	Surrogate	93951-66-7	1.7	5.0
Naphthalene	Target	91-20-3	1.0	5.2
2-Methylnaphthalene	Target	91-57-6	0.70	3.5
1-Methylnaphthalene	Target	90-12-0	0.28	1.4
2-Ethylnaphthalene	Target	939-27-5	0.97	4.8
2,6-Dimethylnaphthalene	Target	581-42-0	0.89	4.4
1,6-Dimethylnaphthalene	Target	575-43-9	0.81	4.1
1,4-Dimethylnaphthalene	Target	571-58-4	1.2	6.2
1,5-Dimethylnaphthalene	Target	571-61-9	1.2	5.9
1,2-Dimethylnaphthalene	Target	573-98-8	0.94	4.7
1,8-Dimethylnaphthalene	Target	569-41-5	0.83	4.2
2,6-Diethylnaphthalene	Target	59919-41-4	0.81	4.1
Acenaphthylene	Target	208-96-8	2.3	12
Acenaphthene	Target	83-32-9	1.1	5.4
Fluorene	Target	86-73-7	0.79	4.0
Dibenzothiophene	Target	132-65-0	0.24	1.2
Phenanthrene	Target	85-01-8	0.46	2.3
Anthracene	Target	120-12-7	1.1	5.2
2-Methylphenanthrene	Target	2531-84-2	0.39	1.9
2-Methylantracene	Target	613-12-7	0.47	2.4
1-Methylphenanthrene	Target	832-69-9	1.1	5.3
9-Methylantracene	Target	779-02-2	0.87	4.4
3,6-Dimethylphenanthrene	Target	1576-67-6	0.42	2.1
2,3-Dimethylantracene	Target	613-06-9	0.34	1.7
Fluoranthene	Target	206-44-0	0.54	2.7

9,10-Dimethylanthracene	Target	781-43-1	0.85	4.2
Pyrene	Target	129-00-0	0.42	2.1
Retene	Target	483-65-8	0.84	4.2
Benzo[a]fluorene	Target	238-84-6	1.7	5.0
Benzo[b]fluorene	Target	243-17-4	1.7	5.0
Benzo[c]fluorene	Target	205-12-9	0.30	1.5
1-Methylpyrene	Target	2381-21-7	0.38	1.9
Benz[a]anthracene	Target	56-55-3	0.75	3.8
Cyclopenta[c,d]pyrene	Target	27208-37-3	0.53	2.7
Triphenylene	Target	217-59-4	0.41	2.0
Chrysene	Target	218-01-9	0.50	2.5
6-Methylchrysene	Target	1705-85-7	0.89	4.4
5-Methylchrysene	Target	3697-24-3	1.7	5.0
Benzo[b]fluoranthene	Target	205-99-2	0.37	1.9
7,12-Dimethylbenz[a]anthracene	Target	57-97-6	0.94	4.7
Benzo[k]fluoranthene	Target	207-08-9	0.53	2.6
Benzo[j]fluoranthene	Target	205-82-3	0.56	2.8
Benz[j]&[e]aceanthrylene	Target	202-33-5 and 199-54-2	1.7	5.0
Benzo[e]pyrene	Target	192-97-2	0.71	3.5
Benzo[a]pyrene	Target	50-32-8	1.2	5.9
Indeno[1,2,3-c,d]pyrene	Target	193-39-5	0.26	1.3
Dibenzo[a,h]pyrene	Target	53-70-3	1.0	5.1
Picene	Target	213-46-7	0.74	3.7
Benzo[ghi]perylene	Target	191-24-2	0.34	1.7
Anthanthrene	Target	191-26-4	0.33	1.7
Naphtho[1,2-b]fluoranthene	Target	5385-22-8	1.7	5.0
Naphtho[2,3-j]fluoranthene	Target	205-83-4	1.7	5.0
Dibenzo[a,e]fluoranthene	Target	5385-75-1	0.47	2.4
Dibenzo[a,l]pyrene	Target	191-30-0	0.48	2.4
Naphtho[2,3-k]fluoranthene	Target	207-18-1	1.7	5.0
Naphtho[2,3-e]pyrene	Target	193-09-9	1.7	5.0
Dibenzo[a,e]pyrene	Target	192-65-4	6.4	32
Coronene	Target	191-07-1	0.7	3.5
Dibenzo[e,l]pyrene	Target	192-51-8	1.7	5.0
Naphtho[2,3-a]pyrene	Target	196-42-9	1.7	5.0
Benzo[b]perylene	Target	197-70-6	1.7	5.0
Dibenzo[a,i]pyrene	Target	189-55-9	1.4	7.1
Dibenz[a,h]anthracene	Target	189-64-0	0.52	2.6

Table S3. List of PAH detection limits in samples. LODs for PAHs measured in water, crayfish tails, and crayfish viscera collected in 2003 and 2013. All tissue concentrations are in ng/g wet weight.

