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Passive samplers accurately predict PAH levels in resident crayfish

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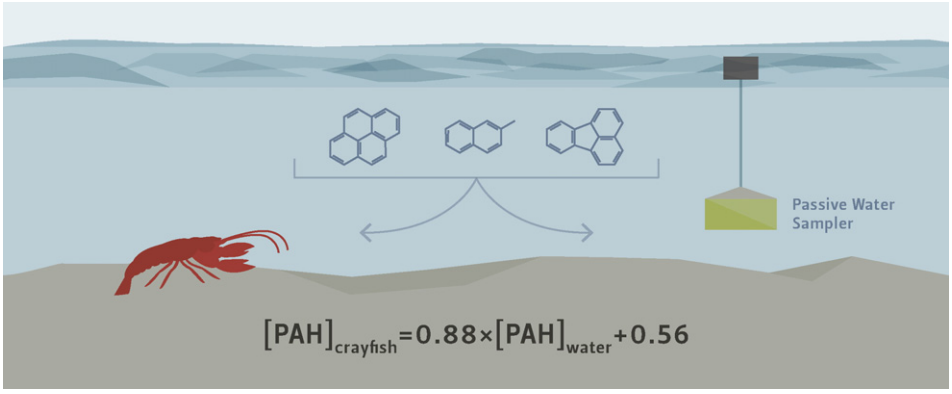
HIGHLIGHTS

- Passive water samplers predict [PAH] in crayfish within a factor of 2, on average.
- The simple model could be adapted to predict risks of eating other shellfish.
- Eating viscera significantly increases cancer risk, compared to eating just tails.
- ∑PAH in crayfish were unchanged after 10 years in Superfund areas pre-remediation.
- ∑PAH in crayfish were higher after 10 years, upriver of an urban Superfund site.

GRAPHICAL ABSTRACT



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ABSTRACT

Contamination of resident aquatic organisms is a major concern for environmental risk assessors. However, collecting organisms to estimate risk is often prohibitively time and resource-intensive. Passive sampling accurately estimates resident organism contamination, and it saves time and resources. This study used low density polyethylene (LDPE) passive water samplers to predict polycyclic aromatic hydrocarbon (PAH) levels in signal crayfish, *Pacifastacus leniusculus*. Resident crayfish were collected at 5 sites within and outside of the Portland Harbor Superfund Megasite (PHSM) in the Willamette River in Portland, Oregon. LDPE deployment was spatially and temporally paired with crayfish collection. Crayfish visceral and tail tissue, as well as water-deployed LDPE, were extracted and analyzed for 62 PAHs using GC–MS/MS. Freely-dissolved concentrations (C_{free}) of PAHs in water were calculated from concentrations in LDPE. Carcinogenic risks were estimated for all crayfish tissues, using benzo[a]pyrene equivalent concentrations (BaP_{eq}). ∑PAH were 5–20 times higher in viscera than in tails, and ∑BaP_{eq} were 6–70 times higher in viscera than in tails. Eating only tail tissue of crayfish would therefore significantly reduce carcinogenic risk compared to also eating viscera. Additionally, PAH levels in crayfish were compared to levels in crayfish collected 10 years earlier. PAH levels in crayfish were higher upriver of the PHSM and unchanged within the PHSM after the 10-year period. Finally, a linear regression model predicted levels of 34 PAHs in crayfish viscera with an associated R-squared value of 0.52 (and a correlation coefficient of 0.72), using only the C_{free} PAHs in water. On average, the model predicted PAH concentrations in crayfish tissue within a factor of 2.4 ± 1.8 of measured concentrations. This affirms that passive water sampling accurately

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estimates PAH contamination in crayfish. Furthermore, the strong predictive ability of this simple model suggests that it could be easily adapted to predict contamination in other shellfish of concern.

1. Introduction

Resident aquatic organism contamination is often of concern at sites with environmental pollution. This is especially true when local communities rely on these organisms for food or income. However, characterizing the risk associated with consuming organisms can be challenging. Collecting enough organisms to assess contamination requires specific knowledge about the organism and the local ecosystem, and it is often prohibitively time and resource-intensive. Harvesting large numbers of organisms can also have adverse impacts on local ecosystems. Accurately assessing resident organism contamination is important for improving both human health risk assessments and ecological risk assessments (Bayen et al., 2009; Boehm et al., 2005). Using a predictive approach to assess organism concentrations is attractive because it requires substantially less time and fewer resources than collecting organisms.

In the past 25 years, passive sampling has been gaining momentum as a useful tool for measuring trace levels of contaminants (O'Connell et al., 2014; Petty et al., 2000). Passive samplers measure time-integrated concentrations of the freely dissolved concentration (C_{free}) of contaminants in water. Passive samplers are relatively low-cost, and they do not require energy or maintenance while deployed. Infusing passive samplers with performance reference compounds (PRCs) before deployment further improves their ability to accurately assess contaminant levels (Huckins et al., 2006).

Low-density polyethylene (LDPE) is a widely-used material for making passive samplers (Anderson et al., 2008). When LDPE is deployed in water, hydrophobic organic contaminants (HOCs) diffuse into LDPE from the water into the hydrophobic polymer. This process is analogous to passive uptake by a phospholipid membrane into an organism's tissues, making LDPE well-suited to serve as a surrogate for contamination in organisms (Allan et al., 2011; Booij et al., 2006; Fernandez and Gschwend, 2015).

Numerous studies have compared uptake of HOCs in passive samplers and aquatic organisms. Many of these investigated the potential for caged organisms to serve as sampling devices, or "biomonitoring organisms" (BMOs) (Joyce et al., 2015). Many studies have used LDPE filled with triolein as a sampling device, known as semi-permeable membrane devices (SPMDs). Anderson et al. (2008) co-deployed SPMDs with triolein-free LDPE samplers and concluded that the two samplers behaved sufficiently similarly. Thus, the two will be directly compared in the present study.

Booij et al. (2006) reviewed nine studies comparing SPMDs and BMO mussels, concluding that SMPDs yield less variable results, while identifying similar spatial trends. In the same year, Huckins et al. (2006) reviewed over 30 studies comparing SPMDs and BMOs, concluding that there are substantial overarching similarities in HOC accumulation in aquatic organisms and SPMDs.

Recent research has continued to assess passive samplers as replacements for BMOs in assessing water quality (Alvarez et al., 2014; Bourgeault and Gourlay-France, 2013; Burgess et al., 2015; Joyce et al., 2015) and as tools to estimate contaminant levels in resident organisms (Allan et al., 2011; Fernandez and Gschwend, 2015; Forsberg et al., 2014). While some studies have highlighted key differences between contaminant accumulation in passive samplers and organisms (Boehm et al., 2005; Bourgeault and Gourlay-France, 2013), the majority report good agreement between contaminant accumulation in passive samplers and organisms (Allan et al., 2011; Alvarez et al., 2014; Burgess et al., 2015; Fernandez and Gschwend, 2015; Forsberg et al., 2014; Joyce et al., 2015).

