Hydrocarbons in Sea Otters (*Enhydra lutris*) and Their Habitat

in coastal British Columbia, Canada

by

Katherine Anne Harris
B.Sc., University of Victoria, 2005

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

in the School of Earth and Ocean Sciences

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The Source, Transport, and Fate of Hydrocarbons

in the Habitat of the British Columbia Sea Otter (Enhydra lutris)

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ABSTRACT

The purpose of this work was to examine the source and fate of hydrocarbons, the primary constituents of oil, in sea otter (Enhydra lutris) habitat on the west coast of British Columbia (BC), Canada and their fate in the sea otter food web. Oil pollution is the primary threat to this recovering population, reflecting their extreme vulnerability as a result of several unique life history characteristics, including the absence of a blubber layer, reliance on their fur for insulation, and the fact that their entire lives can be spent at sea.

While the vulnerability of sea otters to acute oil exposure has been demonstrated, chronic hydrocarbon exposure through dietary processes is not well understood. We measured hydrocarbon (alkane, hopane and sterane biomarker, and polycyclic aromatic) concentrations in sediments, prey items, and live-captured sea otters using high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS). Background signatures were characterized for remote sediment sites, with polycyclic aromatic hydrocarbon (PAH) patterns revealing the predominance of petrogenic sources. However, PAH concentrations were up to three orders of magnitude higher at two small harbour sites, with patterns reflecting weathered petroleum and the combustion of fossil
fuels and biomass. Concentrations at these sites exceeded both national and provincial sediment quality guidelines for the protection of aquatic life.

Despite differences in habitat and feeding ecology, all sea otter prey species sampled exhibited PAH patterns dominated by petrogenic low molecular weight (LMW) compounds, highlighting the likely importance of water as an exposure route. While biota-sediment accumulation factors (BSAFs) generally decreased with increasing octanol-water partitioning coefficients (log Kow) for parent PAHs, BSAFs for alkyl PAHs increased, indicative of bioaccumulation by invertebrates.

Biomagnification factors (BMFs) indicated that while parent PAHs biodiluted in sea otters, consistent with metabolic elimination, some higher alkylated 3- and 4-ring PAHs biomagnified, challenging the commonly held view that PAHs dilute in food webs. This retention was reflected in estimated $\sum$PAH body burdens, in which alkyl PAHs comprised 89 ± 7% and 84 ± 10% of totals in male and female otters, respectively.

While vertebrates are efficient metabolizers of parent PAHs, this apparent retention of some alkyl PAHs in sea otters raises concerns about the potential toxicological effects of these poorly understood compounds. This research suggests that sea otters may be vulnerable to PAH-related health risks as a consequence of their large dietary requirements (~25% of body weight per day), even when prey PAH concentrations are low.
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ACKNOWLEDGEMENTS

I would like first of all to express my gratitude to Dr. Peter Ross for providing me with the opportunity to undertake this project. It has been a privilege to work with such a unique and charismatic species in their incredibly beautiful corner of the world. I would also like to thank my academic supervisor, Dr. Kevin Telmer, for his advice and for his thoughtful comments and questions, Dr. Patrick O’Hara, who was so supportive of my project, and Dr. Jane Watson, for her willingness to offer her expertise in the position of External Examiner. For their financial support, I would like to thank the Species at Risk Act (SARA), especially Laurie Convey, and the Federal Contaminated Sites Action Plan (FCSAP), especially Karen Hutton. I am grateful to AXYS Analytical Services (particularly to Dr. Kalai Pillay), Dr. Mark Yunker, without whose guidance my understanding of hydrocarbon chemistry would still be in serious doubt, Neil Dangerfield, who was so helpful and so patient with my infinite questions (and who managed, for the most part, to keep a straight face), Linda Nichol, who was always willing to share her extensive sea otter expertise, Reet Dhillon, for always being able to put things in perspective, and Tamara Fraser and Norman Crewe, for explaining the mysterious inner workings of the lab. Thanks to my fellow grad students, past and present: Jennie Christensen, Maki Tabuchi, Marie Noël, Tom Child, and Tanya Brown. I appreciate so much all your advice, your willingness to commiserate, and above all, your ability to make me laugh.

Thank you very much to my family for your love and support and for never once suggesting that I get a job, and most of all to my handsome and infinitely patient husband Matthew, who encouraged me when I thought I couldn’t do it anymore. I’m so grateful.
Sea otters

The global sea otter (*Enhydra lutris*) population was devastated by 150 years of commercial exploitation for its fur. Thought to historically number between 150 000 and 300 000 animals [although these estimates are speculative; 1], by the time the International Fur Seal Treaty was signed in 1911, the global population was reduced to fewer than 2000 individuals in small remnant groups in remote areas from Russia to Alaska [1]. Though the population continues to recover, it faces challenges to survival in many parts of its range [2,3]. In British Columbia (BC), the greatest threat to the continued recovery of the sea otter population is oil pollution [4].