PAH	Water (ng/mL)	Crayfish Viscera 2013 (ng/g)	Crayfish Tails 2013 (ng/g)	Crayfish Viscera 2003 (ng/g)
Naphthalene	0.03	0.12	1.0	0.15
2-Methylnaphthalene	0.01	0.08	0.7	0.15
1-Methylnaphthalene	0.002	0.03	0.28	0.15
2-Ethylnaphthalene	0.002	0.11	0.97	0.15
2,6-Dimethylnaphthalene	0.002	0.10	0.89	0.15
1,6-Dimethylnaphthalene	0.002	0.09	0.81	0.15
1,4-Dimethylnaphthalene	0.003	0.14	1.2	0.15
1,5 Dimethylnaphthalene	0.003	0.13	1.2	0.15
1,2-Dimethylnaphthalene	0.003	0.10	0.94	0.15
1,8-Dimethylnaphthalene	0.003	0.09	0.83	0.15
2,6-Diethylnaphthalene	0.002	0.09	0.81	0.15
Acenaphthylene	0.01	0.26	2.3	0.15
Acenaphthene	0.007	0.12	1.1	0.15
Fluorene	0.003	0.09	0.79	0.15
Dibenzothiophene	0.001	0.03	0.24	0.15
Phenanthrene	0.001	0.05	0.46	0.78
Anthracene	0.002	0.12	1.1	0.78
2-Methylphenanthrene	0.001	0.04	0.39	0.78
2-Methylanthracene	0.001	0.05	0.47	0.78
1-Methylphenanthrene	0.001	0.12	1.1	0.78
9-Methylanthracene	0.001	0.10	0.87	0.78
3,6-Dimethylphenanthrene	0.0004	0.05	0.42	0.78
Fluoranthene	0.001	0.06	0.54	0.78
2,3-Dimethylanthracene	0.0003	0.04	0.34	0.78
9,10-Dimethylanthracene	0.001	0.09	0.85	0.78
Pyrene	0.001	0.05	0.42	0.78
Retene	0.001	0.09	0.84	0.78
Benzo[a]fluorene	0.002	0.19	1.7	0.78
Benzo[b]fluorene	0.002	0.19	1.7	0.78
Benzo[c]fluorene	0.0004	0.03	0.30	0.78
1-Methylpyrene	0.0004	0.04	0.38	0.78
Benz[a]anthracene	0.001	0.08	0.75	0.78
Cyclopenta[c,d]pyrene	0.001	0.06	0.53	0.78

Triphenylene	0.0004	0.05	0.41	0.78
Chrysene	0.0005	0.06	0.50	0.78
6-Methylchrysene	0.001	0.10	0.89	0.78
5-Methylchrysene	0.002	0.19	1.7	0.78
Benzo[b]fluoranthene	0.0004	0.04	0.37	0.78
7,12-Dimethylbenz[a]anthracene	0.001	0.10	0.94	0.78
Benzo[k]fluoranthene	0.001	0.06	0.53	0.78
Benzo[j]fluoranthene	0.001	0.06	0.56	0.78
Benz[j]+[e]aceanthrylene	NA	0.19	1.7	0.78
Benzo[e]pyrene	0.001	0.08	0.71	0.78
Benzo[a]pyrene	0.001	0.13	1.2	0.78
Indeno[1,2,3-c,d] pyrene	0.0003	0.03	0.26	0.78
Dibenz[a,h]anthracene	0.001	0.11	1.0	0.78
Picene	0.001	0.08	0.74	0.78
Benzo[ghi]perylene	0.0004	0.04	0.34	0.78
Anthanthrene	0.001	0.04	0.33	0.78
Naptho[1,2-b]fluoranthene	0.003	0.19	1.7	0.78
Naptho[2,3-j]fluoranthene	0.003	0.19	1.7	0.78
Dibenzo[a,e]fluoroanthene	0.001	0.05	0.47	0.78
Dibenzo[a,l]pyrene	0.001	0.05	0.48	0.78
Naptho[2,3-k]fluoranthene	0.003	0.19	1.7	0.78
Naptho[2,3-e]pyrene	0.003	0.19	1.7	0.78
Dibenzo[a,e]pyrene	0.01	0.72	6.4	0.78
Coronene	0.002	0.08	0.7	0.78
Dibenzo[e,l]pyrene	0.002	0.19	1.7	0.78
Naptho[2,3-a]pyrene	0.003	0.19	1.7	0.78
Benzo[b]perylene	0.003	0.19	1.7	0.78
Dibenzo[a,i]pyrene	0.003	0.16	1.4	0.78
Dibenzo[a,h]pyrene	0.001	0.06	0.52	0.78