Only a few studies have used predictive tools to assess human health risks associated with consuming resident organisms (Allan et al., 2011; Forsberg et al., 2014). Many studies have used predictive tools to assess the accumulation of HOCs in aquatic organisms. These predictive tools often require chemical or physical partitioning data such as bioaccumulation factors or partition coefficients between lipid and water, and including these can increase prediction variability (Axelman et al., 1999; Booij et al., 2006; Huckins et al., 2006). Notably, Fernandez and Gschwend showed that using porewater C_{free} predicted more accurate and less variable tissue concentrations in clams than using the traditional biota-sediment accumulation factor (Fernandez and Gschwend, 2015). However, even when predicting based on C_{free}, they suggested that using previously published values (to estimate lipid-water partitioning coefficients and the fraction of lipids in the clams) may have increased variability in their predictions (Fernandez and Gschwend, 2015). Additionally, in their 2006 review, Huckins et al. noted that lipid-normalizing tissue concentrations has been debated in the literature since the early 1980s (Huckins et al., 2006). It is therefore desirable to have a predictive tool that requires as few additional inputs as possible.

Forsberg et al. (2014) demonstrated that mathematical models may work as well or better than physical or chemical partitioning data when predicting organism concentrations using passive samplers. If organismal concentrations could be reliably predicted using only passive sampler data and mathematical models, this would greatly reduce the time and information needed for a risk assessor to estimate contaminant levels in resident organisms.

Polycyclic aromatic hydrocarbons (PAHs) are pervasive environmental contaminants that pose risks to human health. Some PAHs are pro-carcinogens, which can be metabolically activated through oxidation by P450 enzymes, creating reactive intermediates that can form DNA adducts (Baird et al., 2005). Diet is the main pathway by which nonsmokers are exposed to PAHs (Phillips, 1999; Zelinkova and Wenzl, 2015).

The Portland Harbor Superfund Megasite (PHSM) is located in the Willamette River in Portland, Oregon. Since the Industrial Revolution, Portland Harbor has been subjected to countless sources of pollution that left a legacy of pollutants, including PAHs (Allan et al., 2012; ATSDR, 2006). In 2000, the area between river mile (RM) 3.5 and 9.2 was designated the PHSM. The PHSM was later expanded, stretching from RM 2.0 to 11.8 as of 2013 (LWG, 2013). This area is home to many species that are harvested by local fishermen, including the native signal crayfish, *Pacifastacus leniusculus* (ATSDR, 2006). Crayfish consumption was listed as a main route of exposure to pollutants in the Agency for Toxic Substances and Disease Registry (ATSDR)'s Public Health Assessment (PHA) for the PHSM (ATSDR, 2006; ATSDR, 2011).

It has been previously observed that PAHs accumulate in crayfish (Jewell et al., 1997). This is partly due to crayfish having less efficient cytochrome P450 systems than finfish (Levengood and Schaeffer, 2011). Additionally, signal crayfish in this portion of the Willamette likely spend their whole lives in Portland Harbor due to their small home range. This means they are exposed for much more of their lifespans than organisms that only pass through the PHSM (ATSDR, 2006). The combination of reduced metabolism and increased exposure duration may lead to greater bioaccumulation of pollutants in crayfish than in finfish. A similar dynamic would likely be observed in other shellfish

that have similar behavioral and physiological patterns. Thus, a model built using one shellfish species would likely also be easily transferred to predict contaminant levels in other shellfish of interest.

Forsberg et al. (2014) observed that PAH accumulation in *P. leniusculus* correlates well with C_{free} measured by SPMDs. While this study successfully modeled PAH levels in crayfish, the model was limited to 15 PAHs, and crayfish and SPMDs were not directly spatially or temporally paired. These limitations may have necessitated adjustments in model development that reduced modeling accuracy. Thus, the ability of LDPE to predict PAH levels in crayfish and other resident shellfish warrants further investigation. The objective of this study was to use LDPE passive water samplers to predict PAH levels in crayfish, with sufficient accuracy to perform risk assessments.

2. Materials and methods

2.1. Description of study site and sampling locations

The study was conducted in fall 2013, in the lower 18.5 miles of the Willamette River, within and outside of the PHSM. Samples were collected at five sites: upriver of the PHSM (RM 18.5 and 12E), within the PHSM (RM 11E and 3.5W), and downriver of the PHSM (RM 1NW). Although RM 12E is not in the PHSM, it is 0.22 miles upriver of the upper bound of the PHSM, as defined in 2013, and was considered within the "downtown reach" in the 2013 PHSM remedial investigation (LWG, 2013). Site names in this study use approximate RM designations. Comparisons are made with crayfish and passive sampler data from previous sampling campaigns (in 2003 and 2012), which included different RMs, described below and in the SI. A map of the study area is included in SI Fig. S1, and GPS coordinates of sites are available in SI Table S1.

2.2. Crayfish sampling, dissection, and extraction

Resident signal crayfish, *P. leniusculus* were collected from the Willamette River, at the five sites listed above. A total of 130 crayfish were collected during September and October, 2013. Crayfish collection was performed in accordance with Oregon Department of Fish and Wildlife Scientific Taking Permit for Fish, number 18039. Frozen tuna pieces and canned cat food were used as bait, and crayfish were caught in minnow traps that were retrieved within 24 h. Upon trap retrieval, external crayfish surfaces were rinsed with ambient water, rinsed with 18 MΩ∗cm water, and inspected for physical damage. Crayfish were euthanized on site using liquid nitrogen, and wrapped individually in aluminum foil. Crayfish were then transported in coolers with frozen ice packs back to the Food Safety and Environmental Stewardship (FSES) lab at Oregon State University (OSU) in Corvallis, Oregon. At the lab, crayfish were stored at −20 °C until dissection.

Lab processing methods for crayfish are visualized in SI Fig. S2A. Each crayfish was sexed, measured (full body length and carapace length), weighed, and then dissected. Of the 130 crayfish collected, 60 (12 from each of the 5 RMs) were dissected, with viscera and tail (abdominal muscle) tissue being composited separately. This yielded 3 composites of each tissue (viscera and tail) from 12 crayfish, for each RM (with each composite containing tissue from 4 crayfish). Composited tissues were stored at −20 °C until homogenization. Each composited tissue sample was homogenized to a fine powder using liquid nitrogen and a metal mortar and pestle, as described previously (Forsberg et al., 2014).

Homogenized tissue samples were extracted using a slightly modified QuEChERS method (Forsberg et al., 2014; Forsberg et al., 2011). Each tissue sample was spiked with surrogate standards (listed in SI Table S2) immediately before extraction, and 1.0 g (±2%) wet weight of each composited tissue was extracted. Further extraction details are in the SI.

Previous studies assessing the home range of *P. leniusculus*, in ecosystems comparable to the river system in the present study, showed that these crayfish typically migrate less than 225 m in a two-year period (Bubb et al., 2004; Guan and Wiles, 1997). Thus, PAH levels measured in crayfish in the present study are interpreted as site-specific, time-weighted average concentrations.

2.3. LDPE preparation, deployment, cleaning and extraction

Before deployment, LDPE was cleaned using hexanes and prepared as described previously (Anderson et al., 2008). Each LDPE strip was spiked with PRCs to enable calculation of in situ sampling rates and time-integrated water concentrations (Huckins et al., 2006). PRCs used in this study were fluorene-d10, pyrene-d10 and benzo[b]fluoranthene-d12. Slightly different PRCs were used for the 2012 deployment, described in the SI. Between 4 and 100 μg of each PRC was spiked into each LDPE strip. After PRC addition, each LDPE strip was heat-sealed at both ends.

