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EVALUATING POREWATER PAH-RELATED TOXICITY AT A CONTAMINATED SEDIMENT SITE USING A SPIKED FIELD-SEDIMENT APPROACH

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Abstract: Although the complexity of contaminant mixtures in sediments can confound the identification of causative agents of adverse biological response, understanding the contaminant(s) of primary concern at impacted sites is critical to sound environmental management and remediation. In the present study, a stock mixture of 18 polycyclic aromatic hydrocarbon (PAH) compounds was prepared to reflect the variety and relative proportions of PAHs measured in surface sediment samples collected from discrete areas of a historically contaminated industrial estuary. This site-specific PAH stock mixture was spiked into nontoxic in-system and out-of-system field-collected reference sediments in dilution-series spanning the range of previously measured total PAH concentrations from the region. Spiked sediments were evaluated in 10-d *Leptocheirus plumulosus* tests to determine whether toxicity in laboratory-created PAH concentrations was similar to the toxicity found in field-collected samples with equivalent PAH concentrations. Results show that toxicity of contaminated sediments was not explained by PAH exposure, while indicating that toxicity in spiked in-system (fine-grain, high TOC) and out-of-system (course-grain, low TOC) sediments was better explained by porewater PAH concentrations, measured using an antibody-based biosensor that quantified 3-5 ring PAHs, than total sediment PAH concentrations. The study demonstrates the application of site-specific spiking experiments to evaluate sediment toxicity at sites with complex mixtures of multiple contaminant classes, and the utility of the PAH-biosensor for rapid sediment-independent porewater PAH analysis. This article is protected by copyright. All rights reserved

Keywords: Polycyclic aromatic hydrocarbons (PAHs), Sediment toxicity, Sediment porewater, Bioavailability, Biosensor, Novel assessment tool

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are common environmental pollutants that can be released to the atmosphere by incomplete combustion of organic matter (e.g., automobile exhaust, industrial processes, fires) and enter surface water through stormwater runoff, or as discharge from industrial plants and hazardous waste sites (Newman and Unger, 2003; Guo et al, 2011). The PAH class is composed of more than 100 different compounds, 17 of which are considered priority pollutants due to their toxic nature (Boström et al, 2002). Polycyclic aromatic hydrocarbons are environmentally persistent and prone to accumulation in sediments, where they usually occur as a complex mixture of compounds (ATSDR, 1995; Qiao et al, 2006). In addition to mixtures of PAHs, sediments in estuarine environments are often enriched with many other classes of contaminants. The same sources of PAHs to these aquatic systems (e.g., atmospheric deposition of combustion products; industrial effluents; urban runoff) can also contribute significant loads of other organic contaminants and trace metals (Ashley and Baker, 1999; Gunawardana et al, 2012; Mason et al, 2004). Identifying primary contaminant(s) of concern at impacted sites is necessary for sound decision-making concerning environmental management and remediation. However, given the complex nature of sediment contamination, it can often be difficult to link classes of compounds to observed biological effects, especially when multiple contaminant classes correlate with toxic responses (Dévier et al, 2011; Gauthier et al, 2014; Hartzell et al, 2017).

Whereas the majority of PAHs are sorbed to sediment particles, the comparatively small fraction of PAHs dissolved in porewater is commonly considered to be most bioavailable to benthic organisms (ter Laak et al, 2006; Lu et al, 2011; Neff et al, 2005; Di Toro et al, 1991). However, our understanding of the relationship between PAH bioavailability and the various

factors influences PAH partitioning between sediment and porewater (e.g., grain-size, organic matter content. chemical activity) remains incomplete (Chiou et al, 1998; Reichenberg and Mayer, 2006; Semple et al, 2004). Given this knowledge gap, measurement of PAHs in porewater is preferable to extrapolation from whole sediment analysis with corrections for organic carbon content, grain-size and other sediment characteristics. Measurement of PAHs within sediment porewater is traditionally by high-performance liquid or gas chromatography following an elaborate process of filtration, extraction, fractionation, and analysis (Spier et al, 2011). These methods are costly, labor-intensive, and require large volumes of sediment to obtain sufficient porewater volumes for analysis, particularly when low level detection of contaminants is involved (Spier et al, 2011). Portable tools for contaminant detection, including immunoassays, are enabling more rapid site assessment. One such tool, an antibody-based PAH biosensor, was employed in the present study to measure total PAHs in sediment porewater (Li et al, 2016). Previous studies in Bear Creek comparing PAHs in sediment porewater measured by gas chromatography-mass spectroscopy (GC-MS) and the antibody-based biosensor found a strong positive correlation (R^2 = 0.98; slope 0.60; Supplemental Figure S1; Hartzell, 2016).

The present study employed the PAH biosensor technology to investigate contaminant-toxicity relationships in Bear Creek, a tributary of the Patapsco River located near Baltimore Harbor, MD, USA, and adjacent to a former steel manufacturing facility (Figure 1). Sediments within Bear Creek are elevated in PAHs, polychlorinated biphenyls (PCBs), and organochlorine pesticides (Ashley and Baker, 1999; Hartwell and Hameedi, 2007), as well as heavy metals including zinc, chromium, copper, and lead, among others (Ashley and Baker, 1999; Mason et al, 2004; Hartzell et al, 2017). Studies of sediment toxicity in Bear Creek, performed over the past two decades, have demonstrated persistent acute lethality to benthic organisms (McGee et al,

