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HYDROCARBON CONCENTRATIONS AND PATTERNS IN FREE-RANGING SEA OTTERS (*ENHYDRA LUTRIS*) FROM BRITISH COLUMBIA, CANADA

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Abstract—With oil pollution recognized as a major threat to British Columbia's recovering sea otter (*Enhydra lutris*) population, it is important to distinguish acute from chronic exposures to oil constituent groups in this marine mammal. Concentrations and patterns of alkanes and polycyclic aromatic hydrocarbons (PAHs) were determined in blood samples from 29 live-captured sea otters in two coastal areas of British Columbia, as well as in representative samples of their invertebrate prey. Hydrocarbon concentrations in sea otters were similar between areas and among age and sex classes, suggesting that metabolism dominates the fate of these compounds in sea otters. Biomagnification factors derived from PAH ratios in otter:prey supported this notion. Although some higher alkylated three- and four-ring PAHs appeared to biomagnify, the majority of PAHs did not. The apparent retention of alkyl PAHs was reflected in the composition of estimated sea otter body burdens, which provided an alternative way of evaluating hydrocarbon exposure. Alkyl PAHs made up $86 \pm 9\%$ of estimated body burdens ($4{,}340 \pm 2{,}950$ μg), with no differences between males and females ($p = 0.18$). The importance of measuring both parent and alkyl PAHs is underscored by their divergent dynamics in sea otters, with ready depuration of parent PAHs (metabolized or excreted) by sea otters on the one hand and biomagnification of alkyl PAHs on the other. Environ. Toxicol. Chem. 2011;30:2184–2193. © 2011 SETAC

Keywords—PAHs Alkanes Hydrocarbons Sea otters Accumulation

INTRODUCTION

Sea otters (*Enhydra lutris*) are the largest member of the family Mustelidae and the smallest marine mammal. The global population once ranged from the northern Japanese archipelago, through the Aleutian Islands, and along the North American coast as far south as Baja California [1].

Decimated by the maritime fur trade of the 18th and 19th centuries, sea otters existed only in remnant populations by the time of the signing of the International Fur Seal Treaty in 1911 [1], and the British Columbia (BC) population was extirpated by 1929 [2]. After the reintroduction of 89 individuals between 1969 and 1972, the BC population has grown to an approximately 4,700 otters [3]. The majority of these (approximately 4,000) inhabit the western coast of Vancouver Island, and a smaller population (approximately 700) exists adjacent to the central mainland coast [3]. Although no data exist for the province's pre–fur trade sea otter abundance, current numbers are much lower than the estimated carrying capacity of >50,000 otters [4].

Oil is considered the primary threat to BC sea otters because of the relatively small size of the population, geographical constraints to their distribution (sea otters currently occupy only ~ 30% of their original range), proximity of the population to shipping lanes [5], and life history characteristics. The extreme vulnerability of sea otters to acute oil exposure was demonstrated by the 1989 *Exxon Valdez* oil spill in Alaska, in which an estimated 4,000 sea otters (95% confidence interval [CI] = 1,904–11,157) died [6]. Unlike other marine mammals, sea otters do not have a blubber layer, and rely instead on the thickest fur coat in the animal kingdom (~ 100,000 hairs/cm^2) to maintain body temperature [7]. Fouling destroys the fur's insulative capacity, requiring otters to spend a great deal of time grooming (~ 3 h/d) [8]. This renders them vulnerable to the inhalation and ingestion of oil during spill events, which can lead to pulmonary emphysema, gastric erosion, and hemorrhagic diarrhea [9]. In addition, very high metabolic rates require that sea otters eat approximately 25% of their body weight per day [7], which can lead to potentially important contaminant exposures through the consumption of large amounts of prey.

Despite these concerns, only limited hydrocarbon data exist for wild sea otters [10,11]. In other marine mammals, hydrocarbon concentrations have been measured in blubber from both live [12] and dead [13] animals. Furthermore, very little is understood regarding the relative importance of diet as a hydrocarbon exposure route in marine mammals in general and in sea otters in particular. The few studies on hydrocarbon movement through marine food webs either do not include marine mammals [14,15] or do not attempt to construct diet [13].

Although wildlife toxicology studies have inherent value in providing a real-world signal of contaminant transport and fate, they typically lack mechanistic certainty in accurately characterizing trophic transfer. A basic understanding of feeding ecology is typically available for some species, but detailed, lifelong prey preferences for a given (sampled) individual represent a technical impossibility. As such, indirect tools to describe diet for marine mammals include stable isotopes of carbon and nitrogen, stomach content analysis, and scat analysis, among others [16–18]. The design and analysis of food baskets have also provided a more integrated means of describing contaminant exposures through the consumption of prey by a marine mammal [19].

All Supplemental Data may be found in the online version of this article.

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Sea otters occupy a low trophic level, consuming benthic invertebrates from as many as seven phyla [18]. Although the specific dietary preferences of sea otters in BC are not well understood, previous work on patterns of predation following introduction to an area [7], together with studies from other areas of coastal North America [18,20], paint a picture of an adaptable, omnivorous consumer of several species of marine invertebrates. These prey species are generally inefficient metabolizers of hydrocarbons [21] and may therefore act as hydrocarbon reservoirs. By comparing hydrocarbon concentrations and patterns in sea otters and invertebrates, one can explore issues related to metabolism and bioaccumulation.

Oil is made up of thousands of organic compounds, the most abundant of which are hydrocarbons, representing more than 75% of oil by weight [22]. Hydrocarbons are ubiquitous in the marine environment, originating from both natural and anthropogenic sources [22]. Important natural sources of pyrogenic (combustion-derived) hydrocarbons include forest and grass fires, whereas anthropogenic sources include vehicular and industrial emissions. Petrogenic (petroleum-derived) hydrocarbons originate from natural sources including oil seeps and coal and shale deposits, and anthropogenic sources including oil spills and leakage [22]. One class of hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), is known be toxic to biota, including mammals [23].

Marine sediments can act as both a sink and a source of hydrocarbons to the adjacent food web, such that sediment quality guidelines can help to guide risk-based assessments [24]. Variable concentrations and patterns of contaminants over space and time render such risk assessments difficult, but the availability of sediment hydrocarbons for biological uptake into adjacent food webs represents an important concern in the case of contaminated environments, endangered wildlife, and/or human health [21]. Furthermore, a preferential partitioning of some compounds from sediments to food webs may lead to contaminant patterns in biota that differ from those in sediment, rendering risk assessments based on sediment quality alone incomplete.

In the face of port development, increasing tanker traffic, and potential offshore oil and gas exploration and development in BC, we examined hydrocarbon concentrations and patterns in sea otters and their prey, thereby providing insight into ambient hydrocarbon signatures as well as a characterization of exposure, accumulation, and metabolism of this contaminant class.