Passive samplers were deployed in metal cages in the water column. Three PRC-infused LDPE strips were deployed in each cage. At RM 18.5 and 3.5W, three cages were deployed. At RM 12E, 11E and 1NW, one cage was deployed. For the 2012 deployment, three cages were deployed at RM 7E. Additional details and exact sampling dates are in the SI. All samplers were rinsed in ambient water and transported back to the lab in amber glass jars. Samplers were stored at −20 °C until cleaning.

LDPE processing that occurred in the lab after retrieval is visualized in SI Fig. S2B. LDPE were cleaned in 1 N hydrochloric acid, 18 MΩ∗cm water, and two isopropanol baths, stored in amber jars at −20 °C, and extracted as described previously (Anderson et al., 2008). Briefly, extractions were performed using two dialyses of hexane. Immediately before extraction, surrogate standards were spiked onto LDPE to account for extraction efficiency. Extraction surrogates are listed in SI Table S2. Extracts were quantitatively concentrated to 1 mL using TurboVap® closed cell evaporators, transferred to amber chromatography vials, and stored at −20 °C. Details regarding chemicals and solvents are in the SI.

2.4. Chemical analysis

Crayfish and LDPE extracts were quantitatively analyzed for 62 PAHs using an Agilent 7890A gas chromatograph interfaced with a (modified) Agilent 7000 GC–MS/MS, as described elsewhere (Anderson et al., 2015). More information about the analytical method is in the SI. Lists of PAHs, limits of detection (LODs) and limits of quantitation (LOQs) for the instrument method are included in SI Table S2. Lists of LODs for PAHs measured in crayfish tails, crayfish viscera, and water are included in SI Table S3. LODs for this study ranged from 0.0003–0.03 ng/mL in water, 0.03–0.78 ng/g in crayfish viscera, and 0.24–6.4 ng/g in crayfish tails (Table S3).

2.5. Water concentration calculation

Concentrations of PAHs freely dissolved in water (C_{free}) were calculated from concentrations in water-deployed LDPE using SI Eqs. (S1)–(S6), as described by Huckins et al. (2006). Sampling rates (R_s) 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 compounds in variable environmental conditions (Bartkow et al., 2006; Huckins et al., 2002). These calculations are described in detail in the SI. Calculated C_{free} values will be referred to simply as "water" concentrations in the results and discussion.

2.6. Estimation of carcinogenic potency

The carcinogenic potency of the PAH mixture in each crayfish sample was estimated using the EPA's relative potency factor (RPF) approach (EPA, 2010). This approach scales concentrations of PAHs based on their carcinogenic potency relative to benzo[a]pyrene, yielding

a benzo[a]pyrene equivalent concentration, or BaP_{eq}. $\sum BaP_{eq}$ values were calculated using Eq. (S7); a list of RPFs is in SI Table S4.

2.7. Predictive modeling

Examining correlation plots of PAH concentrations in crayfish versus water suggested ordinary linear regression could be used to produce a predictive model with water concentrations predicting crayfish concentrations. Thus, a linear regression model was generated using the paired crayfish and water data for individual PAHs measured in the 2013 sampling campaign. All data were fourth-root transformed prior to modeling, to improve model performance. Other models which included factors such as individual PAH, site, and K_{ow} values were also explored. In the predictive model, any data that was below the LOD (BLOD) in either crayfish or water was excluded. The model was created using JMP Pro, version 11.2.0. Predicted PAH levels in crayfish were calculated using the model, with only the PAH levels measured in water as inputs. Model performance was further investigated by calculating the factor differences between predicted and measured PAH values. To further validate the model, a separate linear regression was created using only 80% of the data. This model was then used to predict crayfish concentrations for the other 20% of the data. These subsets of the data were chosen randomly and non-overlapping. This is described further in the SI. An additional model was made in which the LODs were substituted for all BLOD values. The performance of this model is discussed briefly as well.

2.8. Comparisons of PAHs in crayfish viscera: measured in 2003 and predicted in 2012

A subset of crayfish viscera collected 10 years earlier (fall of 2003) were analyzed for 62 PAHs for comparison with the 2013 crayfish viscera data. The 2003 sampling campaign has been previously described, so only pertinent details are included here (Forsberg et al., 2014). In 2003, crayfish were collected at two sites upriver of the PHSM (RM 17 and 13) and three sites within the PHSM (RM 7W, 7E and 3E). Specific GPS coordinates for sampling locations are presented in SI Table S1B. Importantly, RM 7E is the site of the former McCormick and Baxter Creosoting Company, which was added to the US EPA's National Priorities List in 1994, independently of the PHSM (EPA, 1996). Remedial actions were carried out by the EPA at this site, and were completed in 2005 (EPA, 2015).

Data were separated temporally by the sampling year and spatially relative to the PHSM. The PHSM was divided into RM 7E and the greater PHSM because of RM 7E's independent Superfund status and completed remediation. Additionally, the predictive model from the present study was used to estimate PAH levels in crayfish viscera at RM 7E in 2012, using C_{free} PAH concentrations from RM 7E in 2012 (these water data are presented in the SI). These predicted values enabled comparisons between PAH levels in crayfish at RM 7E before and after remediation (in 2003 and 2012).

2.9. Quantitative human health risk assessment

Given that non-carcinogenic toxicity of PAHs remains understudied, and that carcinogenicity is typically the risk-driving endpoint for PAHs in human health risk assessments (Wickliffe et al., 2014), this study focuses on estimating carcinogenic risk. Eqs. (S8) and (S9), described in the SI, were used to estimate risks associated with consuming crayfish tissues containing the measured PAH mixtures. In data exploration, the crayfish ingestion rate was set at both 3.3 g/day and 18 g/day, which are the average and 95th percentile rates for adults in the United States (ATSDR, 2006). Risk estimates generated using the 95th percentile ingestion rate are presented and discussed here, to estimate a worst -case exposure scenario. All risk estimates would be ~6-fold smaller if the average ingestion rate were used. Excess lifetime cancer risk (ELCR) estimates were used to compare the potential cancer risks across samples. ELCRs exceeding one in a million are discussed, and presented graphically. This was the acceptable risk level used by ATSDR to screen contaminants in their PHA for the PHSM (ATSDR, 2006). Values for all other exposure parameters for the risk assessment calculations were chosen in accordance with EPA or ATSDR guidance, and are available in the SI (ATSDR, 2006).

Measured $\sum BaP_{eq}$ values were used as the tissue concentrations to estimate ELCRs. The predictive model was used to predict $\sum BaP_{eq}$ in 2012 crayfish viscera from RM 7E. This predicted $\sum BaP_{eq}$ was used to estimate an ELCR for 2012 crayfish viscera from RM 7E. Additionally, where tail tissue data were not measured (in the 2003 dataset and in the predicted 2012 data), it was assumed that $\sum BaP_{eq}$ in tail tissue was 10% of $\sum BaP_{eq}$ in viscera. This is health-protective, as $\sum BaP_{eq}$ in tail tissue were as low as 1% of $\sum BaP_{eq}$ in viscera in some crayfish sampled in 2013. Finally, to estimate ELCRs associated with whole crayfish tissue, $\sum BaP_{eq}$ in tail and viscera tissue were summed, scaled relative to each tissue's average percent contribution to total wet weight of sampled tissues (45 and 55%, for tail and viscera, respectively). The resulting estimates are referred to as ELCRs for "whole tissue."