1999, 2004; Hartzell et al, 2017), with the highest toxicity observed in sediments collected nearest to historical sources of industrial activity and diminishing lethality with increasing distances from industrial activity (Hartzell et al, 2017). Concurrent chemical analysis on sediments used in toxicity tests identified multiple and diverse contaminants, many of which correlated with observed toxicity and with one-another, which made assigning causality impossible, and limited the ability to predict effects elsewhere in the system based solely on chemical analysis (Hartzell et al, 2017; Pourabadehei and Mulligan, 2016). Toxicity may be principally the consequence of one contaminant class, or the result of multiple contaminant stressors interacting. Moreover, contributions to toxicity may come from mixtures of contaminants from the same class (e.g., complex PAHs) or from multiple distinct contaminant classes (i.e. metals and persistent organics) (Swarz et al, 1995; Hagopian-Schlekat et al, 2001; Hedfi et al, 2013). Prior attempts to assign causal links between particular contaminants and observed toxicity, either by summing the proportional contributions of individual constituents (McGee et al, 1999, 2004), or by application of a whole-sediment toxicity identification evaluation (TIE) approach (Klosterhaus et al, 2006), yielded inconclusive results. In a recent study of Bear Creek sediments, mortality of test organisms was found to weakly positively correlate with PAHs in both porewater (R^2 = 0.45 p = 0.03) and whole-sediment (R^2 = 0.47 p = 0.025). However, multiple other compounds, including PCBs, total petroleum hydrocarbon (TPH), chromium, and nickel were also positively correlated with mortality (Hartzell et al, 2017).

The present study was designed to determine if PAHs were drivers of sediment toxicity in Bear Creek by investigating whether PAHs alone could account for observed toxicity in field-collected estuarine sediment. The hypothesis tested was that the addition of PAHs to reference

sediment at environmentally-observed concentrations would produce similar toxicity results to those found in field studies. A second objective was to validate the "site-specific" sediment-spiking approach as a tool for future environmental toxicological studies at sites containing complicated contaminant mixtures. The study aimed to assess whether target porewater PAH concentrations could be generated by spiking whole-sediment with a site specific PAH mixture. Quantitation of porewater PAH after spiking was accomplished by employing the antibody-based PAH biosensor (Li et al, 2016).

MATERIALS AND METHODS

Sediment collection

Sediments were collected from two separate sources for the study. The first, referred to as Harbor Sediment, was collected from Bear Creek, within the urban/industrially contaminated Patapsco River system, Baltimore, MD, USA (Figure 1). The particular location of sediment collection within Bear Creek was approximately 2 km north of the region of primary contaminant introduction, and has served as an "in-system" reference sediment with ≥ 90% survival in previous 10-d acute toxicity tests (Hartzell et al, 2017; McGee et al, 1999, 2004). The Harbor Sediment was similar in physical and chemical characteristics to sediments in the historically impacted area in Bear Creek (Table 1). Specifically, the Harbor Sediment was fine-grained (silty-clay), with a high fraction of organic carbon (f_{oc} = 0.056 dry weight), and high porewater content (76% by weight). The second sediment, referred to as Cove Sediment, was collected from Bigwood Cove on the Wye River, MD, USA, an area with no history of industrial activity and low concentrations of PAHs, PCBs and metals (Hartzell et al, 2017). Cove Sediment has served previously as an "out-of-system" reference sediment, with survival of ≥ 98% in multiple 10-d acute sediment toxicity tests (McGee et al, 1999, 2004; Hartzell et al, 2017). Cove

Sediment had a larger average grain-size (sandy-silt), lower f_{oc} (0.016), and smaller porewater fraction (45% by weight) than Harbor Sediment (Table 1).

Sediments from both locations were collected in June 2016, by boat-mounted Ponar® grab sampler, according to standard protocols (ASTM, 2014; USEPA, 1995). In brief, the top 2 cm of approximately 20 grabs at each location were composited in stainless steel bowls and stirred until homogeneous in texture and color and then placed in pre-cleaned 20-L high-density polyethylene HDPE) containers that were capped without headspace. All media was held on ice during collection and transport, and then stored at 4°C until further processed. Sediments were pressed-sieved through a stainless steel 500 μm mesh sieve to further homogenize media, to remove large particles, and to eliminate live organisms, particularly indigenous *Leptocheirus plumulosus*, that could confound sediment toxicity test results (USEPA, 1995).

PAH-stock mixture preparation

Selection of PAH compounds for inclusion in the sediment spike mixture was based on whole-sediment PAH concentrations measured by GC/MS as part of a previous study of Bear Creek sediment contamination and toxicity (Hartzell et al, 2017). Briefly, sediments from 10 sites spanning the historically industrial portion of Bear Creek, and demonstrating acute toxicity ranging from minimal to severe, were analyzed for PAHs by the Geochemical and Environmental Research Group (GERG) at Texas A&M University (Supplemental Table S1). A subset of 18 PAH compounds was selected from the 41 reported PAH analytes (Figure 2). In selecting the suite of individual compounds, criteria included: consistent occurrence at high concentrations in field collected sediments; representative compounds across a wide range of molecular weights, as encountered in field samples; and the availability of pure standards for stock solution creation. A stock mixture was prepared with the 18 constituents added in

approximate proportion to their relative abundance within Bear Creek sediments (Figure 2). Compounds were dissolved in acetone with 5% toluene to a total PAH (ΣPAH) concentration of 43.2 mg mL^{-1}. This stock mixture was then serially diluted (0.5× steps) in acetone to produce stocks decreasing in concentration from 43.2 to 5.4 mg mL^{-1}. Creation of individual stocks allowed introduction of PAHs in geometric series with all treatments receiving equivalent amounts of acetone.