MATERIALS AND METHODS

Sea otter captures

Sea otters (*E. lutris*) were live-captured in September, 2003, on the central mainland coast of BC near Bella Bella (BB) and in September, 2004, in the entrance area of Esperanza/Nuchatlitz Inlets (EN) on the western coast of Vancouver Island (Fig. 1). Permits were obtained from the Fisheries and Oceans Pacific Region Animal Care Committee and section 73 of the Species at Risk Act for scientific research (permits 03-011 for 2003 and 04-017 for 2004). In 2003, nets were set overnight, with captured otters retrieved beginning at approximately 7:30 AM the next morning. Elapsed time

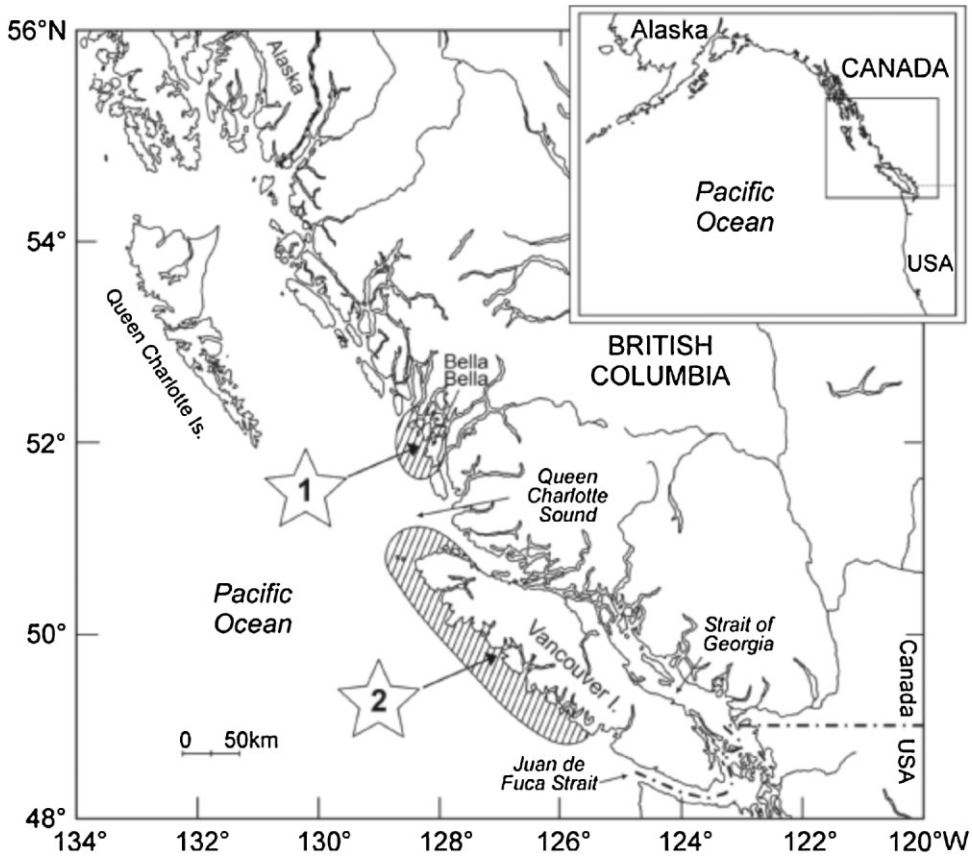

Fig. 1. Sea otters were live captured near Bella Bella (BB) on the mainland central coast of Canada in 2003 (indicated by 1 on map) and in the Esperanza Inlet/Nuchatlitz Inlet (EN) areas on the west coast of Vancouver Island in 2004 (indicated by 2 on map). Prey collection also occurred in the EN area. Sediment samples (results reported by Harris et al. [27]) were also collected in the EN areas. The range of sea otter habitat on the west coast of British Columbia as of 2009 is denoted by the shaded areas [3].

between retrieval and release ranged from 82 to 213 min (average ± standard deviation, 126 ± 43.0). In 2004, elapsed time between retrieval and release ranged from 64 to 208 min (129 ± 36.8).

Sea otters were captured using nylon tangle nets up to 100 m long and 5 m deep, with minimal weighting and suspended by floats. Nets were set by anchoring both ends in an area where sea otters were observed to raft or pass through to access a rafting area. Individual sea otters caught in the net were manually extricated by lifting the net section out of the water and sliding and cutting the net off the animal. The animal was placed in a wooden restraint box with a sliding lid and ventilation holes. Sea otters were administered fentanyl (compounded at Macdonald's Prescriptions) and diazepam (Valium, Sabex) by injection into the hind flipper at a dose of 0.22 mg/kg body weight and 0.07 mg/kg body weight, respectively. Once sedated, the animal was removed from the box to a work surface for measuring and sampling.

Fur samples and 60 to 100 ml of whole blood were collected from each animal (Table 1). Blood was taken from the jugular vein with a 20-gauge one-inch needle and a 20-ml eccentric-tip disposable syringe and immediately transferred to a no-additive Vacutainer® tube (sterile interior) and placed in a cooler (4°C). Blood samples were transferred to a −80°C freezer at the Institute of Ocean Sciences until analysis at Axys Analytical Services. On completion of sampling, the animal was returned to the box, and 0.44 mg/kg body weight naltrexone (compounded at Macdonald's Prescriptions) was injected. The animal was released near the capture site when judged by the veterinarian to be fully alert. Otters were monitored visually as they left the release site.

Prey collection

Sea otter prey items were collected in July and August, 2007, and August, 2008, in the EN areas (Fig. 1). No oil spills were reported in this remote area during the sampling period. Invertebrates representing various combinations of habitat use and feeding ecologies were collected to encompass possible variations in hydrocarbon concentration and patterns likely to be encountered in sea otter prey (Table 1).

Black turban snails (snails; *Tegula funebralis*; intertidal grazer) and California mussels (mussels; *Mytilus califonianus*; intertidal filter feeder) were collected opportunistically in July, 2007, at low tide at 49°48′46″N; 126°57′10″W. Geoduck clams (clams; *Panopea abrupta*; benthic filter feeder) were collected by hand on August 29, 2007, by the Underwater Harvester's Association aboard the Arran Tide II under Research License XR 43 2007 as issued by Fisheries and Oceans Canada. Collection occurred in Pacific Fishery Management Area 25-13-A. Clams were immediately shipped in sea water to the Institute of Ocean Sciences. Finally, 10 red rock crabs (crabs; *Cancer productus*; benthic scavenger/predator) were collected in water depths ranging from 4 to 10 m (49°48′54″N, 126°59′28″W, and 49°49′43″N, 127°58′16″W). Traps were baited with herring in bait jars and set for at least 12 h.

All species were depurated in sea water for at least 18 h to void gastrointestinal contents and to support prey-specific measurements of hydrocarbons. Hydrocarbons in gut contents would only reflect very recent intake by prey, could be biased by considerable temporal and spatial variability, and would preclude an integrated measure of uptake and accumulation by prey over time. Samples were then frozen at −80°C until homogenization. The soft tissue of snails, mussels, and clams and crab hepatopancreas were homogenized to create single samples for each species analyzed. We focused on hepatopancreas in crab because hydrocarbons are lipophilic compounds, and previous work has detected the majority of the hydrocarbon burden in this tissue [25]. Homogenate was stored at −80°C until analysis at Axys Analytical Services.