2.10. Statistical methods

Statistical analyses of PAH data were performed using the statistical software R, version 2.15.3. Wilcoxon Signed-Rank tests were used to compare $\sum$PAH and $\sum BaP_{eq}$ levels in viscera and tails of the same crayfish. Wilcoxon Rank-Sum tests were used to compare $\sum$PAH levels in crayfish viscera collected in different locations in the same year, and to compare $\sum$PAH levels in crayfish viscera collected in similar locations in different years. Any PAH concentrations that were BLOD were treated as zeros. For all comparisons, significant differences were interpreted when $p < 0.05$.

2.11. Quality control (QC)

Numerous quality control measures were taken. Crayfish extraction was duplicated for one sample each of tails and viscera. Additional QC steps taken for crayfish samples included homogenization blanks and extraction blanks for both viscera and tails, as well as an additional SPE blank for the viscera extraction. For the water-deployed LDPE, multiple QC steps were taken. The first was that one LDPE strip was hung in the lab for the entirety of the PRC-infusion process, to account for potential contamination. Samples (containing one LDPE strip each) were taken on each field sampling trip, where they were used as field blanks, to account for potential contamination during LDPE transport or while LDPE were being strung on water cages. LDPE sample deployment was replicated ($n = 3$) at two of the five sampling sites. Before extraction, all samples were spiked with extraction surrogate standards ranging the span of $\log K_{ow}$s of target PAHs (surrogate standards are listed in SI Table S2). The analytical method was validated prior to use using its calibration, precision and accuracy, and detection limits. Before instrumental analysis, perylene-d12 was spiked into all sample extracts at 500 ng/mL to act as an internal standard. During instrument analysis, instrument blanks and continuing calibration verifications were run at the beginning and end of each set of samples. All laboratory and field procedures were performed according to FSES Standard Operating Procedures.

2.12. Quality control results

Carcinogenic PAHs were BLOD in all blank QC samples. Any PAH concentrations that were above the LODs in QC samples were subtracted before data was analyzed. Extraction surrogate recoveries in crayfish tails ranged from 78 ± 5% for chrysene-d12, to 88 ± 5% for naphthalene-d8. For crayfish viscera, extraction surrogate recoveries ranged from 28 ± 11% for naphthalene-d8, to 79 ± 20% for

benzo[a]pyrene-d12. The average differences between individual PAH concentrations for the two duplicate extractions were 0.9 ng/g and 2.5 ng/g for viscera and tails, respectively. For 2003 crayfish samples, quality control results have been previously reported (Forsberg et al., 2014).

For LDPE, extraction surrogate recoveries ranged from 48 ± 4% for naphthalene-d8, to 96 ± 7% for benzo[a]pyrene-d12. Averages of the few target compounds quantified in the field blanks were multiplied by 3 (to convert from one strip of LDPE used as a field blank to 3 strips used in a sample), and the resulting values were used to background-subtract target PAH concentrations in LDPE samples before data analysis. The average RSD among ∑PAH concentrations measured in LDPE replicates in water was 17.5% (n = 3, at two replicate sites).

3. Results and discussion

3.1. PAH levels in crayfish viscera, tails, and water

Average ∑PAH in crayfish viscera, tails, and water are listed in Table S5A. ∑PAH were significantly larger in crayfish viscera than in tails of the same organisms (Wilcoxon Signed-Rank Test, $p < 0.001$). ∑PAH in viscera were 5 to 20 times higher than in tails (Fig. 1A). This is consistent with previous work, which observed that PAH levels are highest in the hepatopancreas and lowest in muscle tissue in crabs (Lee et al., 1976). In crayfish, it has been suggested that oxidative metabolism of PAHs and other xenobiotics occurs in the hepatopancreas and in the green gland (James and Boyle, 1998; Jewell et al., 1997). It has also been observed that PAH uptake rates from water are 11–90 times higher in *P. leniusculus* hepatopancreases than in tail muscle tissue, while elimination rates are comparable or slightly higher in the muscle tissue (Gossiaux and Landrum, 2005). Thus, elevated PAH concentrations in the viscera are not surprising, as the visceral tissue contains both the hepatopancreas and green gland.

3.2. Carcinogenic potency of PAHs in crayfish viscera and tails

$\sum BaP_{eq}$ were significantly higher in viscera than in tails (Wilcoxon Signed-Rank test, $p < 0.001$). Specifically, $\sum BaP_{eq}$ were 6 to 70 times higher in viscera than in tails (Fig. 1B). This is an even more dramatic difference than between viscera and tails for ∑PAH, indicating that the fraction of carcinogenic PAHs is higher in the viscera compared to the tails. This suggests that eating only the tail tissue of a crayfish would yield significantly less carcinogenic risk than eating the viscera, or than eating all of the tissue in the organism. This is important because ingestion is one of the main routes of PAH exposure in nonsmokers (Menzie et al., 1992; Phillips, 1999).

3.3. Predictive modeling

Because the concentrations of ∑PAH were so low in tails, only viscera data were used in predictive modeling. The relationship between PAH levels in crayfish and the C_{free} in water (estimated using LDPE) from 2013 was explored extensively. Individual PAH levels in crayfish viscera and water correlated well across all sites, for both the PAH profile (Fig. S3) and the magnitude of PAH concentration (Fig. S4). This trend was not affected by sampling site or by $\log K_{ow}$ (Fig. S4). Of the 62 PAHs in the analytical method, 18 were BLOD in both crayfish and water at all sites, and were thus not included in the predictive model. The majority of these PAHs were higher molecular weight ($\log K_{ow} \geq 6.75$), and thus would not be expected to be freely dissolved in water at high levels. It is interesting, however, that these higher molecular weight PAHs were BLOD not only in water, but also in crayfish tissues. This suggests that PAH profile is very similar between crayfish and water, providing further support for the use of passive water samplers to predict levels of PAHs, and likely other HOCs, in crayfish. There were instances where a PAH was detected in water and BLOD in crayfish, or vice versa. These PAHs were also excluded from the model. However, it is worth noting that, with the exception of 2,6-Dimethylnaphthalene, or 2,6-DMN (discussed in the SI), the average value measured in one matrix but not the other was 0.09 ppb. Thus, if a PAH is BLOD in water, it is reasonable to predict that it will either be BLOD or very small in crayfish viscera (~1 ppb).