Sediment spiking

Harbor and Cove sediments were divided into equal 1.0 L aliquots in pre-cleaned glass beakers. The quantities of PAHs spiked into beakers were based on calculated dry weight contents of the media (Supplemental Table S2). Briefly, aqueous content for each sediment source was determined by calculating reductions in mass of triplicate samples weighed before and after drying (72 h at 75°C). Results were used to calculate dry weight-equivalents of sediments within beakers by subtracting the calculated mass of aqueous fractions from the total mass. For the Cove Sediment experiment, contents of 1.0 L beakers received 5.0 mL spikes from the stock series. Resulting nominal ΣPAH (ΣPAH_{SED}) concentrations (based on dry wt content) were 254, 127, 642, and 32 mg kg^{-1} (Supplemental Table S3). Two separate Harbor Sediment PAH-spiking experiments were performed. The initial Harbor Sediment experiment (Harbor Sediment I) used 3.5 mL spikes from the stock series to produce nominal ΣPAH_{SED} concentrations of approximately 550, 275, 138, and 69 mg kg^{-1} (Supplemental Table S3). The lower stock volume was required because the Harbor Sediment had a substantially higher water content than the Cove Sediment, so had less solids mass within 1.0 L beakers (Supplemental Table S2). Because even the highest Harbor Sediment exposure produced minimal toxicity in *L.*

plumulosus exposures, a second more concentrated spike series was prepared. In the second experiment (Harbor Sediment II), content from the top stock mixture (i.e., 43.2 mg mL^{-1}) was spiked into 1.0 L beakers at volumes of 10, 5.0, 2.5, and 1.25 mL to achieve nominal ΣPAH_{SED} concentrations of approximately 1,570, 785, 393, and 196 mg kg^{-1} dry weight, respectively (Table 2). One beaker from each series also received an equivalent volume of acetone without PAH amendment (i.e., Cove = 5.0 mL; Harbor I = 3.5 mL; Harbor II = 10 mL) to serve as a solvent control (SC), and another beaker received no acetone to serve as a negative control (CON).

Spiking was achieved by decanting contents of the beakers into a stainless steel laboratory blender and homogenizing continuously while slowly introducing PAH stock solutions by glass pipette. After spiking, contents were returned to their original glass beakers, held covered in the dark at 4°C for 6 d and stirred twice/d with dedicated glass stir-rods. The 6 d holding period with stirring was meant to encourage partitioning of PAHs between sediments and interstitial water. After the mixing period sediment toxicity tests were initiated by apportioning contents of beakers into replicate test beakers as described in the section *Sediment toxicity tests*. At this time triplicate 50 mL samples were also collected to determine porewater ΣPAH (ΣPAH_{PW}) concentrations at the start of each assay (Day 0). An extra replicate beaker was also set up alongside exposure beakers so that triplicate porewater samples could be collected at the end of assays (Day 10) to determine final ΣPAH_{PW} concentrations.

Porewater PAH analysis

Sediment samples were centrifuged in 50-mL conical-bottom HDPE centrifuge tubes (15 min at 3,500 g) to extract porewater. The resulting supernatant was then filtered (0.7 µm glass fiber) to remove remaining particles. The porewater samples were analyzed for total 3-5 ring

PAHs (ΣPAH_{PW}) using the antibody-based PAH biosensor method described in detail by Li et al, 2016. The biosensor utilizes the monoclonal antibody 2G8 to bind and quantify 3-5 ring PAH compounds directly in the aqueous samples with no sample concentration or preparation steps. The KinExA Inline instrument (Sapidyne Instruments, Boise, ID) utilized an automated sample-handling program and was calibrated for total 3-5 ring PAH concentration with phenanthrene standards (ICN Pharmaceuticals) ranging from 0.1 to 2.5 μg L^{-1} made up daily in deionized water by serial dilution from a stock solution. Samples with a detector response outside the calibration curve were diluted with deionized water to bring the instrument response into the most linear portion of the concentration range. Sample handling procedure are described in more detail previously (Spier et al, 2011; Li et al, 2016).

Sediment toxicity tests

Toxicity of spiked-sediment samples was investigated using the benthic estuarine amphipod *Leptocheirus plumulosus* following standard methods (USEPA, 2001). Sediments from each treatment were placed into four replicate 1.0 L glass beakers (175 mL sediment/rep). Dechlorinated, aerated, temperature (23 ± 1°C) and salinity (15 ± 1 ppt; Crystal Sea® Bioassay Formula Marinemix) adjusted municipal water was added to test beakers to a final volume of 1.0 L. Beakers were allowed to sit for 1 d before replacing 90% of the overlying water and adding 20 test organisms/rep (2 to 4 mm length; Chesapeake Cultures, Inc., Hayes, VA, USA). Amphipod survival at the conclusion of the 10-d static exposure period was the only test endpoint. Water quality parameters (temperature, pH, dissolved oxygen, and ammonia) were monitored prior to loading test organisms and daily for the duration of the tests.

Comparison of PAH-spiked and field-collected sediments

Results of *L. plumulosus* exposures to spiked sediments were compared to those of sediment assays performed previously using sediments collected from spatially diverse locations within the historically contaminated portion of Bear Creek (Figure 1). Detailed description of sample locations and methods of collection of field sediments and performance of sediment assays were reported previously (Hartzell et al., 2017). Briefly, toxicity was investigated in 22 surface sediment samples and nine sediment cores (10 cm diameter; sectioned into segments of 0 – 10, 10 – 20, 20 – 30, 30 – 40, 40 – 60, and 60 – 80 cm depth). Porewater from all surface sediments and from all core segments (n = 54) was analyzed for ΣPAH_{PW} by PAH-biosensor. Results of GC-MS analysis of 10 of the surface sediments for ΣPAH_{SED} were used to select the constituents and proportions for the PAH-spike mixture (Supplemental Table S1).

Statistical analysis

Survival effects in *L. plumulosus* 10-d sediment toxicity tests were investigated by calculation of LC50 values using the US EPA Toxicity Relationship Analysis Program (TRAP) tool (Erickson, 2010). Effects levels (LC50 and 95% confidence intervals) were estimated by Maximum Likelihood Tolerance Distribution Analysis with assumption of a Gaussian distribution. For each sediment, separate LC50 values were calculated using ΣPAH_{SED} and ΣPAH_{PW} exposure concentrations, with ΣPAH_{SED} values based on known masses of PAHs spiked into sediments, and ΣPAH_{PW} values calculated as the mean of concentrations measured on Day 0 and Day 10 of exposure periods. Survival data were also tested by one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison. Data not satisfying normality or homogeneity of variance requirements for parametric statistics were tested using Kruskal-Wallis one-way ANOVA on ranks followed by Tukey's multiple comparison. Separate ANOVA were

performed for each test series comparing PAH-spiked treatments to control and to solvent control treatments, with significance level at $\alpha = 0.05$. Relationships between amphipod mortality and PAH concentration in spiked sediment tests were modeled by logistic regression to fit sigmoidal dose-response curves. Statistical analyses and logistic curve fitting were performed using Sigma-Plot version 12.0 (Systat Software, Inc., San Jose, CA, USA).