Tissue hydrocarbon analysis

Whole-blood samples from the 29 sea otters and homogenized soft tissue from four prey species were analyzed for alkanes and PAHs by Axys Analytical Services using high-resolution gas chromatography (GC)/low-resolution mass spectrometry (MS) (Table 2). Blood samples were spiked with a suite of perdeuterated surrogate standards, with d_{50}-tetracosane (n-C_{24}) used as a surrogate for the alkanes, and the following 16 PAH surrogates used for all PAHs: naphthalene (Na), acenaphthylene (Ayl), phenanthrene (Ph), fluoranthene (Fl), benz[*a*]anthracene (B*a*A), chrysene (Ch), benzo[*b*]fluoranthene (B*b*F), benzo[*k*]fluoranthene (B*k*F), benzo[*a*]pyrene (B*a*P), perylene (Per), dibenz[*a,h*]anthracene (D*a,h*A), indeno[1,2,3-*cd*]pyrene (IP), benzo[*ghi*]perylene (B*ghi*P), biphenyl (Bi), 2-methylnaphthalene, and 2,6-dimethylnaphthalene. Samples were then extracted by shaking with a mixture of ethanol,

Table 1. Blood and fur samples were collected from live-captured sea otters (*Enhdyra lutris*) from two sites in coastal British Columbia, Canada: Bella Bella (BB; 2003) and the Esperanza/Nuchatlitz Inlet (EN) areas (2004)[a]

	Body weight (kg)	Matrix	% lipid	$\delta^{15}N$	$\delta^{13}C$
Sea otters (*E. lutris*)					
Bella Bella					
Juveniles ($n = 6$)	20.4 ± 3.3	Blood	0.12 ± 0.034	13.6 ± 0.641	−12.6 ± 0.880
Adult males ($n = 5$)	37.5 ± 0.72	Blood	0.14 ± 0.053	13.2 ± 0.314	−12.2 ± 0.174
Adult female ($n = 1$)	23.8	Blood	0.18	13.1	−13.7
Esperanza/Nuchatlitz inlets					
Juveniles ($n = 5$)	17.6 ± 6.5	Blood	0.21 ± 0.029	14.9 ± 1.09	−12.7 ± 1.15
Adult males ($n = 6$)	31.6 ± 6.4	Blood	0.25 ± 0.10	13.3 ± 1.12	−12.9 ± 1.33
Adult females ($n = 6$)	25.5 ± 1.4	Blood	0.33 ± 0.22	13.9 ± 0.614	−12.3 ± 0.641
Prey samples	Soft tissue weight (g)				
Geoduck clam (*Panopea abrupta*; composite of 6 individuals)	588 ± 215	Soft tissue	0.78	9.95	−14.8
California mussel (*Mytilus californianus*; composite of 6 individuals)	69.7 ± 21.5	Soft tissue	2.00	10.2	−17.2
Turban snail (*Tegula funebralis*; composite of 24 individuals)	2.25 ± 0.55	Soft tissue	2.92	10.1	−13.8
Red rock crab (*Cancer productus*; composite of 10 individuals)	8.92 ± 2.28	Hepatopancreas	9.70	10.9	−19.4

[a] Marine invertebrates were collected in the EN areas in July and August, 2007, and August, 2008. Values represent average ± standard deviation. The juvenile age class ranges from unweaned pups (< one year) to subadults (up to three years). The adult age class encompasses otters > three years.

Table 2. Sea otter blood samples collected from live-captured sea otters from the Bella Bella (BB; $n = 12$) and Esperanza/Nuchatlitz Inlet (EN; $n = 17$) areas in coastal British Columbia, Canada, were analyzed for resolved alkanes ($n = 32$) and polycyclic aromatic hydrocarbons (PAHs; $n = 43$)[a]

	BB juveniles	BB males	BB females	EN juveniles	EN males	EN females
ΣAlkanes ng/g wet wt average ± SD (minimum–maximum range)[b]	713 ± 283 (162–972)	2,010 ± 1,310 (114–3,460)	15.4	488 ± 346 (43.9–899)	293 ± 113 (164–495)	541 ± 375 (249–1.280)
Concentration of top five alkanes (ng/g wet wt)	116 (n-C_{27})	n-C_{26} (324)	1.86 (n-C_{15})	79.7 (n-C_{26})	45.3 (n-C_{26})	89.8 (n-C_{27})
	111 (n-C_{26})	324 (n-C_{27})	1.27 (n-C_{14})	79.0 (n-C_{27})	44.8 (n-C_{28})	88.8 (n-C_{26})
	103 (n-C_{28})	268 (n-C_{28})	1.25 (n-C_{36})	74.2 (n-C_{28})	41.0 (n-C_{27})	80.4 (n-C_{28})
	85.0 (n-C_{25})	261 (n-C_{25})	1.06 (n-C_{35})	54.0 (n-C_{25})	29.4 (n-C_{25})	62.9 (n-C_{25})
	80.4 (n-C_{29})	199 (n-C_{29})	1.04 (n-C_{13})	52.4 (n-C_{29})	27.7 (n-C_{29})	58.4 (n-C_{29})
Percentage contribution of top five	69.5	68.3	42.1	68.6	64.4	70.4
ΣPAH ng/g wet wt average ± SD (minimum–maximum range)[c]	4.26 ± 7.21 (0.14–18.5)	10.5 ± 12.4 (1.07–29.7)	0.45	3.20 ± 2.01 (1.23–6.01)	4.27 ± 1.36 (2.52–6.14)	5.67 ± 3.65 (1.67–13.1)
Concentration of top five PAHs (ng/g wet wt)	1.32 (Na)	1.84 (N)	0.28 (F2)	1.14 (N)	1.20 (N)	2.15 (N)
	0.63 (N1)	1.39 (N1)	0.10 (Ph)	0.80 (F2)	0.60 (F2)	0.57 (F2)
	0.42 (F2)	1.12 (N2)	0.05 (N1)	0.69 (F3)	0.41 (F3)	0.48 (PA4)
	0.23 (Ph)	1.01 (PA4)	0.02 (N2)	0.17 (N2)	0.40 (N1)	0.42 (F3)
	0.22 (Fl)	0.90 (F2)	0.01 (Py)	0.11 (N4)	0.33 (PA4)	0.31 (D2)
Percentage contribution of top five	82.2	66.1	100	75.5	68.1	71.5

[a] Alkane patterns were generally dominated by compounds in the n-C_{25} to n-C_{29} range, whereas PAH patterns, like those in prey species, were generally dominated by low-molecular-weight alkylated PAHs. SD = standard deviation.

[b] Does not include unresolved complex mixture (UCM). Resolved alkanes include n-C_{12} to n-C_{36}, dimethyl undecane, norfarnesane, farnesane, trimethyl tridecane, norpristane, pristane, and phytane.