Sea otters once ranged from the northern Japanese archipelago, through the Aleutian Islands, and along the North American coast as far south as Baja California [5]. The commercial hunt for sea otters in BC began in earnest after Captain James Cook’s third voyage touched at Nootka Sound in 1778 [6]. The BC population was extirpated in 1929, when the last known sea otter was shot in Kyuquot Sound. A confluence of events, including an increasing awareness of the importance of restoring lost species to coastal ecosystems and the testing of nuclear weapons in Alaska, resulted in the transplant of 89 Alaskan sea otters to the west coast of British Columbia between 1969 and 1972 [7]. The reintroductions were successful, and the population currently numbers approximately 4700 individuals [8]. Approximately 4 000 otters inhabit the west coast of Vancouver Island, with another 700 found on the mainland central coast [Figure 1; 8]. In April 2007, primarily as a result of the increase in population size, the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) down-listed the BC sea otter population from ‘threatened’ to ‘special concern’, a listing which was adopted by the Species at Risk
Act (SARA) in March 2009. In BC, sea otters are blue-listed as a species of special concern [9].

Figure 1. The range of sea otter habitat on the west coast of British Columbia as of 2009 (denoted by the shaded areas; [8]). Sea otters were live captured near Bella Bella (BB) on the mainland central coast in 2003 (1) and in the Esperanza Inlet/Nuchatlitz Inlet (EN) areas on the west coast of Vancouver Island in 2004 (2). Prey collection also occurred in the EN areas.

The sea otter is the largest member of the family Mustelidae, and the smallest marine mammal. There are three recognized subspecies: *Enhydra lutris lutris*, which ranges from the Kuril Islands to the Commander Islands, *Enhydra lutris kenyoni*, which
occurred historically from the Aleutian Islands to Oregon, and *Enhydra lutris nereis*, which is found only in California [10].

As a keystone species, sea otters have a disproportionate effect on the ecosystem relative to their biomass or abundance [11]. They shape the abundance and diversity of other species within kelp forest ecosystems, primarily via consumption of sea urchins, which are voracious consumers of kelp [2]. In the absence of sea otters, exploding sea urchin populations create ‘urchin barrens’, areas characterized by the absence of kelp and other species which rely on the kelp forest for camouflage, nursery habitat, and/or foraging. In the presence of sea otters, an abundant kelp canopy flourishes, finfish and other kelp-dependent species are more numerous, and most shellfish species tend to be fewer in number and smaller in size [2]. Sea otters have also been found to exert a strong influence on subtidal, soft substrate prey communities [12]

In addition to their role as a keystone species, sea otters are an effective indicator of coastal marine environmental quality. They forage in nearshore ecosystems, usually at depths of less than 30 m [13], and maintain a relatively narrow home range, such that their contaminant burden may be more representative of a local signal than a regional or global one [2]. They also eat primarily marine invertebrates, which can concentrate and integrate a large suite of chemical contaminants, and may also serve as an intermediary for some of the pathogens and parasites to which sea otters have proved vulnerable [2].

Sea otter prey choice varies by region, with otters generally choosing prey with the highest ratios of caloric value obtained to energy expended in foraging [14]. Dense sea otter populations lead to decreased availability of preferred prey and increased consumption of less common prey items [15,16]. Otters are known to eat prey from over
seven phyla, including clams, snails, chitons, limpets, octopi, crustaceans, starfish, sea urchins, sand dollars, anemones, polychaete worms, echiuriods, tunicates, sea cucumbers, and fish, with occasional reports of predation on seabirds [13,17].

Threats to recovering sea otter populations vary geographically. In heavily developed and densely populated California, the sea otter population faces a variety of threats including starvation, trauma, and parasites and disease [2], while hypotheses to explain the declining sea otter population in southwest Alaska include increased predation by killer whales [3] and/or a major oceanic regime shift [18]. A far more visible impact on the sea otters of Prince William Sound occurred as a result of the Exxon Valdez oil spill (EVOS), which was estimated to have killed up to 4 000 otters [19], equivalent to approximately 85% of the BC population. The EVOS, and the smaller Nestucca spill in 1988 which affected coastal BC [20], underlined the extreme vulnerability of sea otters to oil pollution based on several unique life history characteristics (discussed below). In British Columbia, oil pollution, and more specifically fouling by oil, has been identified as the primary threat to the recovering sea otter population [4], a threat that is likely to increase with port expansion, increased tanker traffic, and the possibility of offshore oil and gas exploration and development.

Hydrocarbons

Oil is made up primarily (>75% by weight) of hydrocarbons [21]. These organic molecules, composed of carbon and hydrogen atoms, are ubiquitous environmental compounds with both natural and anthropogenic sources. Due to their hydrophobic nature, sediments are the primary repository for hydrocarbons in the marine environment
[21,22], making sediment-biota partitioning an important route for hydrocarbon uptake into marine food webs [23].

Hydrocarbons can be divided into two broad categories: 1) aliphatic hydrocarbons, which include alkanes and hopane and sterane petroleum biomarkers; and 2) aromatic hydrocarbons, compounds containing at least one benzene ring (Figure 2).

This study measured a broad suite of compounds from both categories (Table 1).

