

POLYCYCLIC AROMATIC HYDROCARBON BIOACCUMULATION BY MEIOBENTHIC COPEPODS INHABITING A SUPERFUND SITE: TECHNIQUES FOR MICROMASS BODY BURDEN AND TOTAL LIPID ANALYSIS

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Abstract—Microtechniques for polycyclic aromatic hydrocarbon (PAH) body burden and total lipid analysis were developed and applied to determine the first lipid-normalized bioaccumulation factors for a hydrophobic organic toxicant in a meiobenthic organism (0.063–0.500 mm) living in field-contaminated sediments. The total lipid microtechnique combines the standard Bligh–Dyer extraction method with a colorimetric quantification method for analysis of samples containing 1 to 50 µg lipid. The microtechnique for body burden analysis quantifies PAHs from tissue samples containing as little as 10 pg PAH. Fluoranthene, benz[*a*]anthracene, and benzo[*a*]pyrene biota-sediment accumulation factors (BSAFs) were determined for the meiobenthic copepod *Microarthridion littorale* living in an estuarine U.S. Environmental Protection Agency Superfund site. Gravid female, nongravid female, and male BSAFs were 0.82, 0.54, and 0.36, respectively, for fluoranthene; 0.50, 0.44, and 0.40, respectively, for benz[*a*]anthracene; and 0.09, 0.12, and 0.15, respectively, for benzo[*a*]pyrene. Comparison of nonlipid-normalized bioaccumulation factors (BAFs) to BSAFs indicates that *M. littorale* bioaccumulated PAHs on a gram lipid basis. The BSAFs declined consistently with increasing PAH log K_{ow} for all copepod sex and reproductive stages. Sex- and stage-specific comparisons of BSAFs suggest that differences in lipid content and quality may lead to differences in BSAF values depending on PAH molecular weight and/or hydrophobicity.

Keywords—Lipid Copepod Hydrocarbons Meiobenthos Bioaccumulation

INTRODUCTION

Bioaccumulation of hydrocarbons by meiobenthic organisms (sediment-dwelling invertebrates 0.063–0.500 mm in length) living in contaminated field sites has never been assessed. Yet the meiobenthos occur in almost every habitat and sediment worldwide, represent 20% of the standing animal biomass in estuarine sediments [1], and often thrive in the presence of low to moderate levels of sediment-associated organic contaminants common in urbanized estuaries [2]. The meiobenthos are also the most abundant and productive estuarine sediment metazoans [3] and an obligate food source to juveniles of many commercially important fish and shrimp [4]. A few studies have investigated bioaccumulation of hydrophobic organic contaminants by meiobenthos in the laboratory [5–8], but all used laboratory-spiked sediments as the exposure medium. While laboratory exposures can be tightly controlled and often provide information that cannot be obtained from field studies, laboratory-spiked sediments may not reproduce the contaminant bioavailability of field sediments [e.g., 9,10].

Direct chemical analysis of field-collected sediments and their associated fauna provides the most realistic measure of contaminant bioavailability and is essential for determining the extent of bioaccumulation in natural field systems [11]. To date, field bioaccumulation studies of the meiobenthos have been lacking because analytical methods for organisms in their low microgram mass range have not been available. Determination of meiofaunal biota-sediment accumulation factors

(BSAFs) normalized to organic carbon and organism lipid requires microextraction methods for the quantification of organic contaminants and lipids from microgram masses of tissues. Wirth et al. [6] developed a technique for the extraction and measurement of nonlipid-normalized polychlorinated biphenyls (PCBs) from meiobenthic copepods exposed to PCB-spiked sediments; but this method has not been applied to the analysis of other organic contaminants of concern (e.g., polycyclic aromatic hydrocarbons [PAHs]) in meiofauna. The most commonly used method for organism total lipid determination in lipid-normalized pollutant studies [12] cannot be applied to microscale analyses because it is not reliable for tissue sample sizes <5 g [13]. Microtechniques based on the Bligh–Dyer method have been developed [14,15], but they still require sample sizes of at least several milligrams. Adopting these methods for the meiobenthos would require a minimum of 3,000 to 5,000 individuals per sample to attain acceptable precision, and acquisition of such numbers of individuals is logistically difficult and will mask much of the population variance.