[c] PAHs include biphenyl (Bi), napththalene (N), acenaphthylene (Ayl), acenaphthene (Aen), fluorene (F), phenanthrene (Ph), anthracene (An), fluoranthene (Fl), pyrene (Py), benz[*a*]anthracene (B*a*A), chrysene (Ch), benzo[*bjk*]fluoranthene (B*bjk*F), benzo[*e*]pyrene (B*e*P), benzo[*a*]pyrene (B*a*P), perylene (Per), dibenz[*a,h*]anthracene (DahA), picene (Pi), indeno[1,2,3-*cd*]pyrene (IP), benzo[*ghi*]perylene (B*ghi*P), anthanthrene (AA), dibenzothiophene (D), retene (Ret), C1–C4 naphthalenes (N1–N4), C1–C3 fluorenes (F1–F3), C1–C3 dibenzothiophenes (D1–D3), C1–C4 phenanthrene/anthracenes (PA1–PA4), and C1–C3 fluoranthene/pyrenes (FP1–FP3). Compounds designated priority pollutants by the U.S. EPA (ΣU.S. EPA 16) include Na, Ayl, Aen, F, Ph, An, Fl, Py, B*a*A, Ch, B*bjk*F, B*a*P, D*a,h*A, IP, and B*ghi*P.

hexane, and saturated ammonium sulfate solution. The hexane extract was then back-washed by shaking with water and dried over anhydrous sodium sulfate.

To extract prey samples, subsamples of 10 to 30 g were combined with 100 ml methanol and spiked with a suite of perdeuterated surrogate standards (1 alkane and 16 PAH surrogates, as for blood samples). A solution of potassium hydroxide was added, and the mixture was boiled under reflux for 1 h. Ultrapure water was added, and boiling continued for another 1.5 h. Samples were extracted with pentane, and the extract was then washed with ultrapure water and dried over anhydrous sodium sulfate.

Extracted samples were then loaded onto a silica gel column (1 cm × 25 cm; dry packed with 10 g silica gel) and eluted into two fractions using 25 ml pentane (containing the alkanes) followed by 100 ml dichloromethane (containing the PAHs). Samples were then concentrated, spiked with recovery standards, and analyzed using selective ion monitoring GC/MS. A splitless/split injection sequence was used on a Restek Rt_x-5 chromatography column (30 m, 0.25 mm inside diameter [i.d.], 0.25 μm film thickness; Restek Chromatography Products). The MS (Agilent 5973) was operated at a unit mass resolution in the electron impact ionization mode using multiple ion detection acquiring at least one characteristic ion for each target analyte and surrogate standard.

Concentrations of target alkanes and PAHs were calculated using the isotope dilution method of quantification, wherein compounds are quantified by comparing the area of the quantification ion to that of the corresponding deuterium-labeled surrogate and correcting for response factors (response factors are determined daily using authentic PAHs and alkanes).

Quality analysis and quality control

Laboratory blanks and spiked matrix samples were analyzed for each batch (10 samples) and for each analysis. Recovery of each perdeuterated surrogate standard relative to the recovery standard added just before instrumental analysis met established laboratory quality control acceptance criteria of between 30 and 120%, and all data were recovery corrected. Recoveries of alkanes and PAHs from the spiked samples analyzed in each batch were generally within the range of 70 to 130%. Alkane concentrations in laboratory blanks analyzed with prey species ranged from 0.36 to 18.1 ng/g (3.07 ± 5.06 ng/g), whereas PAH concentrations in laboratory blanks analyzed with prey species ranged from 0.007 to 5.98 ng/g (0.30 ± 0.66 ng/g). Alkane concentrations in laboratory blanks analyzed with sea otter blood samples ranged from 0.20 to 18 ng/g (1.50 ± 2.33 ng/g), whereas PAH concentrations in laboratory blanks analyzed with sea otter blood samples ranged from 0.007 to 1.39 ng/g (0.16 ± 0.21 ng/g). All concentrations measured in samples were corrected to the concentration measured in the laboratory blank.

Lipid quantification

Sea otter whole-blood samples and homogenized soft tissue samples from prey species were analyzed for lipid content by gravimetric weight of the extract using two subsamples of the analysis extract.

Stable isotope analysis

Fur samples were washed three times with a 2:1 chloroform:methanol solution to remove any debris, organic matter, and/or surface oils from the fur. They were then freeze dried at −50°C for at least 24 h. Subsamples of whole prey homogenate were freeze dried at −50°C for at least 48 h and then ground into a fine powder using a mortar and pestle.

Stable carbon and nitrogen isotope ratio (^{15}N:^{14}N and ^{13}C:^{12}C) measurements in subsamples (0.5 ± 0.09 mg) were made at the Biogeochemistry Facility in the School of Earth and Ocean Sciences at the University of Victoria, Canada, using

a Fisons NA 1500 elemental analyser-isotope ratio mass-selective interfaced to a FinniganMAT 252 isotope ratio mass spectrometer. Results are reported using standard δ notation as the proportional deviation in parts per thousand (‰) of the isotope ratio in a sample from that in a standard:

$$\delta X = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1{,}000 \quad (1)$$

where δX represents the heavier isotope (e.g., ^{15}N or ^{13}C), R_{sample} is the raw ratio of heavy to light isotope in the sample, and R_{standard} is the raw ratio of heavy to light isotope in an internationally recognized standard (Peedee Belemnite for carbon and atmospheric nitrogen for nitrogen). Both carbon and nitrogen measurements are relative to acetanilide (an in-house standard with known isotopic ratios) and blanks. Replicates were included for random samples to observe within-sample isotopic variation, to measure deviation of values over time, and to measure any differences from one sample rack to another. When deviation occurred, isotope values were corrected to standards.

Data analysis

Sea otters were placed in three age–sex classes: juveniles (male and female), adult males, and adult females. Subadult sea otters were defined as juveniles (0–3 years [26]). For sea otters, total hydrocarbon concentrations were calculated as the sum of the concentrations of compounds that were detectable in at least 70% of samples at each location. Detection limit (DL) substitutions were made for undetected compounds in cases in which at least 70% of animals had detectable values for that compound. When fewer than 70% of the otters had detectable concentrations of a compound, 0 ng/g was substituted for compounds below DLs. In the case of invertebrates, DL substitutions were made for all undetected compounds to ensure that patterns resulting from differences in habitat preference or feeding ecology were conserved. Detection limits for alkanes in prey species ranged from 0.17 to 4.66 ng/g (mean ± standard deviation: 0.70 ± 0.75 ng/g), and DLs for PAHs ranged from 0.01 to 0.29 ng/g (0.05 ± 0.03 ng/g). In sea otters, DLs for alkanes ranged from 0.28 to 4.35 ng/g (0.68 ± 0.49 ng/g), and DLs for PAHs ranged from 0.01 to 0.22 ng/g (0.05 ± 0.03 ng/g). All data are presented as mean ± SD, and all statistical tests are considered significant at $p < 0.05$.

Biota-sediment accumulation factor calculations

Biota-sediment accumulation factors (BSAFs) for prey species were calculated according to the equation

$$\text{BSAF} = \frac{(C_b/L)}{(C_{\text{sed}}/\text{TOC})} \quad (2)$$

where C_b = contaminant concentration in biota (ng/g), C_{sed} = contaminant concentration in sediment (ng/g), L = fraction of lipid in the tissue, and TOC = percentage total organic carbon (OC) content in sediment. Average total PAH concentrations in sediments from seven remote sites in the EN area that are reported in Harris et al. [27] were used for our BSAF calculations. Assuming chemical equilibrium, and that OC and lipids have similar sorption capacities for the compounds, the lipid-normalized concentration in biota should approximate the OC-normalized concentration in sediment; thus, the BSAF should approach 1.0 [28]. The concentrations and patterns of hydrocarbons in sediments in sea otter habitat have been reported and the probable sources discussed in detail in Harris et al. [27].