**Table 1. This study measured concentrations of several hydrocarbon groups in sediments, sea otter prey species, and live-captured sea otters, a brief overview of which is provided here. For full compound names, abbreviations used, and physicochemical properties, see Appendix 1.**

<table>
<thead>
<tr>
<th>Hydrocarbon class</th>
<th># of compounds measured</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1) Aliphatic hydrocarbons</strong></td>
<td></td>
</tr>
<tr>
<td>Resolved alkanes</td>
<td>32</td>
</tr>
<tr>
<td>Unresolved complex mixture (UCM)</td>
<td>n/a</td>
</tr>
<tr>
<td>Tri- and tetracyclic terpane biomarkers</td>
<td>6</td>
</tr>
<tr>
<td>Diagenetic hopane biomarkers</td>
<td>6</td>
</tr>
<tr>
<td>Biogenic hopane biomarkers</td>
<td>5</td>
</tr>
<tr>
<td>Sterane and diasterane biomarkers</td>
<td>10</td>
</tr>
<tr>
<td><strong>2) Aromatic hydrocarbons</strong></td>
<td></td>
</tr>
<tr>
<td>Parent polycyclic aromatic hydrocarbons (PAHs)</td>
<td>16</td>
</tr>
<tr>
<td>Alkyl PAHs</td>
<td>20</td>
</tr>
<tr>
<td>Other PAHs</td>
<td>10</td>
</tr>
</tbody>
</table>

* measured in sediment samples  
  b measured in marine invertebrate whole tissue and sea otter blood samples
1) *Aliphatic hydrocarbons*
   a) Straight-chain alkane
      \((n-C_{12}; \text{dodecane})\)
      ![Straight-chain alkane diagram](image1)
   b) Branched-chain alkane
      \((C_{19}; \text{pristane})\)
      ![Branched-chain alkane diagram](image2)
   c) Hopane (petroleum biomarker)
      ![Hopane diagram](image3)

2) *Aromatic hydrocarbons*
   a) Benzene
      ![Benzene diagram](image4)
   b) Polycyclic aromatic hydrocarbons (PAHs)
      ![Polycyclic aromatic hydrocarbons diagrams](image5)
   c) Alkyl PAHs
      ![Alkyl PAHs diagrams](image6)

Figure 2. This study measured both aliphatic and aromatic hydrocarbons, representatives of which are seen here. For a more complete set of polycyclic aromatic hydrocarbon (PAH) structures, see Appendix 2.
**Aliphatic hydrocarbons**

**Alkanes**

The resolved alkanes include both straight- and branched-chain molecules (Figure 2), while the unresolved complex mixture (UCM) is comprised of cycloalkanes, branched alkanes, and other compounds unresolvable by gas chromatography [24]. Alkanes with a linear arrangement of carbon atoms are called straight-chain or *normal* alkanes (*n*-alkanes), while those with a non-linear arrangement are called branched alkanes. Alkane sources in the marine environment include marine algae, petroleum products, and input by terrestrial plants [24].

Indicators used to determine the primary alkane source include:

1. Cmax: resolved alkane detected at the greatest concentration, where *n*-C$_{18}$ is indicative of an oily sample, C$_{15}$, C$_{17}$, C$_{19}$, or C$_{21}$ indicate a marine algal source, and C$_{23}$ – C$_{33}$ (odd-chain only) indicates input by terrestrial plants [24,25];

2. lower/higher alkanes: ratio of *n*-alkanes of chain length $\geq$ 24 to *n*-alkanes of chain length < 24 ($\geq$24:<24), where values close to one suggest algae, plankton, and petroleum, and higher values indicate input by bacteria, higher land plants, and marine animals [24,26];

3. carbon preference index (CPI): defined as $(25+27+29+31+33)/(26+28+30+32+34)$, where petrogenic sources have values close to one, while values for samples dominated by terrestrial plants and uncontaminated sediments range from three to six [25,27];
4. ratio of the sum of the concentration of resolved alkanes / estimated concentration of the unresolved complex mixture (UCM): this index estimates the degree of degradation in the sample, where low values suggest degradation and higher values suggest the presence of fresh oil [24]. The presence of a UCM is indicative of anthropogenic contamination [24].

**Biomarkers**

Biomarkers are essentially ‘molecular fossils’, derived from once-living organisms, that are found in all petroleum products [28]. They are useful in distinguishing original oil sources, and provide information on environmental conditions during deposition and burial in sediment and the thermal maturity of the oil [28]. Isoprene is the basic structural unit found in all biomarkers, and is composed of five carbon atoms (Figure 3a).

**Hopanes**

Hopanes are C\textsubscript{30} pentacyclic triterpanes (i.e. composed of six isoprene subunits), and consist of four six-membered rings and one five-membered ring. They are derived from precursors in bacterial membranes and commonly contain 27-35 carbon atoms (Figure 3b; [28]). Hopanes with more than 30 carbons are called homohopanes. Hopanes are composed of three stereoisomeric series: 17α(H),21β(H)-, 17β(H), 21β(H)-, and 17β(H),21α(H)-hopanes, with the α and β notations indicating whether the hydrogen atoms are below or above the plane of the rings, respectively. Hopanes with the αβ configuration in the C\textsubscript{27} to C\textsubscript{35} range are characteristic of petroleum because of their greater thermodynamic stability compared to the other (ββ, βα) series. The major precursors for the hopanes in living organisms have ββ or ‘biological’ stereochemistry,
which is almost flat. They are also amphipathic (e.g. possessing both hydrophilic and lipophilic structural components) and this, combined with the flat configuration, appear to be necessary for insertion into lipid membranes. Because this stereochemical arrangement is thermodynamically unstable, diagenesis and catagenesis of the precursors result in transformation of ββ precursors to αβ hopanes.