The objective of this study was to develop microtechniques for the extraction and quantification of PAHs and total lipids in field-collected meiobenthic copepods using samples sizes in the low microgram range. Methods were modified from previously published studies and validated in situ with the harpacticoid copepod *Microarthridion littorale* (Pope) inhabiting PAH-contaminated sediments at a U.S. Environmental Protection Agency Superfund site. *Microarthridion littorale* is ubiquitous in U.S. Atlantic and Gulf coast estuaries and occurs frequently as the predominant copepod species in polluted sediments [2]. *Microarthridion littorale* is an infaunal

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species, continuously exposed to sediment-associated contaminants via its sediment-burrowing lifestyle and ingestion of bulk sediments. Laboratory studies have shown that *M. littorale* bioaccumulates PCBs [6,8] and the organophosphate pesticide azinphosmethyl [7] from spiked sediments. Here, the newly developed PAH body burden and total lipid microtechniques were applied to compare fluoranthene, benz[*a*]anthracene, and benzo[*a*]pyrene BSAFs among adult gravid female, nongravid female, and male *M. littorale* collected from Diesel Creek (Charleston, SC, USA) to investigate the importance of lipid content, sex, and reproductive condition in the bioaccumulation of PAHs. This study provides the first carbon:lipid normalized bioaccumulation factors for any hydrophobic organic contaminant by a meiobenthic organism living in naturally field-contaminated sediments.

MATERIALS AND METHODS

Chemicals

Solvents used in sediment PAH analyses were Ultra Resin-Analyzed grade (J.T. Baker, Phillipsburg, NJ, USA). Solvents used in tissue PAH and total lipid analyses were American Chemical Society grade (99.9% purity) or better. Stable isotope standards were obtained from Cambridge Isotope Laboratories (Andover, MA, USA).

Sample collection and preparation for PAH analyses

Microarthridion littorale (Poppe) and their surrounding sediments were collected from a 0.5-m² mudflat area in the upper reach of Diesel Creek during low tide in December of 1999. Diesel Creek is located within a *Spartina alterniflora* saltmarsh of the Koppers Superfund site, a site adjacent to a former fuel depot and a former creosote wood-treatment facility. In addition to nonpoint source runoff associated with urban and industrial activity surrounding the area, the drainage of fuel oil via surface runoff from the former depot site has served as a chronic source of PAH contamination to the creek for many years. Copepods were collected by sieving the uppermost centimeter of sediment and retaining the 125- to 500- μ m fraction in natural seawater for immediate transport to the laboratory. Whole sediments (~300 g) from this immediate 0.5-m² area used for copepod collection were composited and homogenized in a Teflon[®] jar and then immediately spiked with 200 μ l of a standard containing perdeuterated PAH surrogates (fluoranthene-d10, benz[*a*]anthracene-d12, and benzo[*a*]pyrene-d12 in acetone) for recovery determination. Sediments were then shaken vigorously and flash frozen in liquid nitrogen on site. In the laboratory, sediments were lyophilized, rehomogenized, and split into quadruplicate samples for PAH analysis. Adult gravid female, nongravid female, and male *M. littorale* were sorted from sieved sediments and placed in copepod defecation chambers for 2 to 4 h, allowing sufficient time for complete gut clearance but insufficient time for large-scale elimination of tissue PAHs. Defecation chambers were created by submerging a sieve (83 μ m) into a 200-ml glass beaker containing 100 ml artificial seawater and a stirring bar. Copepods were placed inside the sieve and chambers were gently spun on a magnetic stir plate to allow fecal material to pass through the sieve. This procedure ensured complete gut clearance without reingestion of fecal material. Copepods were then loaded into 0.1-ml conical, screw-capped glass microvials (50 copepods per replicate, four replicates each of gravid females and males, three replicates of nongravid females) for a

total of 55 to 80 μ g dry weight of tissue per sample. Vials were held at -20°C for 20 min to allow copepods to settle to the bottom, and all overlying water was removed. Perdeuterated standard was added to each vial and stored at -70°C until analysis.