RESULTS AND DISCUSSION

Spiked sediment porewater ΣPAH concentrations

Porewater PAH concentrations measured in spiked sediments at the beginning (Day 0) and end (Day 10) of *L. plumulosus* tests, along with treatment means are provided in Table 2. Measured ΣPAH_{PW} concentrations decreased substantially (27% to 81%) over 10-d test periods, but maintained clear distinctions between treatment levels (Supplemental Figure S2). The continued decrease in porewater ΣPAH_{PW} concentrations may indicate that the 6 d mixing and holding time was not sufficient for sediment-porewater partitioning to achieve equilibrium. Porewater PAH concentrations resulting from equivalent spiked amounts of the PAH mixture were substantially lower in Harbor Sediments than Cove Sediments (Table 2; Figure 3), demonstrating the influence of higher organic carbon levels and finer grain size in the Harbor Sediment on PAH sorption to particles (Lee et al, 1999; Wang et al, 2001). Greater amounts of the PAH mixture were required to be spiked into Harbor Sediment to achieve ΣPAH_{PW} concentrations equivalent to those of Cove Sediment.

Relationship between porewater ΣPAH concentrations and mortality

Negative control (CON) and solvent control (SC) treatments had survival of ≥ 97.5% in all 10-d *L. plumulosus* tests (Table 2). Exposure of amphipods to the series of Cove Sediment treatments produced a dose-dependent response with the lowest three treatments not differing significantly from negative and solvent controls. The highest Cove treatment (254 mg kg^{-1} whole-sediment concentration) resulted in 90% mortality and did differ significantly from negative and solvent controls (Kruskal Wallis ANOVA on ranks, Table 2). The top Harbor Sediment I treatment (550 mg kg^{-1} whole-sediment concentration) caused only 22.2% mean mortality with lower treatments producing no apparent effect. The Harbor Sediment II treatments, using an altered spiking regime to achieve higher porewater PAH concentrations, produced a dose-dependent response with the top treatment causing 100% mortality, and the top two treatments differing significantly from controls (Kruskal Wallis ANOVA on ranks, Table 2).

Calculated 10-d LC50 values for amphipod survival in Cove and Harbor II exposures are provided in Table 3. Toxicity measures differed between Cove and Harbor sediments when calculated using ΣPAH_{SED} concentrations (i.e., 3.2× higher in Harbor), but were nearly identical when calculated using ΣPAH_{PW} concentrations (i.e., 1.2× higher in Harbor). These results are similar to earlier sediment studies that show measurement of contaminants exhaustively extracted from whole sediments does not accurately reflect the bioavailable or toxic component (Ehlers and Luthy, 2004; Cui et al, 2013; Ortega-Calvo et al, 2015).

Figure 4 provides mortality response curves (logistic regression) for the three exposure series by plotting mortality against log sediment ΣPAH concentrations and log porewater ΣPAH concentrations. Effects of nominal sediment ΣPAH concentrations differ markedly between tests series with Cove Sediments producing lethality at substantially lower ΣPAH concentrations than

Harbor Sediments (Figure 4, top). By comparison, effects based on porewater ΣPAH concentrations for both sediment types approximately overlay one-another (Figure 4; bottom) and produce a harmonious dose response curve with narrow 95% confidence intervals (R^2 = 0.98; Figure 5). Collectively, all treatments with ΣPAH_{PW} concentrations ≤ 8.2 μg L^{-1} produced minimal mortality (i.e., < 3.8%), treatments with ΣPAH_{PW} concentrations of 11.9 to 20.4 μg L^{-1} produced dose-dependent increases in mortality of 11.2 to 32.5%, and ΣPAH_{PW} concentrations ≥ 35.2 μg L^{-1} produced significant mortality (i.e., 71.2 to 100%). A reasonable threshold for ΣPAH_{PW} before the onset of toxicity appears to be approximately 10 μg L^{-1}.

Comparison of PAH-spiked and field-collected sediments

A comparison of ΣPAH_{PW} concentrations vs. amphipod toxicity for PAH-spiked sediment samples and field collected samples collected from Baltimore Harbor as part of an earlier study (Hartzell et al, 2017) is presented in Figure 5. Three separate datasets are compared; surface sediments (circles; n = 22), sediment obtained from cores (diamonds; n = 54), and the combined results of the three PAH-spiked sediment tests (triangles; n = 15). All three datasets span amphipod toxicity from near 100% survival to 100% mortality and are fitted with logistic mortality response curves. Surface sediments were low in measured ΣPAH_{PW} (range 0.3 to 6.7 μg L^{-1}) compared to the concentrations measured in the core samples (range 0.3 to 40.1 μg L^{-1}). Spiked sediment ΣPAH_{PW} concentrations ranged from 0.5 to 85.1 μg L^{-1}.