To characterize the maturity of hopanes measured in sediment, the following ratio was used:

1. $\frac{22S}{(22S+22R)}$

Isomerization at the C$_{22}$ position in the C$_{31}$ to C$_{35}$ 17α(H)-hopanes occurs earlier than many other biomarker reactions used to assess thermal maturity [28]. Biological hopane precursors have the 22R configuration, which is gradually converted to a mixture of 22R and 22S αβ-homohopanes. In general, C$_{31}$- or C$_{32}$ homohopanes are used to calculate this ratio, which rises from 0 to ~0.6 (equilibrium = 0.57-0.62) during maturation. After equilibrium is reached, this ratio provides no further information as it remains constant [28].

To characterize petroleum composition, the following ratios were used:

1. $\frac{Ts}{(Ts + Tm)}$

During catagenesis, C$_{27}$ 17α(H)-trisnorhopane (Tm) is less stable than C$_{27}$ 18α(H)-trisnorhopane (Ts) [28], so lower ratio values indicate a more mature sample, while higher values indicate a more recent input of petroleum.

2. $\frac{29αβ}{(29αβ + 30αβ)}$

Petroleum derived from organic-rich rocks generally exhibit enhanced concentrations of C$_{29}$- relative to C$_{30}$-hopanes [28].
3. $\Sigma_T/(\Sigma_T + 27\alpha + 29\alpha\beta + 30\alpha\beta)$

This ratio of tricyclic terpanes to 17$\alpha$(H)-hopanes compares lipids derived from bacteria or algae (tricyclics) to markers derived from prokaryotic species (hopanes) [28]. Because tricyclic terpanes and hopanes appear to originate from different biological precursors, this ratio can vary substantially between petroleums from different source rocks [28].

**Steranes**

Steranes are tetracyclic $C_{30}$ compounds (Figure 3c). The sterols in eukaryotic organisms are precursors to the steranes in sediments and petroleum. Like hopane precursors in prokaryotes, the ‘flat’ configuration of sterols allows them to fit into and increase the rigidity of cell membranes. Sterols in living organisms have the following configuration: $8\beta(H),9\alpha(H),10\beta(CH_3),13\beta(CH_3),14\alpha(H),17\alpha(H),20R$.

During diagenesis and catagenesis, the configurations at C-10 and C-13 cannot be changed, and stereoisomerization at C-8 and C-9 does not occur because the biological configuration at these positions is energetically favourable. Thus, C-14, C-17, and C-20 are of most importance in characterizing formation processes. Partly because C-20 is in the sterol side chain and is therefore less impacted by steric effects, the biologically derived 20R isomer is converted to a near-equal mix of 20R and 20S (at equilibrium $20S/(20S+20R) = 0.52-0.55$ for $C_{29}$). Further, the flat configuration imposed by $14\alpha(H),17\alpha(H)$ stereochemistry is lost in favour of the more thermodynamically stable $14\beta(H),17\beta(H)$ form [28].

To determine sample maturity, the following ratios were used:

1. $\beta\beta/(\beta\beta + \alpha\alpha)$ $20R+20S$ $C_{29}$ sterane
Isomerization at the C-14 and C-17 positions in the 20S and 20R C₂₉ regular steranes results in an increase in this ratio from near zero to approximately 0.7 [28]. This ratio appears to be effective at higher maturity levels, as it is slower to reach equilibrium than the following ratio [28].

2. \( \frac{20S}{20S + 20R} \) for 29ααα sterane

Isomerization at C-20 in the C₂₉ 5α(H),14α(H),17α(H)−steranes leads to an increase in this ratio from 0 to approximately 0.5 with increasing maturity. Only the R configuration at C-20 is found in living organisms, and during diageneisis, this is converted to a mixture of R and S configurations [28].

To determine composition, the following ratios were used:

1. \( \frac{27d\beta S}{27d\beta S + 27\alpha R} \) (diasteranes to regular steranes)

Diasterenes are thought to be formed from sterols during diageneisis via catalysis by acidic sites on clays [28], and are then reduced to diasteranes. This ratio is used to distinguish petroleum from various source rock types, where low ratio values indicate anoxic, clay-poor, carbonate source rock, and high values indicate source rocks containing abundant clay [28].

2. \( \frac{27\beta:28\beta}{28\beta:29\beta} \)

The relative abundances of C₂₇, C₂₈, and C₂₉ sterane homologs reflects the carbon number distribution of the sterols in the organic matter in the source rock, and the ratio is primarily used to distinguish groups of petroleums from different source rocks [28].

**Polycyclic aromatic hydrocarbons**

Polycyclic aromatic hydrocarbons (PAHs), distinguished by a structure of two or more fused aromatic rings (Figure 2), are perhaps the most commonly studied group of
hydrocarbons. PAHs collectively comprise a suite of hundreds of individual compounds. Low molecular weight (LMW) PAHs include 2- and 3-ring compounds, while high molecular weight (HMW) PAHs consist of four or more rings (Figure 2).