PAH/organic carbon analyses

Polycyclic aromatic hydrocarbons were extracted from copepod tissues following a method developed by Wirth et al. [6] for the extraction of PCBs from meiobenthic copepods. Briefly, 40 μ l of 4 M sodium hydroxide, 10 μ l of methanol, 50 μ l of iso-octane, and approximately 15 1-mm glass beads were added to each microvial. Copepods were homogenized for 100 s at 3,000 oscillations/min using a Mini-BeadBeater (Biospec Products, Bartlesville, OK, USA) and then centrifuged for 10 min at 3,500 g. The top layer of each vial, containing the iso-octane and PAHs, was transferred to a clean vial and stored at -20°C until analysis. Polycyclic aromatic hydrocarbons were Soxhlet (Millville, NJ, USA) extracted from lyophilized sediments using acetone:hexane (1:1) for 16 h. The extracts were concentrated to 1 ml under nitrogen flow and solvent exchanged to a final volume of 1 ml of hexane. Nonpolar interferences were removed from sediment extracts using a silica gel/alumina column chromatography technique [16], solvent exchanged into iso-octane, and stored at -20°C until analysis.

While the method for tissue extraction is the same as used by Wirth et al. for PCBs [6], a technique for quantification of PAHs was developed here to include low-microgram-range tissue samples. Sediment and tissue extracts were analyzed for fluoranthene, benz[*a*]anthracene, and benzo[*a*]pyrene using a Hewlett-Packard 5890 gas chromatograph (Palo Alto, CA, USA) coupled to a VG70SQ high resolution double-focusing magnetic sector mass spectrometer. Extracts were injected splitless (2 μ l) onto a Restek RTX-5 column (30 m, 0.25 mm i.d., 0.25 μ m film thickness; Bellefonte, PA, USA). Analytes were detected using selective ion monitoring at 4,000 resolution and were quantified by response relative to deuterated analogs. The ramping program consisted of an initial temperature of 90°C (1-min hold), increasing to 210°C at a rate of 10°C/min (12-min hold), and then increasing to 300°C at a rate of 5°C/min (5-min hold). Sediment total organic carbon (6.5 \pm 0.78%; *n* = 4) was determined using a Perkin-Elmer 2400 CHN Elemental analyzer (Wellesley, MA, USA) after sample acidification with 1 M phosphoric acid. At least one sample blank was carried through all extraction and quantification procedures. Tukey's studentized range test was used to detect significant differences in gravid female, nongravid female, and male PAH tissue concentrations.

Lipid analyses

A microtechnique was developed for the analysis of total lipids in tissue samples ranging from approximately 50 to 500 μ g dry weight. A chloroform/methanol extraction based on the Bligh and Dyer [12] method was combined with the colorimetric quantification method used by Van Handel [17], with modifications of solvent and reagent:tissue ratios and apparatus to accommodate smaller sample sizes.

For true measures of lipid-normalized bioaccumulation, total lipid and tissue concentration analyses should be performed on the same organism or, at a minimum, on organisms from the same population on the same day. However, due to copepod size and an insufficient number of copepods at the highly