Table S4. List of EPA Relative Potency Factors (RPFs), or “PAHs with final RPFs based on tumor bioassay data,” from the U.S. EPA’s 2010 Development of a Relative Potency Factor (RPF) Approach for Polycyclic Aromatic Hydrocarbon (PAH) Mixtures (EPA, 2010).

PAH	Relative Potency Factor
Anthanthrene	0.4
Anthracene	0
Benz[a]anthracene	0.2
Benz[b,c]aceanthrylene	0.05
Benzo[b]fluoranthene	0.8
Benzo[c]fluorene	20
Benzo[e]aceanthrylene	0.8
Benzo[g,h,i]perylene	0.009
Benz[j]aceanthrylene	60
Benzo[j]fluoranthene	0.3
Benzo[k]fluoranthene	0.03
Benz[l]aceanthrylene	5
Chrysene	0.1
Cyclopenta[c,d]pyrene	0.4
Cyclopenta[d,e,f]chrysene	0.3
Dibenzo[a,e]fluoranthene	0.9
Dibenzo[a,e]pyrene	0.4
Dibenz[a,h]anthracene	10
Dibenzo[a,h]pyrene	0.9
Dibenzo[a,i]pyrene	0.6
Dibenzo[a,l]pyrene	30
Fluoranthene	0.08
Indeno[1,2,3-c,d]pyrene	0.07
Naphtho[2,3-e]pyrene	0.3
Phenanthrene	0
Pyrene	0
Benzo[a]pyrene	1

Table S5. Average Σ PAH measured in crayfish viscera, tails, and water (using water-deployed LDPE passive samplers), in the Willamette River in Portland, Oregon in 2013 **(A.)** and 2003 **(B.)**. All tissue concentrations are in ng/g wet weight.

A.

2013					
Sample Type	Average Σ PAH \pm Standard Deviation (Sample Size)				
	RM 18.5	RM 12E	RM 11E	RM 3.5W	RM 1NW
Crayfish Viscera (ng/g)	250 \pm 51 (n=3)	290 \pm 44 (n=3)	110 \pm 38 (n=3)	210 \pm 100 (n=3)	390 \pm 90 (n=3)
Crayfish Tails (ng/g)	37 \pm 3.0 (n=3)	38 \pm 14 (n=3)	20 \pm 7.3 (n=3)	26 \pm 3.6 (n=3)	18 \pm 2.0 (n=3)
Water (ng/mL)	8.4 \pm 1.3 (n=3)	4.2 \pm 0.76* (n=1)	3.8 \pm 0.68* (n=1)	20 \pm 4.0 (n=3)	15 \pm 2.7* (n=1)

B.

2003					
Sample Type	Average Σ PAH \pm Standard Deviation (Sample Size)				
	RM 17	RM 13	RM 7W	RM 7E	RM 3E
Crayfish Viscera (ng/g)	31 \pm 14 (n=3)	80 \pm 35 (n=3)	270 \pm 306 (n=3)	2700 \pm 5000 (n=7)	360 \pm 350 (n=3)

* Where n=1, standard deviations were calculated using the average relative standard deviation calculated at sites where sampling was replicated (n=3) in that sampling campaign.

Figure S3. Profiles of PAHs measured in A. crayfish viscera and B. water, organized by $\log K_{ow}$, from the 2013 sampling campaign.