There are four categories of PAH inputs to the marine environment: 1. biogenic (produced by organisms), 2. pyrogenic (derived from combustion processes), 3. petrogenic (derived from fossil fuels), and 4. diagenic (derived from alterations undergone by organic matter during deposition and burial in sediment prior to catagenesis) [21,28]. In Canada, forest fires are the most important natural PAH source, releasing approximately 2 000 tonnes per year [29]. The greatest anthropogenic PAH sources to the atmosphere are aluminum smelters (925 tonnes/year), while major sources to the aquatic environment include creosote-treated products (up to 2 000 tonnes/year) and spills of petroleum products (~75 tonnes/year) [29].
Hopane and sterane petroleum biomarkers

a) isoprene subunit

b) hopane (pentacyclic triterpane; C\textsubscript{30})

c) sterane (tetracyclic; C\textsubscript{30})

Figure 3. Petroleum biomarkers are used to elucidate original oil sources, and to determine the relative maturity of the sample.  
a) Isoprene is the basic structural unit of all biomarkers.  
b) Diagenesis converts hopanetetrols synthesized in prokaryotic organisms to the hopanes that are measured in sediment samples, and 
c) converts sterols synthesized in eukaryotic organisms to the steranes measured in sediment samples.
Differences in formation processes can provide insight into PAH source.

Combustion input can be inferred from an increase in the proportion of less stable, ‘kinetic’ PAHs (e.g. anthracene) of a given molecular mass in relation to the more stable, ‘thermodynamic’ PAHs (e.g. phenanthrene) of the same molecular mass (Figure 4; [30]). Combustion input can also be inferred from a maximum at C0 (parent compound) in the homologue series, as combustion causes the breakdown of organic matter to lower molecular weight radicals followed by reassembly to non-alkylated PAHs [31]. Conversely, petrogenic input can be inferred from a maximum at C1 or higher, as diagenetic processes occurring at relatively low temperatures over geologic time scales result in primarily alkylated PAHs [31]. Ratios used to determine PAH source are calculated within a given molecular mass to minimize differences in volatility, water solubility, adsorption, and other physicochemical properties [reviewed in 32].
Figure 4. Parent PAHs separated into two groups to differentiate between the less stable or kinetic isomer(s) and the more stable or thermodynamic isomer(s). Molecular masses for each compound are indicated at left. Adapted from [30].
In this study, PAH sources were ascertained through the use of:

1. parent PAH ratios of anthracene/(anthracene + phenanthrene) (An/(An+Ph)); where ratio values <0.1 indicate a petroleum source and values >0.1 indicate a combustion source) and fluoranthene/(fluoranthene + pyrene) (Fl/(Fl+Py)); where ratio values <0.4 indicate a petroleum source, values from 0.4-0.5 indicate fossil fuel combustion, and values >0.5 indicate biomass (grass, wood, or coal) combustion;

2. the parent to alkyl ratios of phenanthrene/anthracene (P/A C0/(C0+C1)) and fluoranthene/pyrene (Fl/P C0/(C0+C1)), where ratio values <0.5 indicate a petroleum source and values >0.5 indicate a combustion source;

3. 1,7-dimethylphenanthrene (DMP)/(2,6-DMP + 1,7-DMP) (1,7:(2,6 + 1,7-DMP)) to determine combustion type, where ratio values <0.45 indicate vehicle emissions and/or petroleum inputs and values >0.7 indicate a strong wood combustion component;

4. indeno[1,2,3-cd]pyrene/(indeno[1,2,3-cd]pyrene + benzo[ghi]perylene) (IP/(IP + BgP)), where ratio values <0.2 indicate a petroleum source, values from 0.2-0.5 indicate liquid fossil fuel combustion, and values >0.5 indicate biomass combustion [32].

Differences in bioavailability can occur as a function of source. Pyrogenic (combustion-derived) PAHs have relatively high octanol-water partitioning coefficients (log K_{ow}) and low water solubility and as such are largely associated with particulate matter, thereby significantly decreasing their bioavailability [33]. Conversely, petrogenic (petroleum-derived) PAHs are generally thought to be largely available for uptake by
marine organisms due to their increased water solubility and lower log $K_{ow}$ values [34; Appendix I].

*Alkyl PAHs*

Alkyl-substituted PAHs (alkyl PAHs) have various numbers (usually one to four) of alkyl substituents (*e.g.* methyl groups) attached to the parent molecule (Figure 3). The toxicological significance of alkyl PAHs is largely unknown, yet they comprise a much greater portion of most petroleum deposits and products than unsubstituted PAHs [35]. For example, in crude oil, alkyl PAHs generally account for >90% of the total PAH content [36]. Alkyl PAHs are less water soluble, less volatile, and have higher log $K_{ow}$ values than their respective parent compounds, and as such tend to persist longer in environmental matrices and bioaccumulate to a greater extent [37-39]. They can also be more toxic than their respective parent compounds [38,40,41], and toxicity appears to increase with increasing alkyl substitution on the aromatic nucleus [21,37,42].

*PAH pharmacokinetics*

In vertebrates, the intestines are the interface through which ingested PAHs are generally taken up into the body (Figure 5; [43]). Prior to systemic uptake, the small intestine contributes to the first-pass metabolism of PAHs [reviewed in 43]. However, the liver is ultimately the primary organ for the detoxification of PAHs. Most metabolized PAHs are excreted into the bile and subsequently eliminated via the feces, with a smaller amount excreted via the urine [43]. However, some less polar compounds can undergo enterohepatic circulation, wherein they are reabsorbed into portal circulation and returned to the liver [43,44]. Enterohepatic circulation functions to extend the residence time of PAHs in the body, and continuous enterohepatic recycling may lead to
long half-lives of reactive PAH metabolites [43]. Although the majority of ingested PAHs are metabolized and excreted, in some instances unmetabolized parent compounds pass directly into the lumen of the gastrointestinal tract and are eliminated through the feces [43].