contaminated site to perform both analyses, lipid analyses were performed on individuals from an estuarine population approximately 50 km north of the Diesel Creek site and approximately nine months prior to PAH analyses. *Microarthridion littorale* for total lipid analyses were collected in March of 1999 from a mudflat located in the intracoastal waterway at the Buck Hall Recreational Reserve, 11 km south of McClellanville (SC, USA) (33°2'46"N, 79°31'47"W). Buck Hall Reserve is a largely uncontaminated estuarine site with total PAH <88 ng/g and undetectable levels of PCBs and pesticides. Copepods were collected and handled using procedures described above for chemical analyses. The day prior to analysis, adult copepods were sorted according to sex and reproductive stage and placed in defecation chambers overnight (no longer than 10 h; see chamber description in Sample preparation section). Four replicates each of 50 gravid female, 200 nongravid female, and 300 male copepods were transferred to 0.1-ml conical glass microvials with Teflon-lined septa and placed in the freezer for 20 min. Once copepods settled to the bottom of the vial, all overlying water was removed. Approximately 30 0.5-mm solvent-cleaned zirconia beads (Biospec Products) and 100 μ l chloroform:methanol (1:1) were added to each sample vial. Tissues were homogenized for 100 s at 3,000 oscillations/min using a Biospec Mini-BeadBeater. Sample vials were centrifuged for 10 min at 900 g to remove copepod tissue from the organic phase, and the supernatant was transferred to a 1-ml conical glass vial. The extraction microvial was refilled with 100 μ l of fresh chloroform:methanol and the extraction was repeated twice for a total of three solvent extractions. Nonlipid contaminants were removed from the combined extracts by back extracting against 150 μ l 0.9% sodium chloride solution. The lower layer containing the chloroform and lipid was removed after centrifugation and transferred to a 10- \times 75-mm glass tube. Glass tubes were placed in a 100°C heating block. After the solvent evaporated, 80 μ l of concentrated sulfuric acid were added to each tube and heated at 100°C for 10 min. Samples were allowed to cool, and then 1.92 ml of vanillin reagent (600 mg vanillin dissolved in 100 ml hot water and added to 400 ml 85% phosphoric acid) was added to each tube. After allowing color to develop for 5 min, 1.5 ml of sample was transferred to a polystyrene cuvette, and absorbance of standards and samples was read at 490 nm (against a reagent blank) on a Spectronic Genesys™ 20 spectrophotometer (Thermo Spectronic, Rochester, NY, USA). Total lipid mass per sample was determined from a calibration curve created using 1, 10, 20, 30, and 40 μ g of cod liver oil (1 mg/ml acetone). Total percent lipid for each sex and reproductive stage was calculated using mean dry weights of pooled individuals. Blank replicates were carried through the complete protocol for all analyses. Hamilton Gastight® syringes (Reno, NV, USA) were used for all solvent transfers.

Lipid extraction efficiency was $119.5 \pm 4.15\%$ as determined by carrying five replicates of 9.4 μ g of cod liver oil standard through the complete extraction and quantification protocol. A standard-addition experiment was conducted to determine if the enhanced recovery could be explained by interferences introduced during extraction that were not present in the calibration standards. Using this technique, increasing amounts of the standard solution were added to vials already containing a constant amount of standard solution. This dilution series was carried through the complete extraction procedure and then treated as a calibration curve. The slope

Table 1. *Microarthridion littorale* dry weight and percent total lipid (± 1 standard deviation)^{a,b}

	Dry weight (μ g)	% Total lipid (dry wt)
Gravid female	1.6 (± 0.05)	7.9 (± 0.4)
Nongravid female	1.1 (± 0.06)	5.8 (± 0.3)
Male	1.2 (± 0.04)	3.3 (± 0.2)
Mean	1.3 (± 0.22)	5.6 (± 2.0)

^a Dry weight standard deviations represent the variability among pools of 50 individuals ($n = 4$). The mean was calculated using the individual data (pools of 50 individuals) for each sex and reproductive stage ($n = 12$).

^b Percent total lipid standard deviations represent the variability among pools of individuals (50 gravid females, 200 nongravid females, or 300 males; $n = 4$ for each sex and reproductive stage). The mean was calculated using the individual data (pools of individuals) for each sex and reproductive stage ($n = 12$).

for this standard addition curve was compared with the slope created by a dilution series (i.e., a standard calibration curve) not carried through the extraction protocol. If the enhanced response in the recovery samples was due to interference from the extraction protocol, then the calibration slope for the standard-addition curve should be different from the slope of the calibration curve created using samples not carried through the extraction protocol. Slopes for both curves were the same, however, indicating enhanced recoveries were not due to artifacts in the sample workup. Lipid values were adjusted using a correction factor of 19%. The method detection limit (1.05 μ g) was calculated as three times the standard deviation of blank values ($n = 5$) from method efficiency measurements. Coefficients of variation ranged from 5.0 to 5.4%.