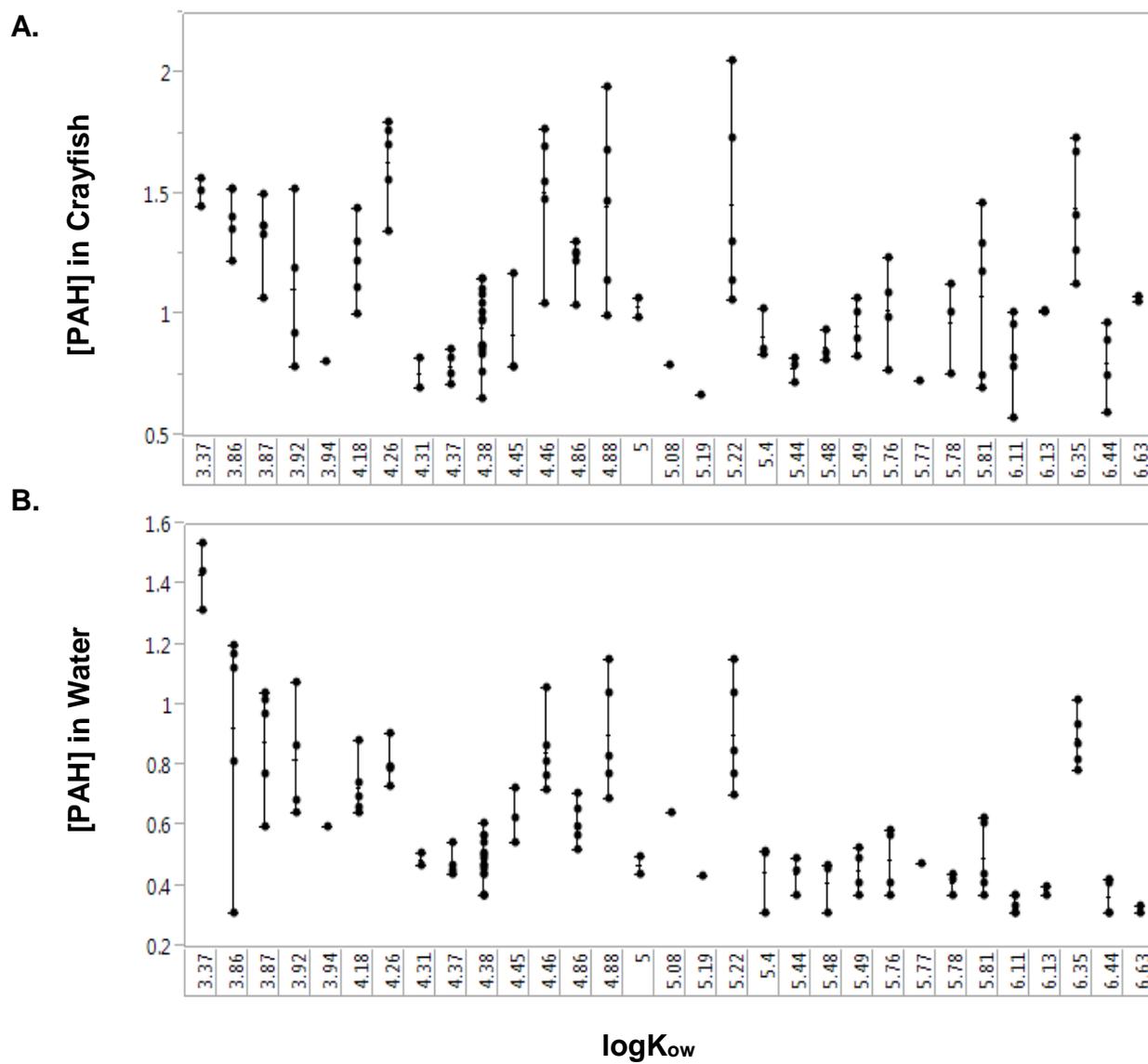


Figure S4. PAHs measured in 2013 crayfish viscera vs. water. Bubble size indicates $\log K_{ow}$ (larger bubbles indicate larger $\log K_{ow}$), while the bubble color indicates the sampling site associated with that data point.