The carcinogenic potential of various PAHs is associated with the formation of reactive phase I metabolites that may either be detoxified through phase II metabolism or bind covalently to other cellular components such as DNA [45].

Metabolism occurs in two phases. Phase I involves sequential oxygenation (mediated by cytochrome P450 1A) and hydration (mediated by epoxide hydrolase) steps, resulting in the formation of several types of reactive intermediates, including epoxides, diols, and diol epoxides [43,46]. Phase II involves the secondary metabolism of phase I intermediates by enzymes including glutathione S-transferases (GST), UDP-glucuronosyltransferases (UDPCT), and sulfotransferases (ST) [43,47]. This step increases the polarity of the phase I metabolites by conjugating them with the compounds in the names of the enzymes (e.g. sulfate), making them more readily excreted [47].
PAH ingestion \rightarrow Gastrointestinal tract \rightarrow Feces \rightarrow Elimination \\
Gastrointestinal tract \rightarrow Liver \rightarrow Bile \rightarrow Enterobiotic circulation \\
Liver \rightarrow Organ/ Tissues \rightarrow Systemic circulation \\
Kidney \rightarrow Elimination \rightarrow Urine

Figure 5. The liver is the primary organ for the detoxification of polycyclic aromatic hydrocarbons (PAHs) in vertebrates [adapted from 43].

**PAH toxicity**

PAHs exert toxicity in a variety of ways according to their size and physicochemical properties. Low molecular weight (LMW) PAHs are generally thought to exert narcotic toxicity, a “reversible anesthetic effect that is caused by hydrophobic chemicals partitioning into cell membranes and nervous tissue that results in disruption of central nervous system function” [48]. The proposed target site of action for narcotic chemicals is the lipid membrane bilayer, and thus the potency of narcotic chemicals is directly related to their lipophilicity (log K_{ow}) [41]. Chemicals that cause narcosis are thought to have minimal toxicity because the critical body residues are substantially higher than those for chemicals with more specific modes of action (e.g. receptor mediated toxicity) [39].
Conversely, high molecular weight (HMW) PAHs exert toxicity via reactive phase I metabolites, which bind to DNA, promoting mutagenesis and carcinogenesis. PAH metabolites have been implicated as causative agents of lung, breast, esophageal, pancreatic, gastric, colorectal, bladder, skin, prostate, and cervical cancers in both human and animal models [reviewed in 43]. PAHs have also been reported to cause hemato-, cardio-, renal, neuro-, immuno-, reproductive, and developmental toxicity in humans and animals [reviewed in 43].

The toxicity of alkyl PAHs appears to depend largely on the placement and number of alkyl groups [reviewed in 49]. For example, while 1,12-dimethylbenz[a]anthracene is inactive as a carcinogen, 7,12-dimethylbenz[a]anthracene is an extremely strong carcinogen [35].

Because environmental exposures are to extremely complex mixtures rather than individual compounds, characterizing and quantifying PAH toxicity to wildlife presents a daunting challenge. Current PAH toxicity models assume dose and/or concentration additivity, with the assumption that effects are mediated by binding to the aryl hydrocarbon receptor [AhR; 41,50]. While some PAHs certainly do bind this receptor, LMW PAHs are poor AhR agonists, and toxicity likely occurs via narcosis. Recent work has suggested that PAH cardiotoxicity in the early life stages of fish is independent of the AhR and that in some cases, CYP1A activity may even be protective against toxicity caused by exposure to some PAHs. Some of the most potent CYP1A inducers (e.g. benzo[k]fluoranthene) were found to be non-toxic to early life-stages of fish, while weak AhR agonists such as alkyl PAHs were highly toxic [41]. Further, some PAHs (e.g. fluoranthene, dibenzothiophene) appear to be CYP1A inhibitors, and have been shown to
increase embryotoxicity when combined with PAHs that are AhR agonists, suggesting that an assumption of additivity may greatly underestimate risk [41].

**Vulnerability of sea otters to oil pollution**

In British Columbia, oil pollution has been identified as the primary threat to the recovering sea otter population as a consequence of the relatively small size of the population and its geographical constraints (sea otters likely occupy only ~30% of their original range), their propensity to aggregate in large numbers, the proximity of the population to shipping lanes, and their life history characteristics [4]. Several of the life history characteristics that make sea otters unique among marine mammals also increase their vulnerability to acute exposure to whole oil, as demonstrated following the EVOS.

Unlike most other marine mammals, sea otters do not have a blubber layer, relying instead on the thickest fur coat in the animal kingdom (\(~100,000\) hairs/cm\(^2\)) [1]. A dense underfur traps air against the body. This air is warmed by body heat and insulates the body [13]. To maintain the air layer, sea otters spend approximately 15% of their day grooming to prevent soiling of the fur and subsequent loss of insulation and reduced buoyancy [51]. If the fur is soiled, water can penetrate to the skin and reduce insulation by up to 70% [52]. Oil destroys the water repellent property of the fur, rendering the otter vulnerable to hypothermia [10]. Oiled otters will groom obsessively, not eating or resting in an attempt to clean their fur, thereby exacerbating hypothermia, spreading the oil, and increasing ingestion of oil [53]. Thus, not only is the fur further compromised by grooming, the otter ingests oil as it grooms.