Dry-weight determination

Dry weights of individual gravid female, nongravid female, and male *M. littorale* were determined by weighing four replicates of 50 adult copepods collected from the Buck Hall site. Copepods were sorted from sediments and placed in defecation chambers (see description in sample preparation section) overnight to allow for complete gut clearance. Copepods were transferred to 0.3-ml conical glass vials and held at -20°C for approximately 20 min. Once copepods had settled to the bottom of the vial, the overlying seawater was removed via syringe and replaced three times in rapid succession with high-pressure liquid chromatography-grade water. Copepods were transferred to tared aluminum weigh boats (precleaned for 96 h at 100°C) using a 100- μ l Wiretrol II glass capillary micropipette (Drummond Scientific, Broomall, PA, USA) and dried for 72 h at 75°C in a covered glass petri dish. Samples were brought to room temperature in a desiccator and weighed on a Sartorius Supermicro S4 balance (0.3- μ g sensitivity; Edgewood, NY, USA).

RESULTS

Total lipid and dry-weight analyses

The total lipid content of adult *M. littorale* was directly associated with sex and reproductive stage (Table 1). Females with extruded/mature eggs (i.e., gravid) contained 1.4 times more lipid than females containing less developed/unextruded eggs (i.e., nongravid). Gravid female lipid content was 2.4 times higher and nongravid female 1.8 times higher than male total lipid. Percent total lipid was determined using the dry

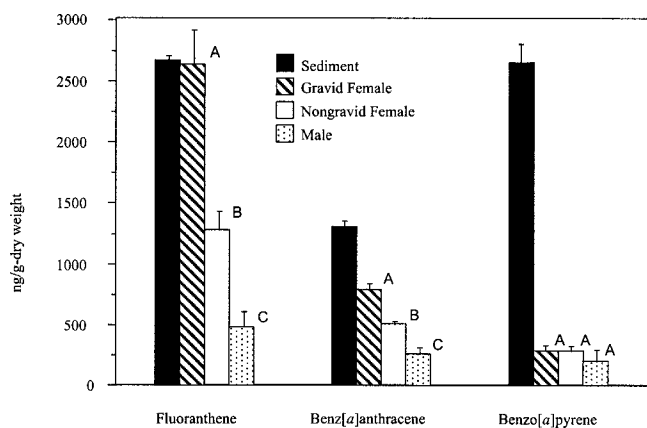


Fig. 1. Mean polycyclic aromatic hydrocarbon concentrations (ng/g dry wt) in Diesel Creek (Charleston, SC, USA) sediments and *Microarthridion littorale*. Means sharing the same letter are not significantly different ($p < 0.05$). Error bars represent one standard deviation of the mean.

weight of individual gravid female, nongravid female, and male *M. littorale* (Table 1).

PAH bioaccumulation

The PAH concentrations in *M. littorale* were consistently lower than their surrounding sediment concentrations for all PAHs (Fig. 1). For all *M. littorale* classes, fluoranthene concentrations were highest, followed by benz[a]anthracene and then benzo[a]pyrene. For both fluoranthene and benz[a]anthracene, concentrations in gravid females were significantly higher than in nongravid females and males, and concentrations in nongravid females were significantly higher than in males ($p < 0.05$). Benzo[a]pyrene concentrations in gravid females, nongravid females, and males were not significantly different from each other, though concentrations in both female stages were higher than in males.

In order to investigate the effects of lipid content on PAH bioaccumulation in *M. littorale*, both bioaccumulation factors (BAFs) and BSAFs were determined for gravid female, nongravid female, and male *M. littorale*. Bioaccumulation factors were calculated by dividing the tissue concentration (ng/g dry wt) by the sediment concentration (ng/g dry wt). Biota-sediment accumulation factors were calculated by dividing the lipid-normalized tissue concentration (ng/g lipid) by the organic carbon-normalized sediment concentration (ng/g organic carbon). In general, BAF trends reflected those observed for concentrations of each PAH (Table 2). For fluoranthene and benz[a]anthracene, differences in mean sex and reproductive-stage BAFs were reduced after lipid normalization to BSAFs (Table 2). The BSAFs decline with increasing log oc-