Mortality in surface sediment toxicity tests correlated only weakly with increasing ΣPAH_{PW} concentration (R^2 = 0.33). Sediments with very low and nearly identical ΣPAH_{PW} concentrations of < 0.5 μg L^{-1} produced mortality ranging from < 10% to > 90%. Moreover, sediments with ΣPAH_{PW} concentrations ranging from 0.3 to 6.7 μg L^{-1} produced mortality of 90 to 100%. This is at odds with results of the PAH-spiked sediment tests that indicate appreciable

PAH-related toxicity should not occur at ΣPAH_{PW} below 10 μg L^{-1}. As ΣPAH_{PW} concentrations in surface sediments were consistently below predicted effects concentrations by more than on order of magnitude, observed toxicity was likely better explained by the abundance and variety of other organic and metal contaminants known to be present in Bear Creek sediments (McGee et al, 1999, 2004; Hartzell et al, 2017). Mortality in sub-surface sediment tests showed a moderate trend of increasing with increasing ΣPAH_{PW} concentration (R^2 = 0.48; Figure 5). Whereas results were generally more in agreement with the PAH-spike response curve than were surface sediments, mortality still occurred in the majority of sub-surface sediments at ΣPAH_{PW} concentrations substantially below predicted effects levels. Improved agreement with the PAH-spike results may indicate that PAHs present in the sub-surface sediments contributed to the overall toxicity of the samples, but that other contaminants were also important contributors to observed toxicity. It is also possible that PAH compounds present in the field sediments but absent from the spiked samples contributed to observed toxicity in field-collected sediments. Previous studies (Unger et al, 2008) have shown that PAH compound toxicity is highly correlated to K_{OW} so the spiked mixture was selected to span the range of molecular weights found in the field sediments. Relative concentrations across the molecular weights were also similar to best simulate the toxicity of the PAH mixtures in the field. Regardless, the occurrence of other PAH contaminants must still be considered as a possible explanation for the increased toxicity seen at lower measured ΣPAH_{PW} concentrations in field collected sediments.

Beyond PAHs, Bear Creek sediments have elevated levels of PCBs, TPH, several organochlorine pesticides, and a number of heavy metals (Ashley and Baker, 1999; Mason et al, 2004; McGee et al, 1999, 2004; Hartwell and Hameedi, 2007; Hartzell et al, 2017). In particular, sediments from Bear Creek are known to contain Cr and Zn, and to a lesser degree, Ni, Cd, and

Pb, in exceedance of screening guidelines (McGee et al, 1999, 2004; Hartzell et al, 2017). Acid-volatile sulfide (AVS) is also abundant in Bear Creek sediments and SEM/AVS ratios are consistently < 1.0 (Mason et al, 2004; Graham et al, 2009), so these and other metals should be largely unavailable (Di Toro et al, 1990). In subsurface sediments, anoxic conditions inhibit sulfur volatilization, increasing the pool of sulfides available to bind metals to the solid-phase and further reduce metal bioavailability (Wang and Chapman 1999; Fang et al, 2005). Assuming metals play a lessor role in the toxicity of sub-surface sediments, mortality in tests with material from cores may have been more attributable to PAHs than was the case for surface sediments. This would explain, in part, the closer agreement of the sub-surface samples to predicted effects.

Total petroleum hydrocarbon also occurs in Bear Creek sediments in exceedance of screening guidelines (Hartzell et al, 2017). Although PAHs are often considered drivers of toxicity within the TPH category, they represent only a small fraction of the large family of legacy environmental contaminants within sediments that originate from crude oil and other petroleum products (Barron et al, 1999; Scarlett et al, 2007). Therefore many hydrocarbons other than those included in the PAH-spike mixture could have contributed to observed toxicity.

CONCLUSIONS

Identifying primary contributors to toxicity at industrial sites can be challenging due to the presence of complex contaminant mixtures and the effects of various physical and chemical factors on bioavailability (Dévier et al, 2011; Gauthier et al, 2014; Ghosh et al, 2003). Methods used previously to better determine the bioavailable toxic fraction of hydrophobic chemicals in sediments and soils include mild solvent extraction of whole sediments and deployment of passive sampling devices (e.g., semi-permeable membrane devices), and normalization of contaminant concentrations to TOC (Cui et al, 2013). Each technique measures or estimates

different portions of the total contaminant pool present, so may have merit identifying potential risks from contaminated sediments. The present study used a new biosensor-based tool to allow rapid quantification of total 3-5 ring PAHs in small volumes of porewater from two distinct sediment types, a fine grained sediment with high TOC, and a course-grained sediment with low TOC. Biosensor results allowed generation of a ΣPAH_{PW} mortality response curve with narrow 95% confidence intervals that was independent of sediment type, i.e., did not require correction for TOC or other sediment characteristics.

The potential for near real-time assessment of sediment toxicity by this approach could provide rapid and economical initial assessment of PAH-contaminated sites to help guide remediation, and could be incorporated into a tiered regulatory framework that takes into consideration the importance of contaminant partitioning and bioavailability (Ortega-Calvo et al, 2015). While the PAH-biosensor approach shows promise, additional research is needed to confirm the utility of the technique for rapid field assessment. Most beneficial would be studies using field-collected sediments from sites contaminated primarily with PAHs and investigating correlation with additional species and toxicological endpoints.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.xxxx.

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Data availability—Data can be accessed by contacting the corresponding author (lyonkos@umd.edu).

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Figure 1. Bear Creek, near Baltimore, MD within the Chesapeake Bay (rectangle on inset map); hashed area indicates historically contaminated region; star indicates in-system reference site.

Figure 2. Mean concentrations of individual PAH congeners in surface sediments from 10 locations within the historically contaminated region of Bear Creek, Baltimore, MD, USA (light bars; left y-axis; error bars are standard deviation); and concentrations of 18 PAHs in the spike mixture (dark bars; right y-axis).

Figure 3. Relationships between whole sediment ΣPAH and porewater ΣPAH concentrations in spiked Cove and Harbor sediments (linear regression with adjusted R^2 reported); sediment ΣPAH concentrations calculated as the mass of PAH spike to dry weight equivalent sediment mass (excludes PAHs present in sediment prior to mixture spike); porewater ΣPAH concentrations measured using the antibody-based PAH biosensor (Li et al, 2016).