Previous studies have observed that the relationship between contaminant uptake in organisms and passive samplers is not one to one (Booij et al., 2006). Thus, a linear regression model was generated to predict crayfish PAH levels using individual PAH levels measured by LDPE in water (Fig. 2). The R-squared value for the line of best fit was 0.52, which corresponds to a correlation coefficient of 0.72. The narrow confidence interval (darker shading) indicates that the average response is modeled well (Fig. 2). The prediction interval (lighter shading) is wider, because it characterizes uncertainty associated with individual observations, rather than averages (Fig. 2). The regression slope of 0.88 indicates that, in transformed units, a change of 1 in water corresponds to a change of 0.88 for crayfish in transformed units. The equation of the line of best fit is:

$$[PAH]_{crayfish} = 0.88 \times [PAH]_{water} + 0.56. \quad (1)$$

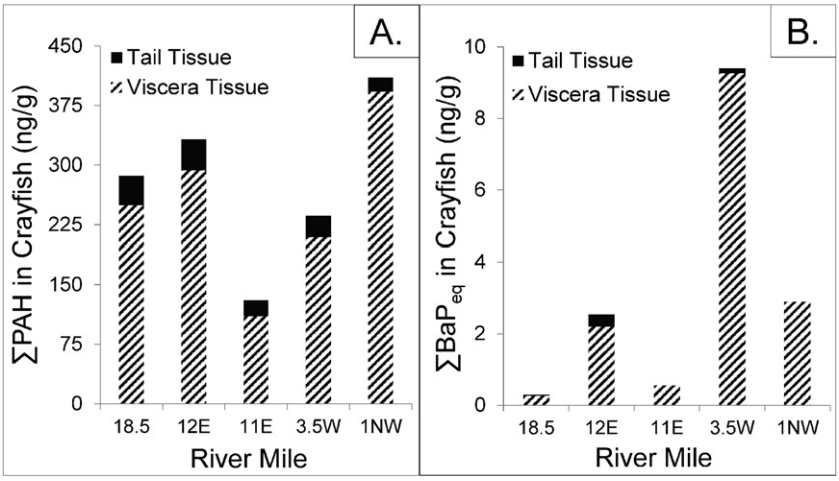

Fig. 1. Average (A) ∑PAH and (B) $\sum BaP_{eq}$, in tail and viscera tissue from crayfish collected in the Willamette River in Portland, Oregon (n = 3) in 2013. Crayfish were collected upriver (RM 18.5 and 12E), within (RM 11E and 3.5W), and downriver (RM 1NW) of the Portland Harbor Superfund Megasite. Three composited samples of each tissue were analyzed from each river mile. All tissue concentrations are in ng/g wet weight.

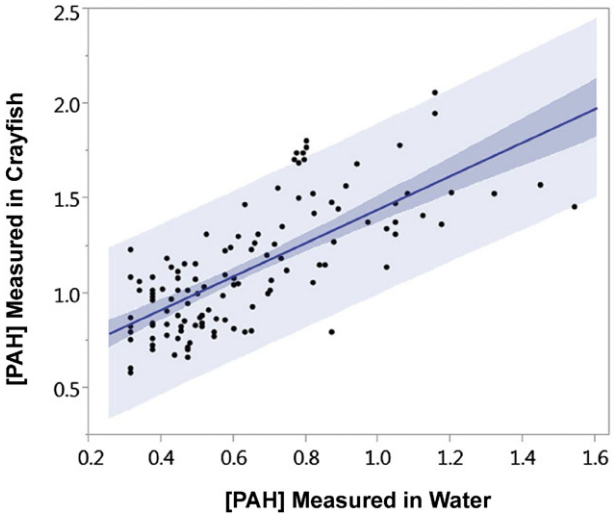

Fig. 2. Linear regression of 34 individual [PAH], measured in spatially and temporally paired crayfish viscera ($\sqrt[4]{(ng/g)}$) vs. water ($\sqrt[4]{(ng/mL)}$). Samples were collected upriver, within, and downriver of the Portland Harbor Superfund Megasite (PHSM) in the Willamette River in Portland, Oregon. R-Squared = 0.52 and correlation coefficient = 0.72. Dark shading represents confidence intervals and light shading represents prediction intervals. The slope of the line of best fit is 0.88 ± 0.078 and the intercept is 0.56 ± 0.054.

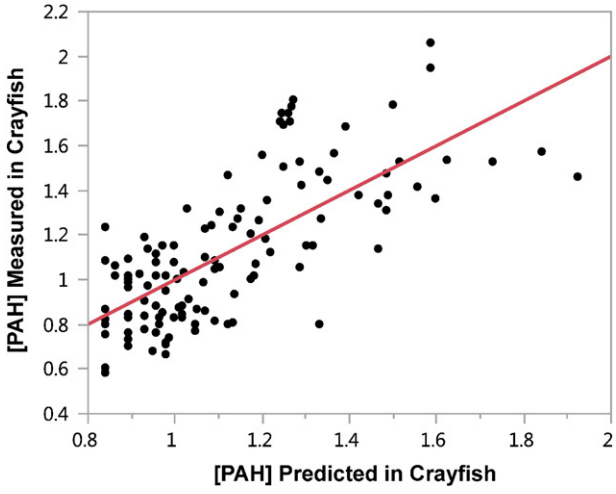

Fig. 3. [PAH] measured in crayfish viscera ($\sqrt[4]{(ng/g)}$), vs. [PAH] predicted in crayfish viscera ($\sqrt[4]{(ng/g)}$) from freely dissolved [PAH] in water. [PAH] were predicted in crayfish viscera using Eq. (1), from the linear regression model displayed in Fig. 2. The diagonal reference line indicates where predicted and measured values are 1:1. The average untransformed concentrations predicted by the model were within a factor of 2.4 ± 1.8 of measured concentrations.

Eq. (1) can be used to predict PAH concentrations in crayfish viscera, using only C_{free} PAH concentrations in water. The standard error for intercept is 0.054, and standard error for slope is 0.078. Additionally, the training model (built using 80% of the data) was almost identical to the model built using all of the data, and it accurately predicted crayfish concentrations for the other 20% of the data (Fig. S5). Further details are provided in the SI.

When LODs were substituted for BLOD data (instead of excluding them from the model as was done in the first model), the equation of the line of best fit was:

$$[PAH]_{crayfish} = 1.0 \times [PAH]_{water} + 0.39. \quad (2)$$

3.4. Comparison of predictive capability of model to previously reported predictive tools

The model predicts PAH concentrations in crayfish using C_{free} concentrations of PAHs (measured using LDPE passive samplers) well (Fig. 3). For the 34 PAHs included in the model, crayfish concentrations were predicted by the model within a factor of 2.4 ± 1.8 of measured concentrations (Table S6). The BLOD-substitution model (Eq. (2)) predicted PAH levels in crayfish within a factor of 2.7 ± 2.4 of measured concentrations. This suggests that these two models are performing very similarly. The former model (Eq. (1), in which any data that were BLOD were excluded when creating the model) is used for the remainder of the analyses. Due to the relatively small concentrations of many PAHs measured in crayfish, even seemingly larger predicted/measured ratios can be practically insignificant. The average difference between the predicted and measured concentration for the 34 PAHs included in the model at all sites was 1.2 ng/g. There were only 3 PAHs for which this difference was greater than 5.0 ng/g at any site. Measured concentrations, predicted concentrations, and the factor differences between predicted and measured concentrations for all PAHs are in SI Table S6.

Predicted/measured factor differences for individual PAHs are equal to or better than those presented for previously published models, and the present model includes more PAHs than comparable models. In a recent study, Fernandez and Gschwend compared traditional methods of predicting PAH levels in clams (using biota-sediment accumulation factors (BSAF), the concentration in the sediment, and the fraction of organic carbon in the sediment) to predicting PAH levels in clams using LDPE passive pore-water samplers. The authors reported average predicted/measured ratios of 0.43, 3.7, and 1.1 for phenanthrene, pyrene and chrysene, respectively, with standard deviations that are close to an order of magnitude larger than the averages (Fernandez and Gschwend, 2015). Forsberg et al. (2014) reported a mathematical model predicting concentrations of 15 PAHs in crayfish viscera using C_{free} PAH concentrations in water (from SPMDs) within a factor of 3. The authors stated that this was an improvement over previous prediction techniques (Forsberg et al., 2014). Thus, the average predicted/measured ratio of 2.4 ± 1.8 in the present study, from a model including 34 PAHs, is a substantial improvement over what has been previously observed.