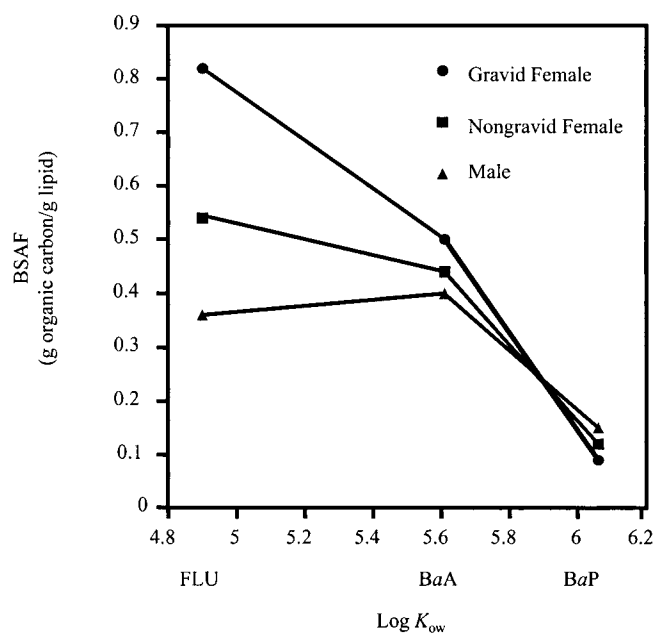


Fig. 2. Biota-sediment accumulation factors (BSAF) versus log K_{ow} (fluoranthene, 4.90; benz[a]anthracene, 5.61; benzo[a]pyrene, 6.06) for *Microarthridion littorale* living in Diesel Creek (Charleston, SC, USA).

anol-water partition coefficient (K_{ow}) [18] for both sexes and reproductive stages (Fig. 2).

DISCUSSION

Total lipid microtechnique

Microarthridion littorale total lipid (mean of all sex and reproductive stages $5.6 \pm 2.0\%$ dry wt) was within range of other infaunal marine crustaceans, including the amphipods *Rhepoxinius abronius* (5.3% dry wt) [19] and *Ampelisca abdita* (10.4% dry wt) [20]. *Microarthridion littorale* total lipid values were lower than values reported for two other meiobenthic harpacticoid copepods, *Schizopera knabeni* (22.2% dry wt) and *Coullana* sp. (31.7% dry wt) [5]. Methods used to determine the total lipid values reported above all utilized chloroform and methanol as extraction solvents. However, total lipids in *S. knabeni* and *Coullana* sp. were quantified by combining individual lipid class measurements using an Iatroscan® (Bioscan, Washington, DC), which combines thin layer chromatography and flame ionization detection, and may have resulted in the higher percent lipid values for those species.

Because hydrophobic neutral organic contaminants are known to associate with the neutral and/or membrane-bound lipid pools and chloroform/methanol extraction targets both pools [21], the Bligh-Dyer total lipid method has been rec-

Table 2. Means and ranges of polycyclic aromatic hydrocarbon (PAH) bioaccumulation factors (BAFs) and biota-sediment accumulation factors (BSAFs) for *Microarthridion littorale* living in Diesel Creek^a (Charleston, SC, USA)

PAH	BAF			BSAF (g carbon/g lipid)		
	Gravid female	Nongravid female	Male	Gravid female	Nongravid female	Male
Fluoranthene	0.99 (0.87–1.14)	0.48 (0.43–0.54)	0.18 (0.14–0.22)	0.82 (0.58–1.13)	0.54 (0.39–0.72)	0.36 (0.23–0.53)
Benz[a]anthracene	0.61 (0.55–0.67)	0.39 (0.36–0.42)	0.20 (0.15–0.29)	0.50 (0.37–0.66)	0.44 (0.33–0.56)	0.40 (0.25–0.68)
Benzo[a]pyrene	0.11 (0.09–0.16)	0.11 (0.08–0.14)	0.08 (0.05–0.13)	0.09 (0.06–0.15)	0.12 (0.08–0.18)	0.15 (0.07–0.32)