Figure 4. Mortality response curves in 10-d *Leptocheirus plumulosus* sediment toxicity tests plotted against log sediment ΣPAH concentrations (top) and log porewater ΣPAH concentrations (bottom); lines fitted by logistic regression model; sediment ΣPAH concentrations calculated as the mass of PAH spike to dry weight equivalent sediment mass (excludes PAHs present in sediment prior to mixture spike); porewater ΣPAH concentrations measured using the antibody-based PAH biosensor (Li et al, 2016).

Figure 5. Comparison between mortality in 10-d *Leptocheirus plumulosus* sediment toxicity tests and measured log concentrations of porewater PAHs (ΣPAH) in Bear Creek surface sediments (circles), sediments collect at depths of 0 to 80 cm from cores (diamonds), and combined results of Cove Sediment and Harbor Sediment I and II spiked sediment assays (triangles); surface and core sediment test results modified from Hartzell et al, 2017; PAH constituents and proportions used in the spike mixture were selected based on measured whole sediment PAHs in a subset of

ten of the surface sediments; porewater ΣPAH concentrations measured using the antibody-based PAH biosensor (Li et al, 2016); logistic mortality response curves (solid lines) and 95% confidence intervals (dashed lines, PAH-spike data only).

Table 1. Location of sediment collection and sediment characteristics

Site Name	Location		Porewater (%)	TOC (%)	Grain size (%)				Shepard's classification
	Latitude (north)	Longitude (west)			Gravel	Sand	Silt	Clay	
Harbor Sediment	39.25329	76.47597	76	5.6	0	7.1	55.8	37.1	Silty-Clay
Cove Sediment	38.89314	76.16551	45	1.6	0	30.1	55.1	14.8	Sandy-Silt

Table 2. Nominal sediment ΣPAH concentrations, porewater ΣPAH concentrations, and *Leptocheirus plumulosus* survival in spiked Cove Sediment and Harbor Sediment experiments

Treatment ID	Nominal sediment ΣPAH[a] (mg kg^{-1})	Measured porewater ΣPAH[b] (μg mL^{-1})			Mortality[c] (%)
		Day 0	**Day 10**	**Mean**	
Cove Sediment					
CON	0	nd	nd	nd	1.2 ± 2.5
SC	0	0.7	0.6	0.6	1.2 ± 2.5
31	31.7	4.2	1.1	2.6	1.2 ± 2.5
62	63.5	11.8	4.7	8.2	3.8 ± 2.5
125	126.9	17.9	10.9	14.4	18.2 ± 14.4
250	253.8	45.1	25.5	35.2	90.0 ± 11.5*
Harbor Sediment I					
CON	0	nd	nd	nd	1.2 ± 2.5
SC	0	0.6	0.5	0.5	1.2 ± 2.5
62	68.7	1.8	0.6	1.2	0
125	137.4	5.3	1.0	3.2	1.2 ± 2.5
250	274.8	9.7	6.2	6.2	3.8 ± 7.5
500	549.6	23.0	8.2	15.6	22.2 ± 10.4
Harbor Sediment II					
CON	0	0.6	0.4	0.5	2.5± 2.9
SC	0	0.6	0.4	0.5	0
200	196.4	14.5	9.2	11.9	11.2 ± 8.5
400	392.7	23.6	17.2	20.4	32.5 ± 14.4
800	785.1	48.8	26.4	37.6	71.2 ± 14.9*
1600	1570.5	109.6	60.5	85.1	100*

[a] Nominal sediment ΣPAH concentration calculated as mass of PAH spike to dry weight equivalent sediment mass (excludes PAHs present prior to mixture spike).
[b] Porewater ΣPAH calculated by PAH-biosensor (Li et al, 2016).
[c] Mean mortality ± standard deviation.
* Treatment differs significantly from CON and SC (Kruskal Wallis ANOVA on ranks; $p < 0.05$).
CON = negative control; SC = solvent control; nd = no data

Table 3. *Leptocheirus plumulosus* survival effects (10-d LC50 and 95% confidence interval) in Cove Sediment and Harbor Sediment II experiments calculated based on sediment total PAH concentrations (ΣPAH_{SED}) and porewater ΣPAH concentrations (ΣPAH_{PW}), and ratio of Harbor Sediment II to Cove Sediment LC50 results

	LC50 (95% confidence intervals)	
Treatment Series	ΣPAH_{SED}[a] (mg kg^{-1})	ΣPAH_{PW}[b] (μg L^{-1})
Cove Sediment	182 (168 – 196)	23.9 (21.7 – 26.1)
Harbor Sediment II	583 (524 – 642)	28.7 (26.0 – 31.4)
Ratio: Harbor / Cove	3.2	1.2

[a] ΣPAH_{SED} concentration calculated as mass of PAH spike to dry weight equivalent sediment mass (excludes PAHs present prior to mixture spike).

[b] ΣPAH_{PW} calculated as the mean of initial and final measured concentrations.