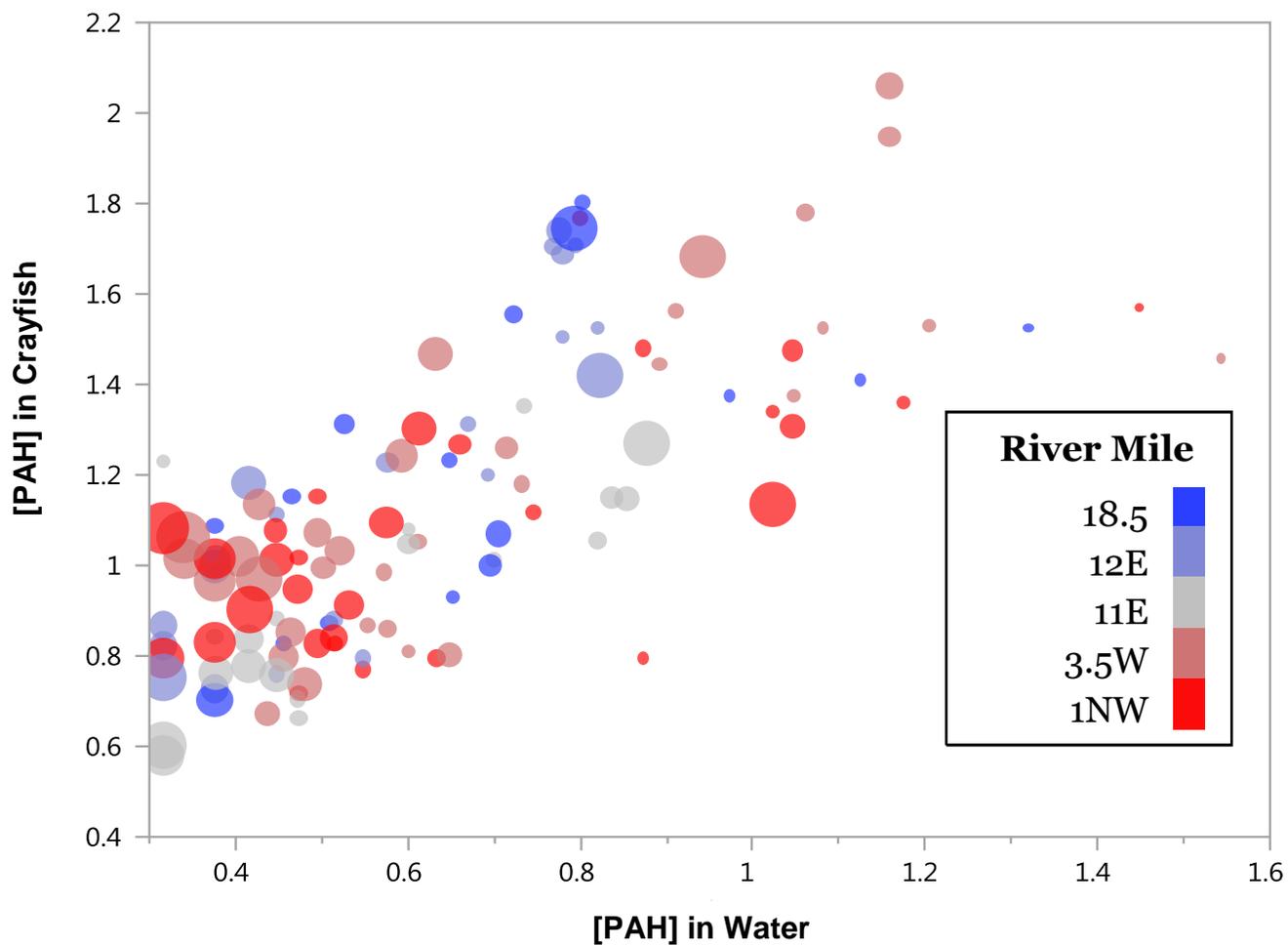


Figure S5. Predictions generated using test set (20% of the data). A model built only with a training set (80% of the paired 2013 crayfish and water data), was used to predict these PAH levels in crayfish for a test set (the other 20% of the data). These predicted values are compared to PAH levels measured in the crayfish. The diagonal reference line represents a predicted:measured ratio of 1:1.