^a Means and ranges were determined from replicates of pooled individuals for tissue concentration (50 copepods/replicate, $n = 3$ or 4) and total lipid (50–300 copepods/replicate, $n = 4$), and from replicate sediment and organic carbon samples ($n = 4$).

ommended for use in lipid-normalized pollutant bioaccumulation studies [22]. The Bligh–Dyer extraction method was adapted here for meiobenthic copepods by scaling down solvent:tissue ratios and developing new homogenization and extraction techniques. However, preliminary work using a Sartorius Supermicro S4 balance (0.3- μg precision) indicated that lipid residues from meiobenthic copepods could not be quantified gravimetrically, as in the Bligh–Dyer method, due to the difficulty in weighing such small masses with precision. Thus, a more sensitive colorimetric method originally used to quantify total lipids in individual mosquitoes [17] was adapted here for meiobenthic copepods. Previous work has shown that the Van Handel method yields comparable results to the Bligh–Dyer method [23]. The total lipid method developed here is logistically simple, sensitive (method detection limit $\sim 1 \mu\text{g}$ lipid), and precise (mean coefficient of variation 5.2%). Excluding sample preparation, actual lipid analysis of 10 to 15 samples takes only 3 to 4 h. Total lipid content can be determined with precision from $<100 \mu\text{g}$ of dry tissue, thus allowing inclusion of microinvertebrates and their larval stages in lipid-normalized pollutant bioaccumulation studies.

PAH bioaccumulation

The reduction in the range of mean bioaccumulation factors among copepod sex and reproductive stages for fluoranthene and benz[*a*]anthracene after lipid normalization to BSAFs indicates that *M. littorale* bioaccumulated these sediment-associated PAHs on a gram lipid basis as predicted by the equilibrium partitioning bioaccumulation model [24]. The PAH tissue concentration was associated with reproductive status (i.e., total lipid content), with lipids providing a mechanism for higher PAH accumulation in females over males and gravid females (including eggs) over nongravid females. Similar results [5] were observed for the meiobenthic copepod *Coullana* sp., with gravid females accumulating more than twice the tissue concentration of fluoranthene than nongravid females after 24-h exposures to ^{14}C -fluoranthene-spiked sediments in the laboratory. Because females can trap higher burdens of total PAHs in their more lipid-rich tissue, the approximate twofold difference in total lipids between female and male *M. littorale* may enable females to tolerate higher concentrations of these and other sediment-associated nonionic organic contaminants as well. The PCB Aroclor 1254 (Monsanto, St. Louis, MO, USA) median lethal concentration for *M. littorale* is twice as high for females than males [25], and after 24-h aqueous exposures to mixtures of the pesticides DDT and chlorpyrifos, 14% of females survived compared with 1% of males [26]. Furthermore, the association of PCBs within lipid-rich eggs enabled females of the marine planktonic copepod *Acartia tonsa* to eliminate PCBs twice as rapidly as males following exposure to ^{14}C -PCB-spiked seawater [27].

Tissue concentrations substantially lower than surrounding sediment concentrations (Fig. 1) are likely due to the occurrence of metabolic pathways enabling *M. littorale* to eliminate PAHs. The meiobenthic copepods *S. knabeni* and *Coullana* sp. have limited ability to metabolize fluoranthene [5], and several amphipods have demonstrated some ability to metabolize PAHs with various efficiencies [e.g., 20,28–30]. Alternatively, because the turnover of interstitial water is often faster than the desorption rates of PAHs in oxic sediments [31], *M. littorale* may be at equilibrium with interstitial water concentrations yet maintain tissue concentrations lower than surrounding sediment concentrations.