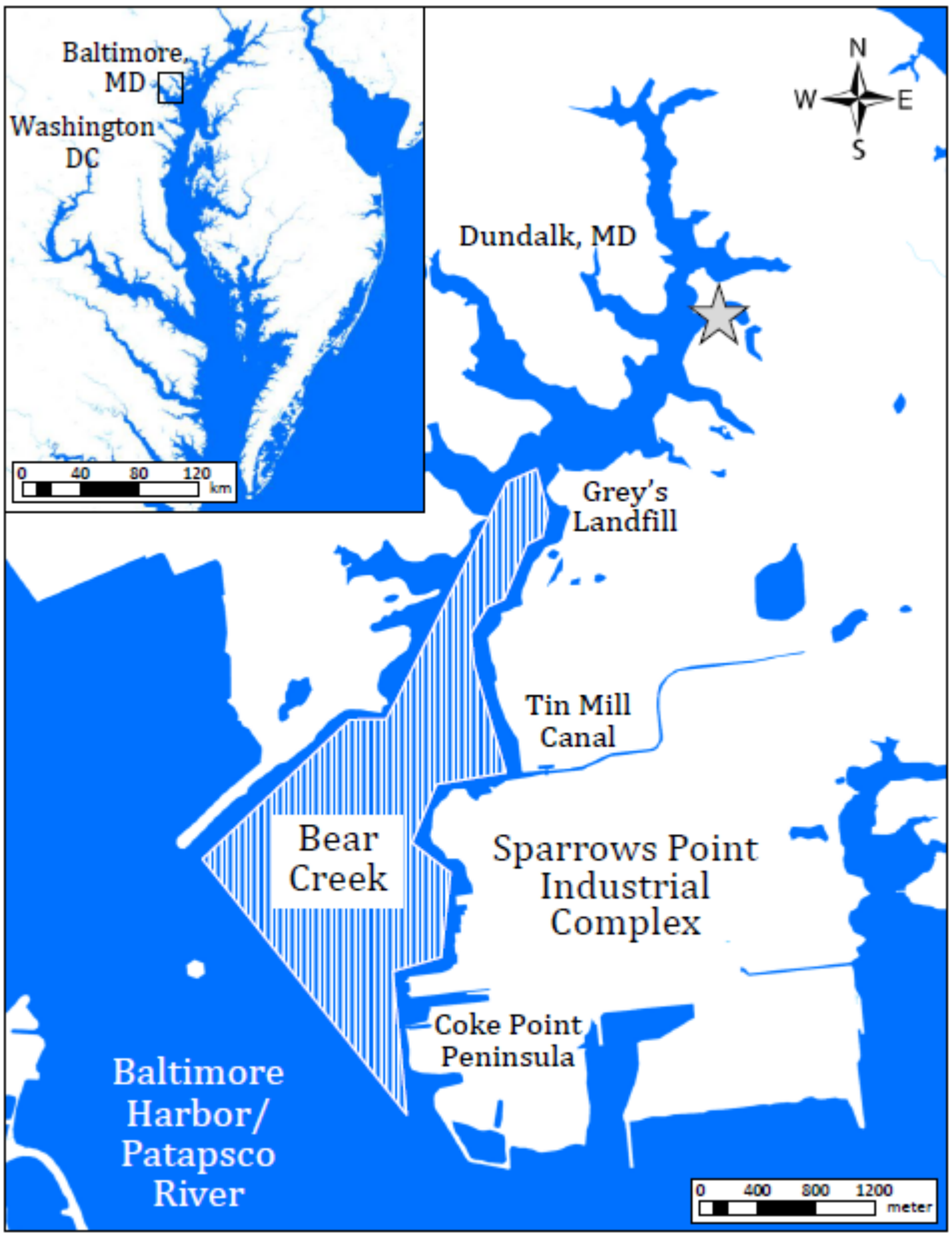


Figure 1

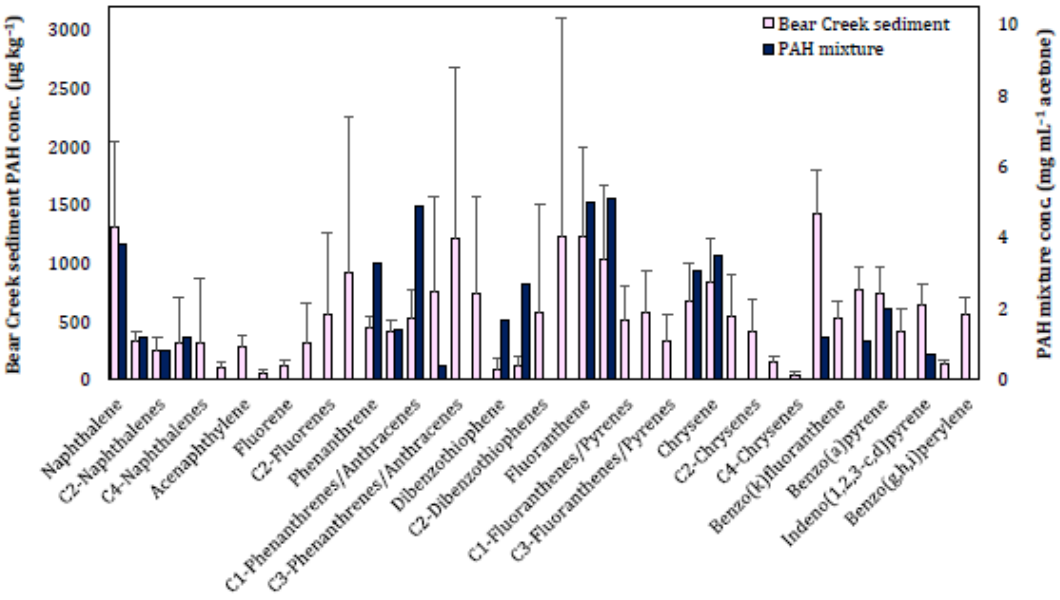


Figure 2

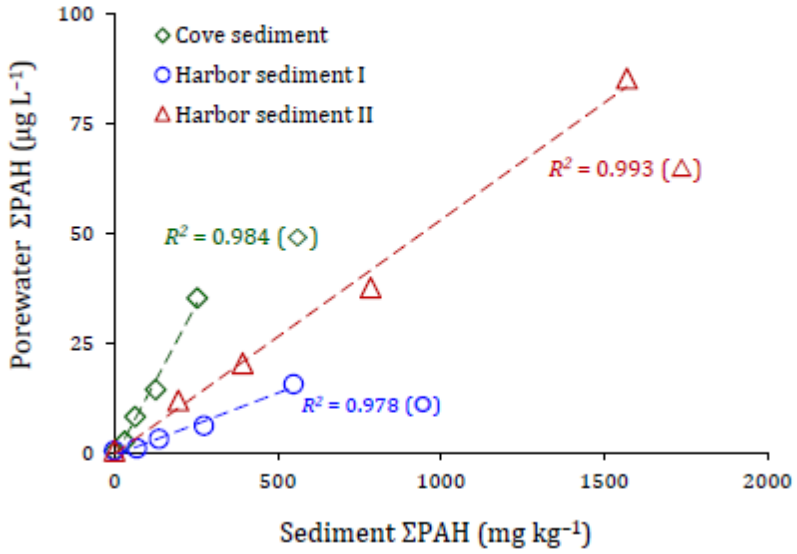