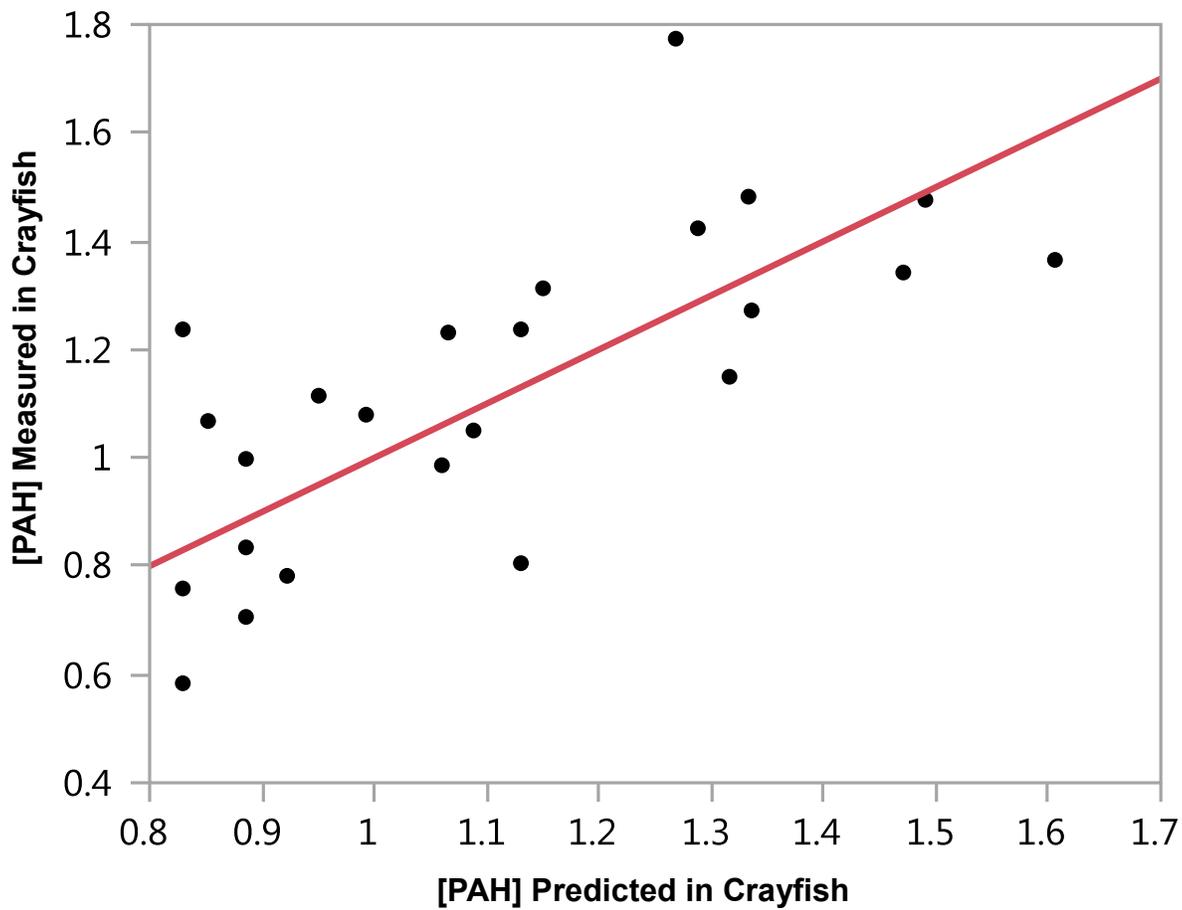
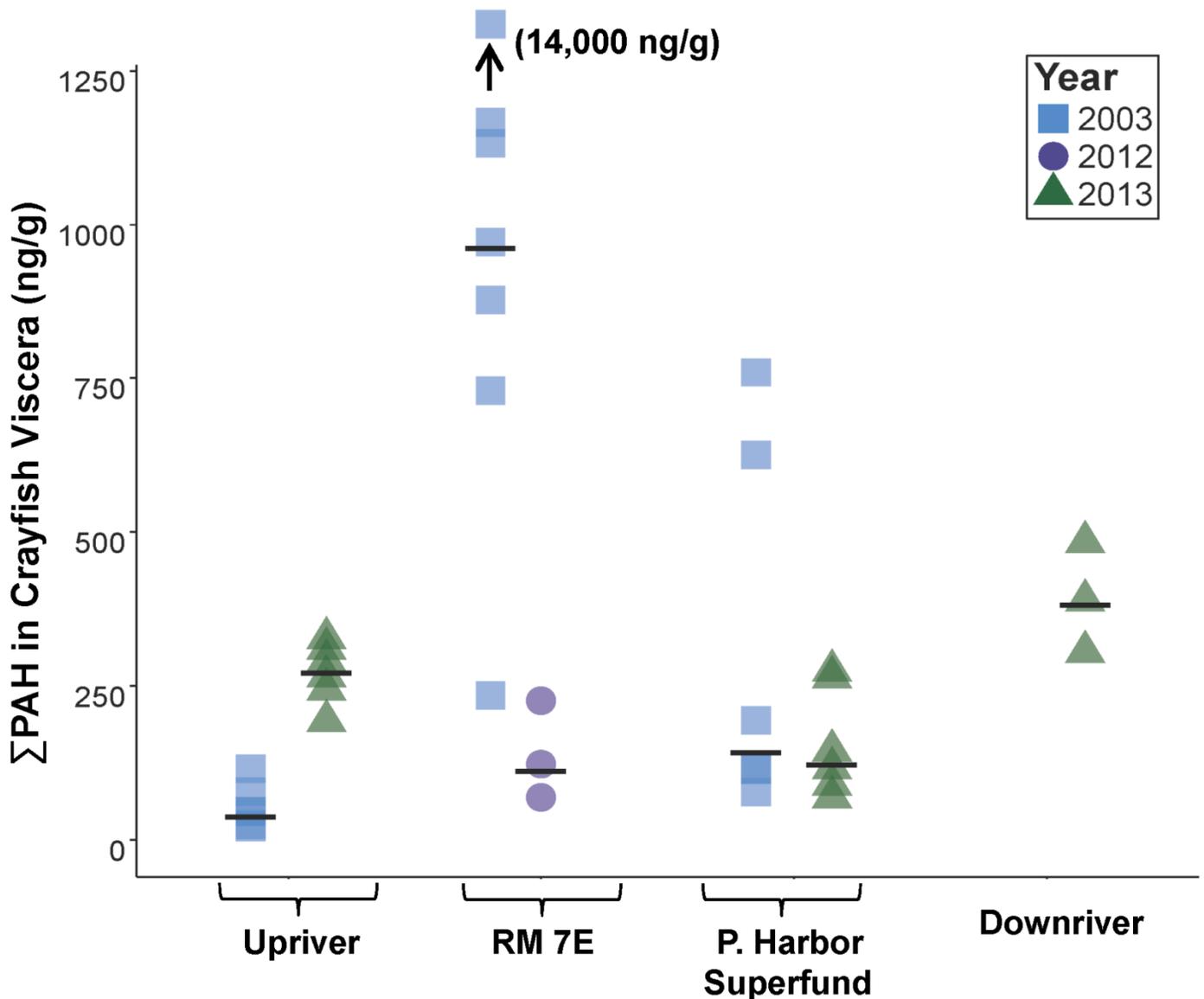