Biomagnification factor calculations

Biomagnification factors (BMFs), used to measure changes in tissue contaminant concentrations with increasing trophic level, were calculated according to the equation

$$\text{BMF} = \frac{C_{\text{pred}}/L}{C_{\text{prey}}/L} \quad (3)$$

where C_{pred} = contaminant concentration in the predator (ng/g), C_{prey} = contaminant concentration in the prey (ng/g), and L = fraction of lipid in the tissue. Because no information on the dietary preferences of sea otters in the study area was available, BMFs were based on five modelled dietary scenarios. Four of these scenarios assumed that a single prey species made up 100% of the diet, whereas the fifth used a food basket approach, with each prey item contributing an equal proportion (25%) to the diet. The BMFs were calculated based on contaminant concentrations observed in the average EN sea otter (excluding unweaned juveniles).

RESULTS AND DISCUSSION

The very high energetic requirements of sea otters relative to other marine mammals [29] predispose them to heightened exposure to some environmental contaminants through the consumption of prey (Fig. 2). However, legal and ethical considerations have typically limited sampling to dead or diseased sea otters. Furthermore, studies have been hampered by the difficult environments inhabited by sea otters (exposed, rocky, remote sections of the coast), and challenges in capturing these large (up to ~ 40 kg) and able divers.

Stable isotope ratios of carbon and nitrogen in sea otters did not reveal any major differences in trophic level or feeding ecology between the two sampling locations, although EN juveniles had higher $\delta^{15}N$ values than males (Table 1; $p = 0.04$). We found no relationship between hydrocarbon concentrations and C or N values for any of the age, sex, or location groups, perhaps reflecting rapid metabolic elimination of many compounds (results not shown).

Ratios of $\delta^{15}N$ in invertebrate samples were generally approximately 3‰ lower than values observed in sea otters (Table 1), consistent with observations in other predators and their prey and supporting their use as sea otter prey items in our evaluation. Higher variability in observed $\delta^{13}C$ ratio values among prey (Table 1) very likely is due to a wider range of carbon sources associated with nearshore marine habitats and benthic food webs [30].

Alkanes in sea otters

Alkane concentrations in sea otters from both EN and BB locations were generally lower than those reported previously in this species [10,11] (Table 2). Within sites, BB males had higher total alkane concentrations than BB juveniles ($p = 0.05$), but no differences were observed among EN groups (juveniles vs males, $p = 0.14$; juveniles vs females, $p = 0.41$; males vs females, $p = 0.09$).

Between sites, BB males exhibited higher total alkane concentrations than EN males ($p = 0.02$), but there was no difference between BB and EN juveniles ($p = 0.14$). Differences remained following lipid correction, indicating that body condition did not underlie these observations. Furthermore, the absence of $\delta^{15}N$ differences between sites ruled out trophic position as a reason for higher alkane concentrations in BB otters. Thus, the higher total alkane concentrations in BB males

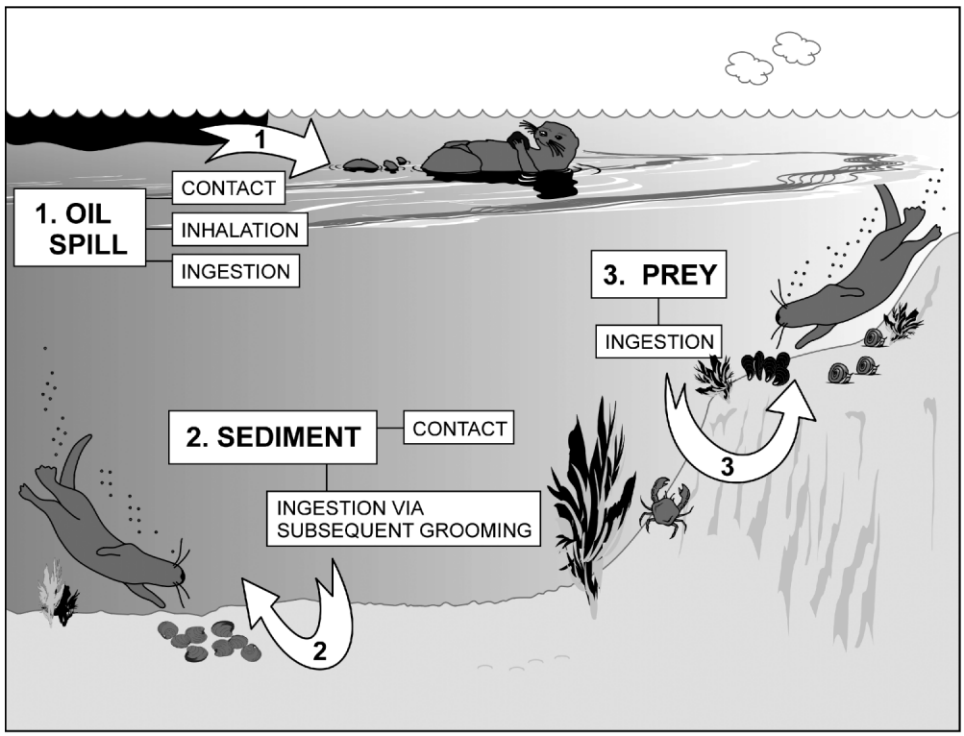

Fig. 2. Schematic of generalized hydrocarbon exposure routes for sea otters. Although the vulnerability of sea otters to acute exposure to whole oil (1) and, to a lesser extent, contact with contaminated sediments (2) [46] has been documented in the case of catastrophic oil spills, little is known about chronic dietary exposure to hydrocarbons (3), the primary constituent of oil. This exposure route may be particularly important in sea otters, which consume up to 25% of their body weight per day.

were likely due to differences in diet of the same trophic level or to differences in local contaminant levels in prey. Greater numbers of compounds were detected in BB males than EN males ($76.9 \pm 2.8\%$ vs $62.5 \pm 9.8\%$, respectively; $p = 0.002$), suggesting higher exposure.

Despite these intersite differences in total alkane concentrations in sea otters, patterns in all age and sex classes exhibited a similar parabolic shape with a peak at *n*-C_{26} to *n*-C_{27} (the top five alkanes by concentration are listed in Table 2). This likely results from the preferential metabolism or excretion of shorter chain alkanes and restricted uptake of longer chain alkanes (Table 2), as observed previously in sea otters [10]. Alkanes appear to be readily metabolized by vertebrates [31,32] and may also be excreted unchanged via feces [32].