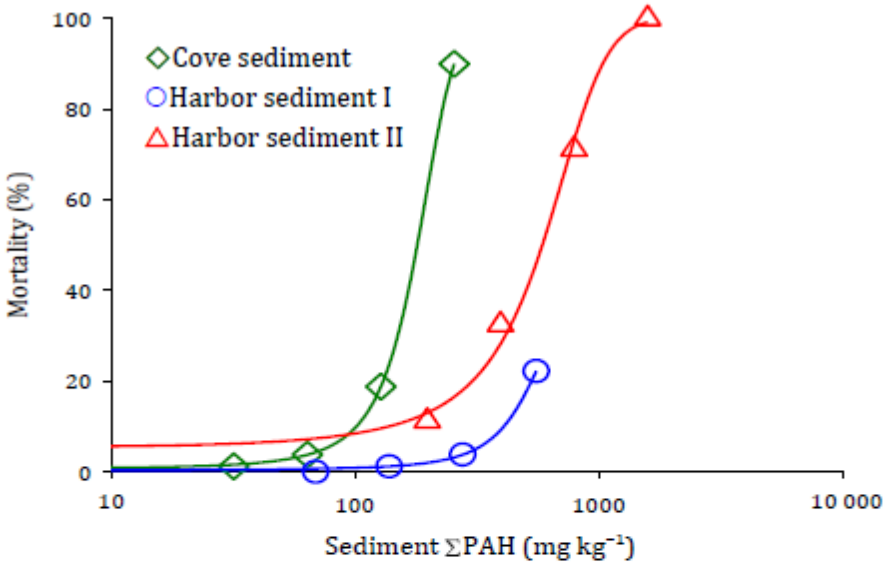


Figure 3



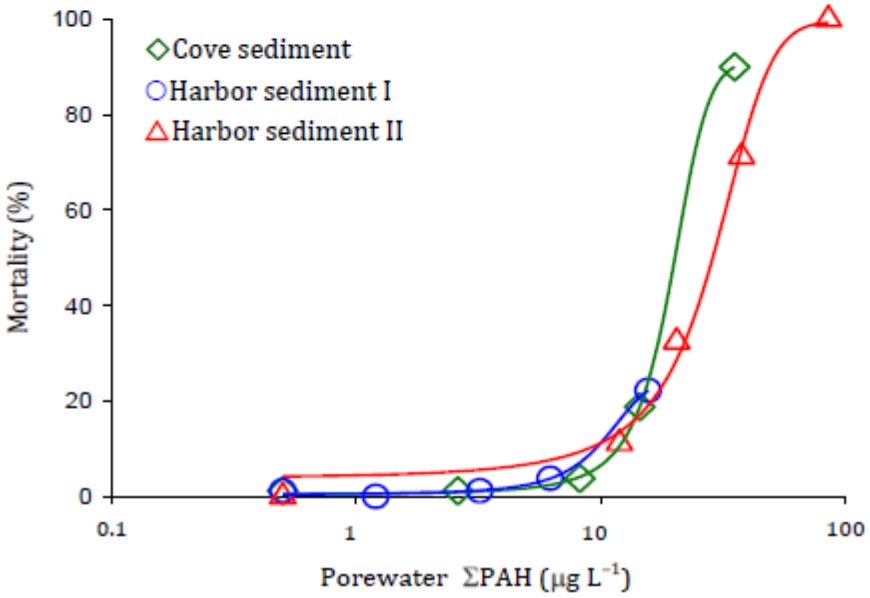


Figure 4

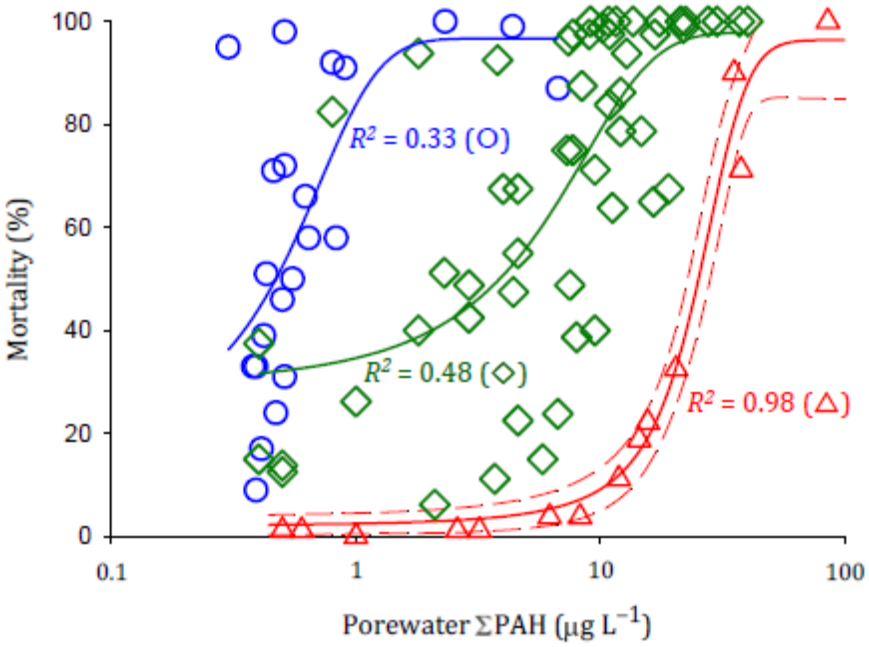


Figure 5