Furthermore, sea otters in BC are highly aggregated. They may forage as solitary individuals but spend the greatest proportion of their day in rafts, which are generally
sex-segregated and have been observed to include up to 200 otters [53,54]. This rafting habit means that a large segment of the population (e.g. up to 200 females) may be exposed to oil at once. Compounding the problem, sea otters can spend their entire lives at sea, where they rest, mate, give birth, and forage, and the mortality associated with the EVOS indicated that thousands of sea otters did not or could not avoid the oil.

While sea otters are highly vulnerable to acute exposure to whole oil, their large dietary requirements underscore the potential for prey to act as an important route for chronic hydrocarbon exposure, even when hydrocarbon concentrations in prey are low. The high metabolic rate of sea otters [two to three times that of similarly-sized land mammals; 51]) requires that they consume approximately 25% of their body weight every day [1]. This is of concern because sea otters consume primarily marine invertebrates which act as hydrocarbon reservoirs because of their inability to metabolize these compounds.

Their reliance on benthic invertebrates may also require frequent and prolonged contact with potentially contaminated sediments. As a hydrocarbon sink, sediments may play an important role as a route of uptake for these compounds into marine foodwebs. It has been estimated that sea otters foraging in certain sections of Prince William Sound, Alaska may encounter oil from the EVOS approximately once every 200 sediment disturbances, which, at a conservative rate of digging three pits per day, would lead to exposure to residual oil at least once every two months [55].

*Thesis summary*

Most research on hydrocarbon exposure has focused, perhaps understandably, on acute exposure to high environmental hydrocarbon concentrations following catastrophic
oil spills. Thus, the consequences of acute exposure to oil are generally better understood than the risks associated with chronic dietary exposure to ambient hydrocarbons of natural and anthropogenic origin. This study complements the dominance of acute and spill-related hydrocarbon research, and provides a first look at the sources and fate of hydrocarbons in the habitat of sea otters in BC and new insights into the threat that chronic dietary exposure to hydrocarbons may pose to sea otters.

In view of the increasing likelihood of offshore oil and gas exploration and development, increased tanker traffic plying the BC coast, and the extreme vulnerability of sea otters to oil pollution, this work characterized hydrocarbon source, transport, and fate in the habitat and food web of sea otters in BC. Chapter 2 examines the sediment concentrations and patterns of hydrocarbons in remote BC sea otter habitat on the west coast of Vancouver Island and compares them to those in an urban/industrial positive reference site on the province’s south coast. Chapter 3 examines the concentrations and patterns of hydrocarbons in sea otter prey and compares and contrasts these with those measured in the sea otters. Chapter 4 synthesizes the results of previous chapters and discusses potential future research directions.
CHAPTER 2: COMPOSITION AND SOURCES OF ALIPHATIC AND AROMATIC HYDROCARBONS IN SEDIMENTS FROM SEA OTTER (ENHYDRA LUTRIS) HABITAT IN BRITISH COLUMBIA, CANADA

This chapter has been submitted under the following citation:

Kate A. Harris, Mark B. Yunker, Neil Dangerfield, and Peter S. Ross. 2010. Composition and sources of aliphatic and aromatic hydrocarbons in sediments from sea otter (Enhydra lutris) habitat in British Columbia, Canada. Environmental Pollution.
Introduction

As the primary repository for hydrocarbons in the marine environment, sediments also represent a source of these compounds for adjacent food webs [34]. Benthic invertebrates, for example, accumulate hydrocarbons [34] which may then be available to higher trophic level species. Sea otters (*Enhydra lutris*) represent one keystone wildlife species that may be especially vulnerable because of their heavy consumption of invertebrates (up to 25% of their body weight per day), for which they forage at the substrate-water interface [13,56].

Hydrocarbons are the primary constituents of crude and refined oil, generally comprising more than 75% by weight [21]. While the 1989 *Exxon Valdez* oil spill (EVOS) in Alaska clearly illustrated the vulnerability of sea otters to whole oil [e.g. 57,58], chronic exposure to oil constituents (e.g. hydrocarbons) from multiple sources represents an ongoing and poorly understood risk. With oil regarded as the primary threat to sea otters in British Columbia [BC; 4], it is important to distinguish between natural and anthropogenic hydrocarbon sources, and to characterize exposure levels and associated risks.

Hydrocarbons are ubiquitous in the environment, with both natural and anthropogenic sources. Combustion-derived (pyrogenic) hydrocarbons are formed as a result of the incomplete combustion of organic matter at relatively high temperatures, while petroleum-derived (petrogenic) hydrocarbons are formed from organic material at relatively low temperatures over geologic time scales [59]. Natural pyrogenic hydrocarbon sources include forest and grass fires, while anthropogenic pyrogenic sources include vehicular and industrial emissions [21,22,32]. Natural petrogenic
hydrocarbons sources include crude oil seeps and coal and shale deposits, while anthropogenic sources include oil spills, chronic petroleum discharges and coal [60]. Due to the lipophilic, hydrophobic nature of hydrocarbons, sediments are the primary repository in the marine environment [22,61].