PAHs in sea otters

No differences in total PAH concentrations were observed among sea otter age–sex classes within or between locations. Total PAH concentrations ranged from 0.14 to 29.2 ng/g wet weight in BB otters and from 1.2 to 13.1 ng/g wet weight in EN otters (Table 2), with $31.1 \pm 15.7\%$ of the 43 compounds measured detected in the otters. Concentrations were lower than those reported previously for both sea otters and other marine mammals [11,12,33], but different matrices, analytical approaches, and reporting formats make such comparisons difficult. Most studies report only the 16 PAHs designated by the U.S. Environmental Protection Agency (U.S. EPA) as priority pollutants (ΣU.S. EPA 16; see Table 2 footnotes). In the present study, the mean ΣU.S. EPA 16 was 1.93 ± 3.22 ng/g wet weight in BB otters and 1.72 ± 2.93 ng/g wet weight in EN otters, accounting for just $25.8 \pm 13.7\%$ and $26.4 \pm 31.1\%$ of measured ΣPAH concentrations, respectively.

The absence of relationships between total PAH concentrations and age, sex, and trophic position ($\delta^{15}N$) of our sea otters again indicates a dominant role for metabolism in driving PAH concentrations, as described for other vertebrates [13,14]. Polycyclic aromatic hydrocarbon half-lives in marine invertebrates are on the order of days to weeks [34] and are thought to be even shorter in sea otters [33]. Because adipose tissue in sea otters provides a source of energy but does not serve as a storage depot or insulative barrier, a rapid turnover of hydrocarbon burden might be expected [10].

Low-molecular-weight alkyl PAHs dominated PAH profiles in the sea otters, with F1 to F3 and PA1 to PA4 representing major constituents (average contribution to ΣPAH: 48.7 and 17.3%, respectively; Fig. 3). Interestingly, BB males exhibited a higher contribution of the pyrogenic high-molecular-weight PAHs than EN males ($p = 0.05$, data not shown). Together with alkane results, this suggests that BB otters are exposed to higher hydrocarbon concentrations and that these PAHs are anthropogenic in origin. These results provide a net exposure and accumulation signal, but the examination of hydrocarbon levels in prey can provide additional insight into the putative role that metabolism plays in shaping hydrocarbon patterns in sea otters.

Alkanes in sea otter prey

Alkane concentrations measured in our invertebrate samples, the choice of which was supported by stable isotope data (Table 1), were generally lower than those reported elsewhere [35] (Table 3). Among the 32 compounds measured in our sampled prey species, 100% were detected, underscoring the general ubiquity of alkanes in the present study area.

Although alkane concentrations and patterns were generally similar across the four invertebrate sample pools, the benthic species (clam and crab) were dominated by alkanes in the *n*-C_{15}

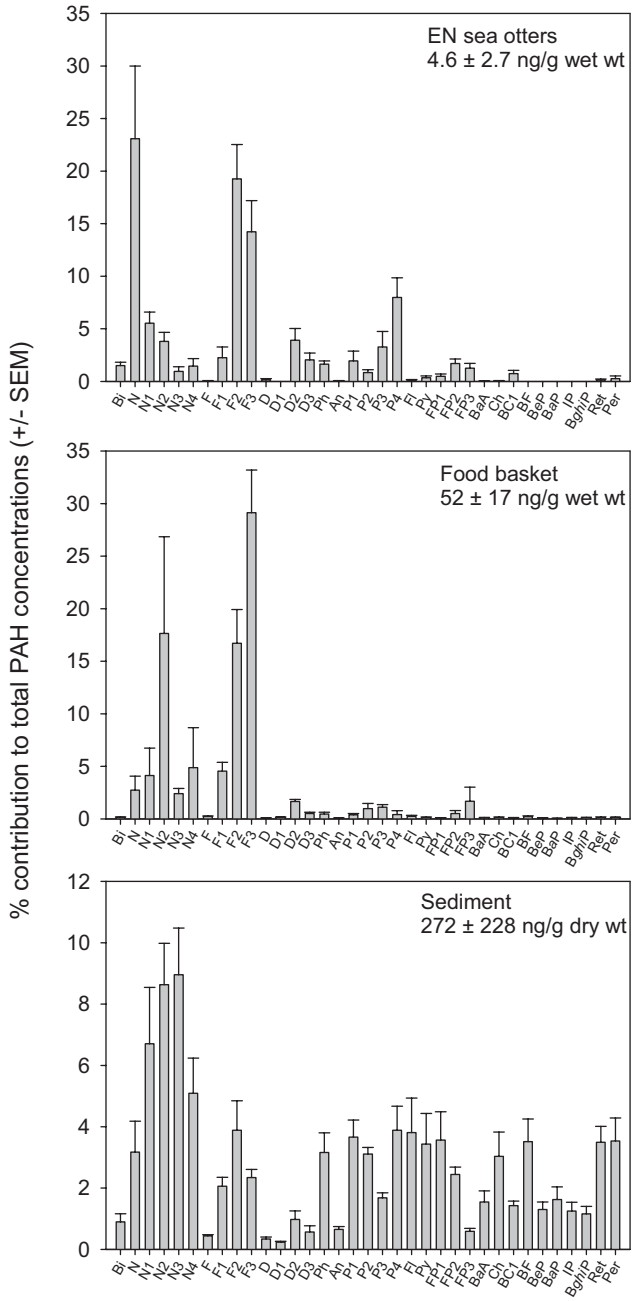

Fig. 3. The percentage contribution (±standard error of measurement) of individual polycyclic aromatic hydrocarbons (PAHs) to total PAH concentrations revealed an overarching petrogenic signature in sediment from remote sites [27]. As a proxy for sea otter prey, the food basket revealed a predominance of two- and three-ring PAHs with relatively little variation across marine invertebrate prey species; and patterns in the Esperanza/Nuchatlitz Inlets (EN) sea otters were similar to those in prey, likely reflecting dietary uptake. Values presented in each figure indicate average (±standard error of measurement) of total PAH concentrations measured in each matrix.

to n-C_{21} range, whereas the intertidal species (mussels and snails) exhibited a peak in the n-C_{27} to n-C_{31} range in addition to important contributions by n-C_{15} and n-C_{17} (Table 3). Marine invertebrates have little or no capacity to metabolize n-alkanes (for review see Geiszler et al. [31]), so the slight pattern differences observed may indicate a divergence in feeding ecology or habitat use (the influence of terrestrial plant-derived, odd-chain n-C_{27} to n-C_{31} alkanes in intertidal species).

PAHs in sea otter prey

Different feeding ecologies also appeared to contribute to differences in total PAH concentrations in the four invertebrate species (Table 3). For example, turban snails, which are grazers, had higher PAH concentrations than filter-feeding mussels. Grazers can exhibit high contaminant concentrations as a consequence of preferential feeding on organic films that can concentrate contaminants [36]. Conversely, metabolic capacity likely influenced PAH composition in our crab (for review see Meador et al. [37]): only 54.2% of measured compounds were detected, compared with 93.8 to 95.8% of measured PAH compounds detected in clam, snails, and mussels. Total PAH concentrations in our invertebrates were relatively low compared with those reported elsewhere (for review see Meador [21]).

Patterns of PAHs among our study species were similar (Fig. 3) and were dominated by low-molecular-weight alkyl naphthalenes and alkyl fluorenes. The top five PAHs accounted for 79 to 91% of total concentrations (Table 3). The predominance of low-molecular-weight PAHs, coupled with low concentrations of high-molecular-weight PAHs that are typically sediment-associated points to water as likely to be an important PAH exposure route [34] in the invertebrates.