Table S6. Measured and predicted PAH concentrations (ng/g) in 2013 crayfish viscera, within and outside of the Portland Harbor Superfund Megasite. Predicted:measured factor differences shaded light green are less than a factor of 1.5, ratios shaded yellow are between a factor of 1.5 and a factor of 4, and ratios shaded light red are more than a factor of 4. BLOD = below limit of detection.

	Inside Superfund			Outside Superfund		
	Measured (ng/g) (\pm Standard Deviation)	Predicted (ng/g)	Factor difference between predicted & measured	Measured (ng/g) (\pm Standard Deviation)	Predicted (ng/g)	Factor difference between predicted & measured
Naphthalene	3.3 (1.7)	14	4.2	5.5 (0.49)	10	1.9
2-Methylnaphthalene	3.9 (2.2)	3.7	1.0	4.3 (1.0)	5.1	1.2
1-Methylnaphthalene	2.5 (1.6)	3.2	1.3	4.0 (1.0)	3.7	1.1
2-Ethylnaphthalene	0.37 (0.25)	1.1	3.0	0.51 (0.14)	1.1	2.2
2,6-Dimethylnaphthalene	95 (10)	NA	NA	250 (73)	NA	NA
1,6-Dimethylnaphthalene	4.7 (1.9)	2.8	1.6	9.6 (1.0)	2.6	3.7
1,4-Dimethylnaphthalene	0.28 (0.40)	1.2	4.3	0.35 (0.10)	0.88	2.5
1,5-Dimethylnaphthalene	0.78 (0.24)	1.1	1.4	1.7 (0.13)	0.91	1.9
1,2-Dimethylnaphthalene	0.12 (0.17)	0.92	7.5	0.16 (0.27)	1.1	6.9
1,8-Dimethylnaphthalene	BLOD	NA	NA	BLOD	NA	NA
2,6-Diethylnaphthalene	BLOD	NA	NA	BLOD	NA	NA
Acenaphthylene	0.22 (0.31)	1.4	6.6	BLOD	NA	NA
Acenaphthene	2.7 (3.8)	5.3	2.0	1.1 (0.88)	2.2	NA
Fluorene	2.7 (2.3)	2.6	1.0	2.3 (0.70)	1.9	1.2
Dibenzothiophene	0.87 (0.51)	1.1	1.2	1.1 (0.24)	0.73	1.5
Phenanthrene	5.6 (6.2)	3.9	1.4	6.4 (1.9)	2.5	2.5
Anthracene	1.0 (1.4)	2.1	2.2	0.27 (0.23)	1.4	5.2
2-Methylphenanthrene	1.9 (0.93)	1.7	1.1	2.6 (0.35)	1.4	1.9
2-Methylanthracene	0.62 (0.52)	1.0	1.6	1.3 (0.25)	0.8	1.5
1-Methylphenanthrene	0.21 (0.29)	1.7	8.0	BLOD	NA	NA
9-Methylanthracene	BLOD	NA	NA	BLOD	NA	NA
3,6-Dimethylphenanthrene	0.20 (0.29)	0.87	4.3	0.25 (0.24)	0.8	3.3
2,3-Dimethylanthracene	BLOD	NA	NA	BLOD	NA	NA
Fluoranthene	9.9 (12)	4.7	2.1	4.5 (4.1)	3.1	1.4
9,10-Dimethylanthracene	BLOD	NA	NA	BLOD	NA	NA
Pyrene	8.1 (9.0)	4.6	1.8	4.6 (3.6)	3.1	1.5
Retene	5.3 (3.8)	3.5	1.5	5.0 (3.9)	3.3	1.5
Benzo[a]fluorene	0.57 (0.80)	1.1	1.9	0.36 (0.31)	0.79	2.2
Benzo[b]fluorene	0.15 (0.21)	0.95	6.5	BLOD	NA	NA
Benzo[c]fluorene	0.10 (0.15)	0.81	7.8	BLOD	NA	NA
1-Methylpyrene	0.26 (0.37)	0.90	3.4	0.42 (0.40)	0.71	1.7
Benzo[a]anthracene	1.4 (1.4)	1.1	1.3	0.81 (0.74)	1.0	1.2