Part of what is reducing variability in the present model may be that it does not require inputs for the physical or chemical parameters of the system (lipid–water partitioning coefficient, bioaccumulation factor, etc.). These have been observed to increase prediction variability, due to variability of factors including chemical bioaccumulation, organism behavior, and organism anatomy and physiology (Fernandez and Gschwend, 2015; Huckins et al., 2004). An additional benefit of the model described here is that it includes 34 PAHs, some of which are both relevant to human health and rarely included in analyses.

3.5. 10 years later — comparison of PAH levels in crayfish viscera in 2003 and 2013

Average ∑PAH in 2003 crayfish viscera are listed in SI Table S5B. In 2003, upriver ∑PAH were significantly lower than at RM 7E, and significantly lower than in the PHSM (Wilcoxon Rank-Sum tests, p = 0.001 and 0.026). This is consistent with crayfish within the Superfund sites being exposed to higher contaminant levels than those upriver (Forsberg et al., 2014). In 2013, the trend is reversed, with ∑PAH levels in 2013 crayfish viscera significantly higher *outside* the PHSM than inside (Fig. S6). This was true when comparing both crayfish collected upriver and downriver to those within the PHSM (Wilcoxon Rank-Sum tests, p = 0.041 and 0.024). Additionally, ∑PAH in crayfish upriver of the PHSM were significantly higher in 2013 than in 2003, while ∑PAH in PHSM crayfish were not significantly different in the two years (Wilcoxon Rank-Sum tests, p = 0.002 and 0.700). The 2003 crayfish data also has much more variability than the 2013 data, both within and among sites. This is discussed further in the SI.

The increased PAH levels in crayfish upriver of the PHSM, along with the shift from highest PAH levels being measured within to outside of the PHSM, shows that the distribution of pollution in the river has changed over this 10-year period. This could be due in part to remediation efforts within the PHSM, such as the early-action remediation at RM 7E and RM 7W. Outside the PHSM, storm-water runoff is the predominant source of PAHs (Sower and Anderson, 2008). Thus, the relative PAH increase upriver of the PHSM could be due in part to increasing inputs from nonpoint sources from the growing Portland metropolitan area. Differences between these two time periods may also have been affected by variation in the locations where crayfish were captured.

Average PAH levels in crayfish at RM 7E in 2003 were higher than at any other location in either year (Fig. S6). RM 7E is unique because it was the site of the former McCormick and Baxter Creosoting Company. This was historically one of the most PAH-laden sites in this area of the river, but remediation was completed in 2005. Average predicted $\sum$PAH in RM 7E crayfish viscera in 2012 was 150 ng/g. This is 18-fold smaller than average $\sum$PAH measured at this site in 2003. This predicted decrease is significant (Wilcoxon Rank-Sum test, $p = 0.017$). There were no significant differences between $\sum$PAH predicted in crayfish viscera at RM 7E in 2012 and $\sum$PAH measured in crayfish viscera in the PHSM in 2003 or in 2013 (Wilcoxon Rank-Sum tests, $p = 0.714$ and 0.714) (Fig. S6). It is worth considering that 2012 samples were deployed during a rainier part of the year, when lower PAH levels have been observed in Willamette River (Sower and Anderson, 2008). However, even if predicted PAH levels were two-fold higher at RM 7E in 2012, this would still be a substantial reduction in pollutants compared to 2003. This suggests that remedial efforts at RM 7E substantially reduced the pollutant levels available for movement from the sediment into the overlying water, reducing PAH levels in crayfish.

3.6. PAH profiling — $\sum$PAH vs. $\sum BaP_{eq}$

PAH profiling indicated that PAHs contributing most to $\sum$PAH are different from PAHs contributing most to $\sum BaP_{eq}$ (Fig. 4). In fact, fluoranthene is the only PAH in the top 10 PAHs for both $\sum$PAH and $\sum BaP_{eq}$ in 2013 viscera (Fig. 4B, D), and only fluoranthene and chrysene are on both of these lists in 2003 viscera (Fig. 4A, C). This illustrates that $\sum$PAH can present a very different picture than $\sum BaP_{eq}$. Therefore, measuring $\sum$PAH alone may not be a useful metric for comparing health risks among sites.

$\sum BaP_{eq}$ and $\sum$PAH also present different pictures of contamination levels at different sites throughout the river. For instance, in 2013, viscera from RM 3.5 had the highest average $\sum BaP_{eq}$ (Fig. 1B), while the same viscera only had the 4th highest average $\sum$PAH of the 5 sites (Fig. 1A). This is another demonstration of the importance of using the appropriate metric to estimate risk.

2,6-DMN was the most abundant PAH contributing to average $\sum$PAH in crayfish viscera (Fig. 4B) and tails at all sites in 2013, but does not affect $\sum BaP_{eq}$ because it does not have an RPF value. The prevalence of 2,6-DMN is discussed further in the SI.

3.7. PAH profiling — 2003 vs. 2013

PAH profiles are similar in crayfish viscera collected in the PHSM in 2003 and 2013 (Fig. 4). Four of the top five PAHs contributing to $\sum$PAH in both 2003 and 2013 crayfish viscera collected in the PHSM are fluoranthene, phenanthrene, pyrene and retene (Fig. 4A, B). The profile of PAHs driving carcinogenic risk in crayfish viscera in the PHSM was well conserved after this 10-year period, despite crayfish being collected at different sites within the PHSM. While the magnitude of $\sum BaP_{eq}$ in viscera collected in the PHSM is much lower in 2013 than 2003, BaP_{eq} profiles in 2003 and 2013 are even more similar than PAH profiles. Nine of the top 10 PAHs contributing to $\sum BaP_{eq}$, are the same in 2003 and 2013 (Fig. 4C, D). Additionally, the percent contribution of each of these PAHs to $\sum BaP_{eq}$ is almost identical in 2003 and 2013 (Fig. 4C, D).

3.8. Human health risk assessment based on crayfish consumption

The trend for risk estimates associated with 2013 crayfish tissue is opposite of the trend for $\sum$PAH concentrations for the same samples. Estimated risk associated with consuming whole crayfish tissue is highest within the PHSM and lowest upriver of the PHSM, suggesting that risk-driving carcinogenic PAHs are relatively more abundant inside the PHSM (Fig. 5, where ELCR estimates <1 in a million are not shown). $\sum BaP_{eq}$ for crayfish viscera from the highest and lowest RMs are just