Characterizing hydrocarbons in the food web of sea otters

The lack of toxicological information on alkanes constrains our interpretation of the low concentrations observed in both sea otters and their prey. However, the ubiquity of alkanes as natural compounds in the marine environment, coupled with the low concentrations and limited trophic transfer in the sea otter food web (mean ± SD: BSAF 0.37 ± 0.33, BMF 1.19 ± 1.68; Supplemental Data, Tables 1 and 2), suggests a limited health risk for this class of compounds. These compounds are readily used in lipid synthesis pathways or undergo rapid elimination in vertebrates [38,39]. Conversely, PAHs are known to exert toxicity via multiple mechanisms in vertebrates [40], so we explored the movement of these compounds in a series of dietary scenarios for sea otters in BC.

Biota-sediment accumulation factors (Eqn. 2) provide insight into the movement of PAHs between sediments and sea otter prey. Sediment PAH concentrations reported elsewhere for the EN study area [27] were used to derive BSAFs (range, 0.0–2.5) for hydrocarbons in invertebrates, which were in general agreement with those reported for other marine invertebrates [13,21]. The BSAFs were generally about one order of magnitude lower in crabs than clams, mussels, and snails, providing additional support for the notion that crabs have a greater capacity to metabolize PAHs [37]. Only two compounds (F3, FP3) had BSAFs >1.0, and both were observed in clams. In clams, snails, and crabs, BSAFs for alkyl PAHs were higher than those for parent compounds (results not shown; $p = 0.02$, 0.02, and 0.01, respectively).

A quadratic relationship was observed for BSAFs and log K_{OW} for parent PAHs in clams, snails, and mussels, but not in crabs (food basket average; Fig. 4a). Similar patterns in clams, mussels, and snails likely reflect water as an exposure route, with bioavailability decreasing with decreasing solubility in water, with some dietary uptake of high-molecular-weight (higher log K_{OW}) PAHs. Conversely, increasing BSAFs (though still generally <1.0) were observed with increasing alkylation within alkyl PAH groups, suggesting some degree of bioaccumulation (Fig. 4b). Increasing log K_{OW} with increasing alkyl groups would be expected to reduce bioavailability, so this

Table 3. Pooled homogenates (from 46 individuals) of four important sea otter prey species were analyzed for resolved alkane ($n = 32$) and polycyclic aromatic hydrocarbon (PAH; $n = 43$)[a]

	Geoduck clam	California mussel	Turban snail	Red rock crab	Food basket
ΣAlkanes (ng/g wet wt)[b]	171	290	510	513	371
Concentrations of top five alkanes (ng/g wet wt)	*n*-C_{17} (59.3)	*n*-C_{15} (52.7)	*n*-C_{17} (69.2)	Pristane (152)	*n*-C_{17} (56.3)
	n-C_{15} (28.6)	*n*-C_{29} (26.9)	*n*-C_{29} (50.8)	*n*-C_{17} (73.5)	Pristane (48.6)
	Pristane (22.6)	*n*-C_{30} (23.4)	*n*-C_{27} (47.7)	*n*-C_{15} (27.5)	*n*-C_{15} (35.3)
	n-C_{16} (16.1)	*n*-C_{17} (23.0)	*n*-C_{28} (41.9)	*n*-C_{16} (26.9)	*n*-C_{29} (24.9)
	n-C_{21} (5.69)	*n*-C_{27} (22.5)	*n*-C_{31} (39.2)	*n*-C_{27} (25.9)	*n*-C_{27} (23.9)
Percentage contribution of top five alkanes	77.3	51.2	48.7	59.0	50.9
ΣPAH (ng/g wet wt)[b]	40.2	36.2	61.5	73.0	45.7
Concentration of top five PAHs (ng/g wet wt)	F3 (16.9)	F3 (11.7)	F3 (14.5)	N2 (32.5)	F3 (14.4)
	F2 (10.8)	F2 (5.00)	N4 (10.8)	F3 (19.4)	F2 (8.84)
	FP3 (2.37)	N1 (4.98)	F2 (10.8)	F2 (9.52)	N2 (4.94)
	F1 (2.10)	N2 (4.37)	N2 (9.86)	F1 (4.58)	N4 (3.99)
	N3 (1.01)	N (2.62)	N3 (2.49)	PA4 (1.11)	N1 (2.39)
Percentage contribution of top five	82.8	79.7	79.2	92.5	75.6

[a] Alkane patterns exhibited a peak in the *n*-C_{15} to *n*-C_{17} range for all species, with a second peak in the *n*-C_{27} to *n*-C_{31} range for California mussels and turban snails. PAH patterns were dominated by low-molecular-weight alkylated PAHs in all species.
[b] See Table 2 footnotes for a list of resolved alkanes and PAHs measured in the present study.

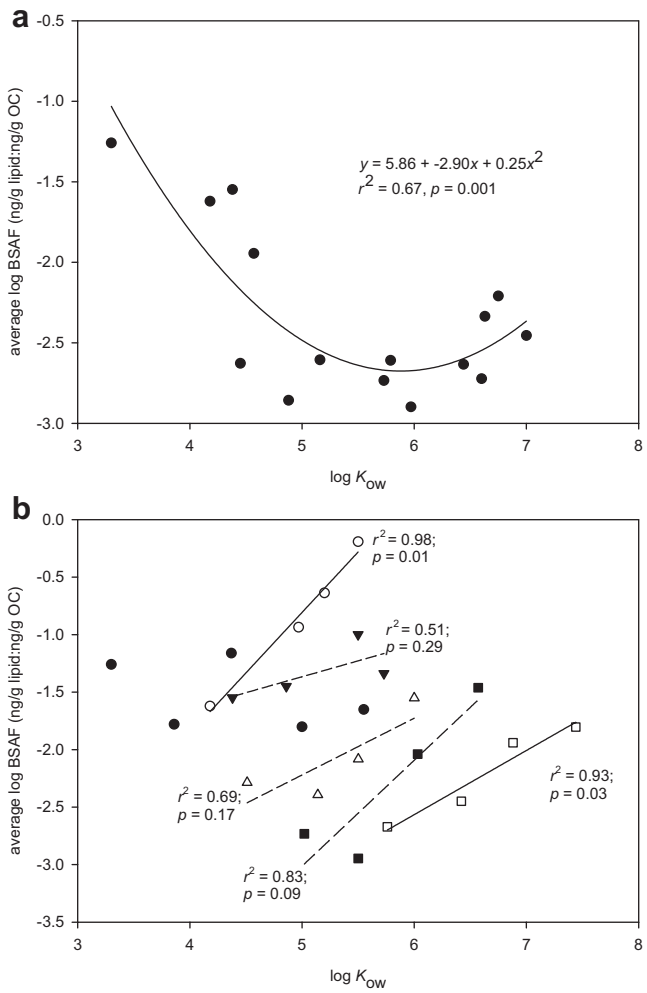

Fig. 4. Biota-sediment accumulation factors (BSAFs) exhibited a quadratic relationship with increasing log K_{OW} values for parent PAHs (**a**). Interestingly, alkyl polycyclic aromatic hydrocarbons (PAHs) within groups exhibited increasing BSAFs with increasing log K_{OW} values, suggesting that the addition of alkyl groups led to greater retention (**b**). The BSAFs were calculated as the ratio of lipid-corrected PAH concentrations in a food-basket prey composite to organic carbon (OC)-corrected PAH concentrations reported elsewhere for sediments in the same area [27]. Solid circles = alkyl naphthalenes; open circles = alkyl fluorenes; solid triangles = alkyl dibenzothiophenes; open triangles = alkyl phenanthrene/anthracenes; solid squares = alkyl fluoranthene/pyrenes; open squares = alkyl benz[*a*]anthrancenes/chrysenes.

relationship likely reflects decreased depuration or excretion. In previous work, alkyl PAHs have been observed to bioaccumulate to a greater degree than parent PAHs [41], but, to the best of our knowledge, the present study is the first to indicate divergent relationships for alkyl and parent PAHs with log K_{OW}.