Cyclopenta[cd]pyrene	BLOD	NA	NA	BLOD	NA	NA
Triphenylene	0.91 (0.59)	0.87	1.0	0.58 (0.54)	0.89	1.5
Chrysene	2.5 (3.1)	1.2	2.1	1.7 (1.3)	1.0	1.8
6-Methyl chrysene	BLOD	NA	NA	0.08 (0.14)	NA	NA
5-Methylchrysene	BLOD	NA	NA	BLOD	NA	NA
Benzo [b] fluoranthene	1.0 (0.94)	0.71	1.4	0.69 (0.46)	0.8	1.2
7,12-Dimethylbenz[a]anthracene	BLOD	NA	NA	0.06 (0.11)	NA	NA
Benzo [k] fluoranthene	0.61 (0.65)	0.55	1.1	0.22 (0.20)	0.50	2.3
Benzo [j] fluoranthene	0.49 (0.53)	0.57	1.2	0.26 (0.24)	0.64	2.5
Benz[j]and[e]aceanthrylene	BLOD	NA	NA	BLOD	NA	NA
Benzo [e] pyrene	0.51 (0.53)	0.64	1.3	0.33 (0.33)	0.62	1.9
Benzo [a] pyrene	0.54 (0.77)	0.71	1.3	0.45 (0.55)	0.64	1.4
Indeno [1,2,3-c,d] pyrene	0.23 (0.33)	NA	NA	BLOD	NA	NA
Dibenzo [a,h] anthracene	BLOD	NA	NA	BLOD	NA	NA
Benzo [a] chrysene	BLOD	NA	NA	BLOD	NA	NA
Benzo [g,h,i] perylene	0.75 (0.74)	0.55	1.4	0.65 (0.69)	0.50	1.3
Anthanthrene	BLOD	NA	NA	BLOD	NA	NA
Naphtho[1,2-b]fluoranthene	BLOD	NA	NA	BLOD	NA	NA
Naphtho[2,3-j]fluoranthene	BLOD	NA	NA	BLOD	NA	NA
Dibenzo [a,e] fluoranthene	BLOD	NA	NA	BLOD	NA	NA
Dibenzo [a,l] pyrene	BLOD	NA	NA	BLOD	NA	NA
Naphtho[2,3-k]fluoranthene	BLOD	NA	NA	BLOD	NA	NA
Naphtho[2,3-e]pyrene	BLOD	NA	NA	BLOD	NA	NA
Dibenzo [a,e] pyrene	BLOD	NA	NA	BLOD	NA	NA
Coronene	0.10 (0.14)	NA	NA	0.09 (0.15)	NA	NA
Dibenzo[e,l]pyrene	BLOD	NA	NA	BLOD	NA	NA
Naphtho[2,3-a]pyrene	BLOD	NA	NA	BLOD	NA	NA
Benzo [b] perylene	BLOD	NA	NA	BLOD	NA	NA
Dibenzo [a,i] pyrene	BLOD	NA	NA	BLOD	NA	NA
Dibenzo [a,h] pyrene	BLOD	NA	NA	BLOD	NA	NA

Figure S6. Spatial and temporal comparison of Σ PAH in crayfish viscera. Σ PAH are presented in crayfish viscera (ng/g) that were collected upriver of Superfund sites, at RM 7E (the McCormick and Baxter Superfund site), in the greater Portland Harbor Superfund Megasite (PHSM), and downriver of the Superfund sites, in the Willamette River in Portland, Oregon. Σ PAH in crayfish were measured in crayfish collected in 2003 (blue squares), and in 2013 (green triangles). For 2012, Σ PAH in crayfish were predicted from water data, using the linear regression described in this study (purple circles). Horizontal black lines represent the median of Σ PAH in each spatiotemporal group of samples.



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