Microarthridion littorale field-derived BSAFs for fluoranthene (Table 2) were comparable with laboratory-derived BSAFs determined for the meiobenthic copepods *S. knabeni* (0.51–0.80) and *Coullana* sp. (0.22–0.67) [5]. Fluoranthene BSAFs for *M. littorale* were also comparable with the freshwater amphipods *Diporeia* sp. (0.345–0.818) and *Hyaella azteca* (0.161–0.612) [28]. *Microarthridion littorale* BSAFs were similar to that of the marine amphipod *A. abdita* for benzo[*a*]pyrene (0.147) and three to four times higher than *A. abdita* for benz[*a*]anthracene (0.126) [20]. The BSAFs for the marine amphipod *R. abronius* exposed to field-contaminated sediments in the laboratory were lower than *M. littorale* BSAFs; however, the trend in bioaccumulation was the same as in this study (Fig. 2) relative to compound hydrophobicity [32]. *Microarthridion littorale* BSAFs were also within the two- to threefold range of those expected under equilibrium partitioning conditions [33].

As stated previously, for logistical reasons, our reported lipid values were not measured on the same copepod population or during the same month as PAH body burdens. Sampled populations were in relatively close proximity to one another and were likely exposed to a food supply of similar quality and quantity [34]; therefore, significant differences in total lipid values between populations were not expected. It is possible that mean lipid levels may have changed over the three-month winter time differential even though harpacticoid food production is normally at an ebb during these coldest winter months [35,36]. The agreement of *M. littorale* BSAFs with other estuarine benthic species and the observation that total lipid values fell within the range of other infaunal crustaceans suggest that the sampling differential is not a major concern in these BSAF measurements, but we cannot be certain without additional seasonal profiling of lipid content and character.

In addition to the benthic crustaceans *M. littorale* and *R. abronius*, the decline in BSAFs with increasing log octanol–water partition coefficient (K_{ow}) has been observed for other freshwater and marine species in field studies of PAH bioaccumulation [e.g., 37,38], including a study performed at the same site sampled in this study [10]. It has been suggested that this relationship is likely a result of reduced bioavailability that occurs when PAH sorption to sediment organic carbon increases as compound hydrophobicity increases [e.g., 37]. Reduced PAH bioavailability with increasing log K_{ow} may explain the low bioaccumulation of benzo[*a*]pyrene by all *M. littorale* in this study, regardless of lipid content. Lower accumulation of very high log K_{ow} PAHs (log $K_{ow} > 5.5$) in fish was suggested to be the result of increased incompatibility in lipid phases/membranes with increasing solute size/ K_{ow} [39] and may explain the low accumulation of benzo[*a*]pyrene in this study as well. Preferential binding of higher log K_{ow} PAHs to soot particles in sediments may also contribute to decreased bioavailability [40]; however, soot content of Diesel Creek sediments was not determined. Diesel Creek is located in a highly urban and industrialized area, and the sampling site is located in the high intertidal zone where high levels of soot particles may be expected to accumulate as a result of surface runoff, atmospheric deposition, and/or tidal forcing.

Lipid normalization reduced the range of mean bioaccumulation factors (BAFs to BSAFs) among sex and reproductive stages, yet small differences in BSAFs still exist. Under lipid:carbon normalization and equilibrium partitioning conditions, BSAFs for each PAH should not vary by sex or re-

productive stage. Instead, in this study, the range of mean BSAFs for each PAH among the different sex and reproductive stages decreased with increasing log K_{ow} . Higher BSAFs were associated with increased lipid content for fluoranthene and possibly benz[*a*]anthracene (Fig. 2). This trend may have resulted from compound-specific differences (e.g., molecular structure/weight and/or partitioning behavior) in the diffusion of PAHs into organism lipid in general and/or enhanced diffusion into specific types or classes of lipid. More lipid-rich organisms may contain discrete droplets of storage lipid and likely have differing amounts of specific lipid classes. Smaller, low molecular weight PAHs such as fluoranthene may have a greater ability to permeate higher molecular weight lipids such as egg yolk vitellin, leading to enhanced body burdens in lipid-rich females. Thus, even after lipid normalization, differences in PAH molecular structure and weight as well as variations in organism lipid content may contribute to differences in BSAF values that are directly related to extrinsic but often overlooked factors such as sex and reproductive condition.

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