Estimated biomagnification factors (Eqn. 3) suggested that most individual PAHs did not biomagnify between prey and sea otters. Although this likely is due primarily to metabolism, it may also be due in part to low gut assimilation efficiency relative to other, more recalcitrant contaminants in the same log K_{OW} range [15].

Despite our evidence for metabolism, an average of 14 of the 38 PAHs used in BMF calculations appeared to biomagnify (BMF >1.0; Table 4). Among these, 10 were alkyl PAHs. The absence of biomagnification observed for most PAHs in sea otters underscores the metabolic vulnerability of these compounds compared with more recalcitrant compounds such as the polychlorinated biphenyls (PCBs), despite similar ranges for molecular weights and log K_{OW}. Conversely, the more complex structures of the alkyl PAHs may, in part, explain their greater retention in sea otters compared with parent PAHs.

The apparent biomagnification of many alkyl PAHs in the present study has not been previously reported and highlights the added value in measuring a more complete suite of PAHs. For example, a reliance on measuring the ΣU.S. EPA 16 PAHs may result in a continued, and seemingly flawed, notion that no PAHs biomagnify [13–15]. Alkyl PAHs have higher log K_{OW} than their parent PAHs and may be more persistent and bioaccumulative [42].

Risks associated with prey consumption

Although uptake and metabolism can be explored using BMFs, concurrent estimates of dietary intake and body burden provide additional means of characterizing exposure and retention by the sea otters. We estimated daily dietary intakes of total PAHs by adult sea otters using prey concentrations and an assumed daily consumption of 25% body weight (average otter weight 28.6 ± 5.5 kg). Intake estimates were 374 ± 124 μg wet weight/d (or 17,400 ± 13,400 μg lipid wt/d), corresponding to 13.1 ± 4.34 μg/kg body weight/d. No comparable data are available for marine mammals, but average daily intakes for humans have been estimated to range from 1 to 5 μg/d [43,44] or 0.014 to 0.071 μg/kg body weight/d based on an average body weight of 70 kg [43]. Parent PAHs were estimated to

Table 4. Biomagnification factors (BMFs) were calculated for the 38 (individual alkyl homologs were grouped by number of methyl groups)[a] measured polyaromatic hydrocarbons using the average concentration (ng/g lipid wt) in adult Esperanza/Nuchatlitz Inlet sea otters ($n = 12$) relative to the concentration (ng/g lipid weight) in each of four individual prey species and a prey composite (equally weighted food basket)[b]

	Geoduck clam	California mussel	Black turban snails	Red rock crabs	Food basket
Bi	3.22	1.91	2.38	51.6	14.8
Na	3.97	1.36	2.29	48.1	13.9
N1	3.14	0.44	1.66	12.4	4.41
N2	0.55	0.19	0.12	0.12	0.25
N3	0.27	0.84	0.41	3.16	1.17
N4	0.13	0.40	0.03	3.04	0.90
Ace	0	0	0	0	0
Aen	0	0	0	0	0
F	0	0	0	0	0
F1	0.19	0.51	1.01	1.07	0.69
F2	0.24	1.31	0.89	3.35	1.45
F3	0.12	0.45	0.53	1.33	0.61
Ph	1.47	2.23	3.14	NA	NA
An	0	0	0	NA	NA
PA1	0.51	1.06	0.50	27.8	7.71
PA2	0.14	1.53	0.70	67.7	17.5
PA3	1.76	11.35	3.09	15.2	7.85
PA4	NA	NA	51.3	16.8	NA
D	0.30	0.78	0.95	NA	NA
D1	0	0	0	0	0
D2	1.10	3.22	3.10	14.4	5.44
D3	2.33	16.2	4.28	19.9	10.7
Fl	0.05	0.08	0.13	NA	NA
Py	0.29	0.64	0.35	NA	NA
FP1	3.03	9.04	5.07	NA	NA
FP2	0.69	6.03	6.12	NA	NA
FP3	0.16	7.70	6.64	16.5	7.76
B*a*A	0.10	0.09	0.21	NA	NA
Ch	0.07	0.16	0.12	NA	NA
BC1	1.59	10.46	NA	NA	NA
BC3	0.31	7.61	11.2	NA	NA
B*bjk*F	0	0	0	NA	NA
B*e*P	0	0	0	NA	NA
B*a*P	0	0	0	NA	NA
IP	0	0	0	NA	NA
B*ghi*P	0	0	0	NA	NA
Ret	0.12	NA	1.19	3.40	NA
Per	0.47	1.99	1.92	9.89	3.57

[a] See Table 2 footnotes for a list of resolved alkanes and PAHs measured in the present study.
[b] NA = BMF could not be calculated as the concentration in the prey was 0 ng/g.

account for only $11.1 \pm 6.7\%$ of the total estimated PAH body burden in males ($5{,}120 \pm 3{,}600$ μg) and $16.5 \pm 10.0\%$ of the total in females ($3{,}710 \pm 2{,}560$ μg), whereas alkyl PAHs made up $88.9 \pm 6.7\%$ and $83.5 \pm 10.0\%$, respectively.

Alkyl PAHs, in addition to being more persistent and bioaccumulative than parent PAHs [41], have also been shown to be more toxic to early life stages of fish [45]. Furthermore, toxicity appears to increase with increasing alkyl substitution [22]. Risk assessments focusing primarily on subsets of parent PAHs may therefore underestimate risk to other aquatic organisms [42], including sea otters. In addition, studies typically characterize risk on the basis of exposure or accumulation. However, the loss of parent PAHs (BMFs generally <1.0) in sea otters means that highly reactive intermediates are formed, which is of concern given that these metabolites are generally more toxic than their parent compounds [45].

Although the vulnerability of sea otters to acute oil exposure has been clearly established [9], the present study provides a first examination of chronic dietary exposure to natural and anthropogenic hydrocarbons. High-resolution analysis of a broad suite of PAHs ($n = 43$) allowed a comparative evaluation of parent and alkyl PAHs and demonstrated the apparent retention and biomagnification of the latter.

SUPPLEMENTAL DATA

Table S1. BSAFs for resolved alkanes in four sea otter prey species.

Table S2. BMFs for resolved alkanes in EN sea otters (44 KB DOC).

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