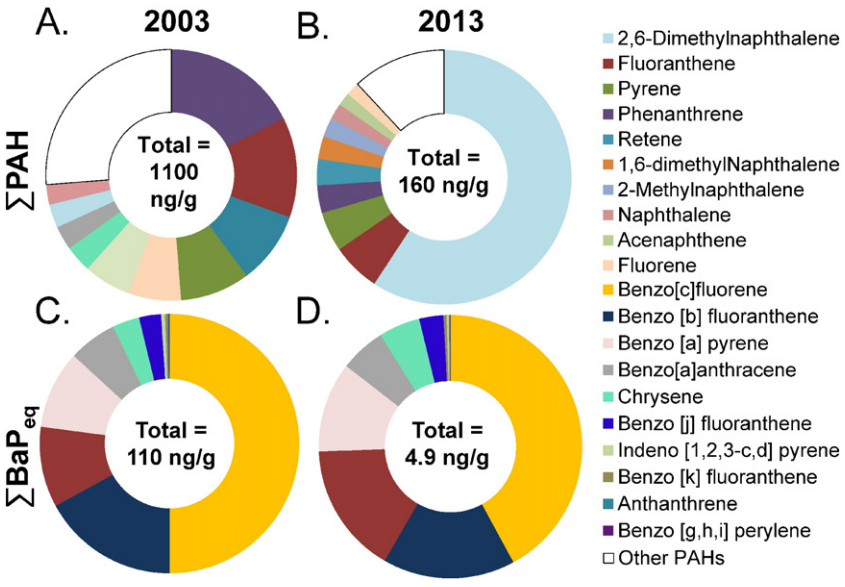

Fig. 4. Percent contribution of the 10 most abundant PAHs to $\sum$PAH in 2003 (A), $\sum$PAH in 2013 (B), $\sum BaP_{eq}$ in 2003 (C), and $\sum BaP_{eq}$ in 2013 (D). PAHs were measured in crayfish viscera collected in the Portland Harbor Superfund Megasite (PHSM) in the Willamette River in Portland, Oregon. The top 10 PAHs contributing to $\sum BaP_{eq}$ in PHSM crayfish viscera are nearly identical in 2003 and 2013. The only PAHs that contribute substantially to both $\sum$PAH and $\sum BaP_{eq}$ are fluoranthene (2003 and 2013), and chrysene (2003). All tissue concentrations are in ng/g wet weight.

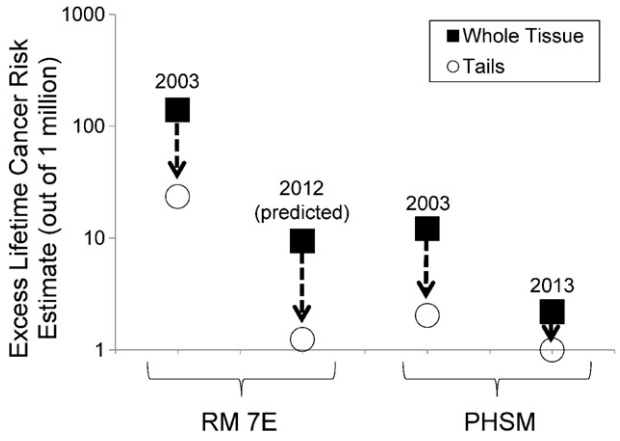

Fig. 5. Average excess lifetime cancer risk (ELCR) estimates associated with eating whole tissue (solid squares) or just tail meat (open circles) of crayfish. ELCRs are presented for crayfish collected at RM 7E (the McCormick and Baxter Superfund site), and in the rest of the Portland Harbor Superfund Megasite (PHSM), in the Willamette River in Portland, Oregon. The 2012 ELC Rs were estimated using PAH concentrations predicted from water concentrations, using the predictive model described in this study and shown in Fig. 2 and presented in Eq. (1). ELCRs for "whole tissue" assume the consumer eats both the viscera and tail tissue. ELCRs less than 1 in a million are not shown.

shy of significantly different (Wilcoxon Rank-Sum test, $p = 0.10$). This illustrates that elevated $\sum$PAH does not necessarily indicate elevated carcinogenic risk, as individual PAHs have different mechanisms of toxicity and potencies.

Even at the 95th percentile ingestion rate, risk related to consuming whole 2013 crayfish is barely above the acceptable level of 1 in a million, with the highest ELCR at an individual site only being 4 in a million (at 3.5W, within the PHSM). All estimated risks are below 1 in 100,000, which is the acceptable risk level the Oregon Department of Environmental Quality applies when evaluating cumulative exposure to all carcinogenic chemicals (DEQ, 2010).

ELCR estimates were one to two orders of magnitude lower in 2013 crayfish tails than in viscera from the same crayfish. Thus, ELCRs estimated based on consuming whole tissue are substantially higher than ELCRs estimated just based on consuming just tails (Fig. 5). Average ELCR estimates for consuming tails were lowest downriver of the PHSM and highest upriver, with ELCR estimates within the PHSM falling in between these two. The highest ELCR for crayfish tails was found in crayfish collected slightly upstream of the PHSM, at RM 12E (LWG, 2013). This site sits right next to a large storm-water effluent pipe, and beneath an overpass of a major interstate highway. This suggests that nonpoint sources (such as storm-water runoff) may be contributing risk-driving PAHs to crayfish tails. This deserves consideration, as point sources are often the focus of remediation efforts. However, these ELCRs estimate that risk associated with consuming crayfish tails is less than 1 in a million at all sites where crayfish were collected in 2013, even at the 95th percentile ingestion rate. Thus, risks associated with eating crayfish tails from sites within or near the PHSM appear to be minimal.

The substantially elevated ELCRs when viscera is included in consumption-based risk estimates show that eating only tails would significantly lower PAH-related carcinogenic risk (Fig. 5). This has important implications for human health. The message to human health risk assessors and consumers is that even if crayfish are being collected from a contaminated area, eating only the tail meat offers a means of reducing personal exposure to carcinogenic PAHs. In the US, crayfish's whole bodies are often cooked in liquid along with other foods (such as a traditional crayfish "boil"). A common practice is to suck the fat and juice from the cephalothorax after a crayfish is boiled, before eating its tail (Ogburn, 2013). These practices may inadvertently extract PAHs from the viscera, increasing the associated risk above that of eating just the tails.

3.9. Comparison of estimated risks in 2013 crayfish, to those for previously collected crayfish

ELCR estimates for PAH mixtures in whole 2003 crayfish spanned three orders of magnitude among sites. ELCRs were substantially higher at RM 7E and within the PHSM than upriver (Fig. 5, where ELCR estimates <1 in a million are not shown). The highest ELCR from 2003 (RM 7E) was more than a factor of 30 larger than the highest ELCR from 2013 (RM 3.5W). However, if RM 7E is removed from the comparison, the highest ELCR from 2013 is the same as the highest ELCR from 2003. This suggests that remedial action has been successfully removing some of the worst sources of contamination from the PHSM over the past 10 years. It also suggests background contamination has remained roughly the same within the PHSM.

ATSDR's PHA for the PHSM concluded that all crayfish contaminant levels produced potential carcinogenic risk estimates below 1 in 10,000, for both average and 95% ingestion rates, and thus that adverse health risks were not anticipated related to crayfish consumption (ATSDR, 2006). This is slightly different from the conclusions reached in the present risk assessment. When the 95% ingestion rate is used, the present assessment estimated an ELCR slightly above 1 in 10,000 in whole tissue from RM 7E in 2003, and ELCR estimates above 1 in a million at RM 7W and 3E in whole crayfish from 2003, and at RM 3.5W in 2013.

The ATSDR PHA notes that there is limited data on crayfish consumption from the PHSM, either commercially or recreationally (ATSDR, 2006). Crayfish ingestion rates may vary among different ethnic or cultural groups. Outside of the PHSM, crayfish consumption is traditionally higher among communities in the southeastern US, especially in Louisiana, than in most other groups in the US. Thus, higher ingestion rates may be needed to be protective of health risk if this were the population of concern.

One factor likely increasing the present estimates relative to those performed in the PHA is that the present analysis uses the EPA's (2010) RPF approach, combined with an analytical method that quantifies 23 of the 26 PAHs with RPFs. The PAH analysis in the PHA only included 5 PAHs, and it was published before the EPA (2010) RPF guidance document. Thus, the present study likely used higher carcinogenic potency values to estimate risk.

Another factor affecting comparisons of these risk estimates is that the PHA analyzed entire crayfish for their risk estimates, while the present study analyzed isolated viscera and tail tissue. Because PAH levels are so much higher in viscera than in tails, incorporating the entire body (including carapace) would dilute visceral PAH levels, reducing risk estimates. Additionally, viscera only contributed 13 ± 4% to total body weight (when carapace is included in total weight) for crayfish used in the present analysis, suggesting that this dilution may be dramatic. In contrast, viscera contributed 55 ± 7% to the whole soft tissue (viscera and tail muscle) included in the present risk estimates. Thus, analyzing the whole organism would likely substantially reduce risk estimates, relative to analyzing the isolated viscera.

This illustrates that risk estimates, and therefore risk management strategies, may be affected heavily by which PAHs are included in analysis, by what toxicity information is available at the time of the assessment, and by which tissues are analyzed to estimate risk. There are many PAHs, and many contaminants, for which toxicity information remains sparse. There are also organisms of interest for which data on PAH levels in the environment are sparse. The more toxicity data risk assessors have, the better they will be able to make risk management decisions that are protective of public health.

3.10. Ability of the model to predict risk estimates

Using the 95th percentile ingestion rate, ELCR estimates predicted in whole crayfish were 9 in a million within the PHSM, and 1 in a million outside of the PHSM. When compared with ELCRs calculated using

measured data (2 in a million within the PHSM and 1 in a million outside of the PHSM), the model predicts risk of crayfish consumption relatively well (predicting within a factor of 5 of measured concentrations within the PHSM, and within a factor of 1 outside of the PHSM).

Additionally, the model was used to predict PAH levels in crayfish in 2012, using only water data as inputs. These data were then used to estimate ELCRs associated with consuming crayfish from RM 7E in 2012 (Fig. 5). The predicted reduction in risk estimates is consistent with crayfish posing less consumption risk post-remediation. These estimates seem reasonable, as they are similar to what was measured at other sites within the PHSM in the following year. This demonstrates that the model can be used to estimate tissue concentrations, which can be used to estimate risk associated with crayfish consumption, based only on water concentrations derived from passive samplers.

3.11. Additional considerations

Uptake and elimination of contaminants by organisms is a complex phenomenon, with various behaviors potentially altering organisms' contaminant levels (Bayen et al., 2009). Bayen et al. (2009) proposed that for passive samplers to most accurately predict contaminant levels in organisms, information is needed about contaminant uptake and elimination in the species of interest, and with the proposed type of passive sampler. LDPE passive samplers are relatively well-studied, and the present model is already performing well, but continued study of crayfish uptake and elimination of PAHs could further improve the predictive capabilities.

Including EPA's (2010) extended list of PAHs with RPFs in human health risk assessments will increase estimates, compared to using EPA's previous 16 priority pollutants, where RPFs were only given for 7 PAHs (EPA, 1993; Zelinkova and Wenzl, 2015). This is discussed further in the SI. It is also important to consider that current methods of estimating risk associated with consuming a mixture of PAHs assume that the toxicities of each of the PAHs in the mixture are additive. However, it has been observed that interactions between individual compounds in a mixture can lead to a greater or less than additive toxic response (Cedergreen et al., 2008). One way to improve risk estimates would be to directly test the PAH mixtures that are repeatedly measured in the environment in toxicity assays. While testing the endless list of potential mixtures would be prohibitively challenging, a starting place may be to assess the combined toxicities of mixtures that commonly occur in the environment. For instance, in the present study, the profile of risk-driving carcinogenic PAHs in PHSM crayfish viscera was almost identical in 2003 and 2013 (Fig. 4C, D). Characterizing the toxicity of this PAH mixture could provide useful information about a real-world exposure. Given that this mixture was conserved in these two sampling events 10 years apart, it may also be similar to the mixture in shellfish at other sites with similar sources of contamination.

The selection of an organism's tissues for analysis can be a major source of variation when directly measuring contaminants in organisms to assess risk. The present work illustrates this issue clearly, because crayfish viscera contained significantly higher $\sum BaP_{eq}$ than tails (Fig. 1B). Risk assessors have traditionally included different tissues in analyses to estimate risk. For instance, the ATSDR's PHA for Portland Harbor used whole bodies, while the EPA's guidance document for fish advisory data suggests only using tails (ATSDR, 2006; EPA, 2000). Given that this decision can substantially affect measured PAH levels, and subsequently risk estimates, care must be taken to ensure that the tissues being analyzed fit the question being asked.

Importantly, the agreement seen in the predictive model suggests that, with only a little more data, it could be used to predict contaminant levels (and subsequently to estimate associated consumption risk) in other shellfish, and perhaps even finfish. Additionally, assessing risks associated with dermal exposure requires only knowledge of water concentrations. Thus, the passive sampling approach described here provides risk assessors a way to estimate risks associated with multiple exposure routes, all from one set of data collected by passive samplers. Thus, only deploying passive samplers in the water would enable practitioners to estimate risks associated with multiple exposure pathways.

4. Conclusions

This work presents a model predicting levels of 34 PAHs in crayfish viscera, using only C_{free} PAH levels in water (measured by LDPE passive samplers). The model predicts PAH levels in crayfish within a factor of 2 of measured values, on average. Analyses revealed that $\sum$PAH are 5–20 times higher in crayfish viscera than in tails, and $\sum BaP_{eq}$ are 6 to 70 times higher in viscera than in tails. This suggests that eating viscera significantly increases cancer risk, compared to eating just tail meat. Additionally, PAH levels in crayfish were not significantly different after 10 years in the PHSM, at sites that have not been remediated, while a significant decrease in PAH levels is predicted in crayfish at a site within the PHSM that has been remediated. Finally, PAH levels in crayfish were significantly higher after 10 years in a suburban area upriver of the PHSM. Additionally, the highest average $\sum BaP_{eq}$ in crayfish tails was measured just upriver of the PHSM, near a stormwater effluent pipe and under a highway overpass. This suggests that nonpoint sources in metropolitan areas, and not just point sources that typically receive remedial attention, may contribute substantially to PAH load in shellfish.

Abbreviations

PAH	polycyclic aromatic hydrocarbon
LDPE	low density polyethylene
PHSM	Portland Harbor Superfund Megasite
C_{free}	freely dissolved concentration of contaminants
BaP_{eq}	benzo[a]pyrene equivalent
HOCs	hydrophobic organic contaminants
PRCs	performance reference compounds
BMOs	biomonitoring organisms
RM	river mile
FSES	Food Safety and Environmental Stewardship
OSU	Oregon State University
LOD	limits of detection
RPF	relative potency factor
ELCR	excess lifetime cancer risk
SPE	solid phase extraction
BLOD	below limits of detection
QC	quality control
RSD	relative standard deviation
2,6-DMN	2,6-Dimethylnaphthalene
USGS	United States Geological Survey

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2015.11.142. Supplemental material includes additional information about sampling, lab processing, calculations, data exploration, and interpretation of results.

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