Hydrocarbon classes reported here include the resolved alkanes and the unresolved complex mixture (UCM), hopane and sterane biomarkers, and the polycyclic aromatic hydrocarbons. The resolved alkanes, which can be either straight (n-alkanes) or branched chain, are commonly used to obtain a broad overview of hydrocarbon sources ranging from terrestrial plant material to marine algae to petroleum. Hopanes and steranes, derived from precursors in bacterial cell membranes and eukaryotic cell membranes, respectively, can often provide more specific information on hydrocarbon sources and maturity [28].

PAHs comprise perhaps the most comprehensively studied hydrocarbon class, since they are widespread in the aquatic environment [30], and are detected everywhere from Arctic ice and snow to deep sea sediment [61]. Anthropogenic PAHs are considered persistent organic pollutants (POPs), but differ from other POPs in their relative ease of metabolism, multiple possible structures, and widespread and continuing sources [31].

Petrogenic and pyrogenic processes generate very different PAH mixtures. A predominance of low molecular weight (LMW) alkyl PAHs generally indicates a petrogenic origin, while a greater contribution of high molecular weight (HMW) parent PAHs often suggests the importance of pyrogenic sources from anthropogenic combustion [62]. Pyrogenic PAHs form in soots as a result of free radical reactions in
the flames typical of fossil fuel combustion, and in chars as the result of flaming and
smouldering of the cellulose-rich solid residues of plant tissues during biomass
combustion. The distinction is important because smaller combustion particulates, such
as the soot black carbon from fossil fuel combustion, transport easily in air and water,
while larger particles, like wood chars, undergo limited atmospheric transport but can
move extended distances with water or ice [32,63-65].

Conversely, petroleum-derived (petrogenic) PAHs are formed by diagenetic
processes at relatively low temperatures over geologic time scales, leading to the
formation of primarily alkylated PAHs [31]. Thus, PAH series with a maximum at C_0
generally indicate the predominance of pyrogenic PAHs, while an alkyl series maximum
at C_1 or higher indicates petrogenic input [30]. PAHs are of concern from a health
perspective as LMW PAH often are acutely toxic [21], while many HMW PAH are
known carcinogens and mutagens [66-69].

The objectives of this study were to characterise the concentrations and sources of
natural and anthropogenic hydrocarbons in BC sea otter habitat. In order to provide a
broader context to hydrocarbons in sea otter habitat, and illuminate the relative
contributions of natural and anthropogenic sources, we also examined impacted sediment
sites in the heavily urbanized Burrard Inlet, adjacent to the City of Vancouver. In
addition, the potential toxicological significance of hydrocarbon concentrations at each
location was evaluated against effects-based sediment quality guidelines for the
protection of aquatic biota.

Methods

Study area
This study focused on the Esperanza Inlet/Nuchatlit Inlet (EN) areas on the west coast of Vancouver Island (WCVI; Figure 6a, b). Forestry is the primary commercial venture in the area. Logging has long occurred in the watershed to supply materials for the former Tahsis Company Ltd. sawmill at west Tahsis harbour (closed in 2001), and the company’s pulp and paper mill at Gold River [70]. The deep, glacially-carved fjords in the inlet provide ideal transportation corridors for ocean-going freighters and other marine vessels [70]. Small towns and camps in the area are reached by water, air, or the extensive network of logging roads.

Figure 6. a) Surficial sediments (~10 cm) were sampled at two locations on the British Columbia (BC) coast. Dashed lines indicate current sea otter range. Sediments were sampled: b) in sea otter habitat in the relatively remote Esperanza Inlet/Nuchatlit Inlet (EN) areas, and c) in Burrard Inlet, an urban/industrial area on the province’s south coast. Numbers on the map correspond to the following sample sites: 1. Nuchatlit Inlet (NI); 2. Port Lanford (PL); 3. Louie Bay (LB); 4. NE Catala Island (CI); 5. Zeballos fuel dock (ZD); 6. Little Zeballos River (ZR); 7. Saltery Bay (SB); 8. S Centre Island (SC); 9. E Nootka Island (EI); 10. west Tahsis harbour (WT); 11. Shell Oil dock (SO); 12. oil spill boom (OB); 13. Indian Arm (IA); 14. Shell Oil terminal (OT); 15. narrows to Moody Arm (MA); 16. Reid Point marina (RP); 17. Port Moody log sort (LS); 18. Port Moody sawmill boom (PM). d) Total PAH concentrations on ng/g dry weight basis are presented for the 18 sites at our two study locations.
By contrast, Burrard Inlet (BI) is a highly developed urban/industrial area on BC’s south coast and is the location for Vancouver’s main harbour. Nearshore development includes rail yards, container and bulk cargo ship terminals, and oil refineries [71]. The most commonly reported spills into the Inlet include hydrocarbons (bunker oil, gasoline and diesel fuel) and plant derived oils (canola oil). Nearly 1.4 million people live in urban centres on the shores of this 25 km long inlet [71].

Sampling

In July 2007, 11 surficial sediment samples (0-10 cm) were collected in and around Esperanza and Nuchatlitz Inlets (Figure 6). Samples were taken manually using a Petit Ponar® surface grab sampler (Ponar, Buffalo, NY, USA) from a rigid hull inflatable boat. Water depths ranged from 7.9-15.2 m (average 11.6 m). Samples were homogenized immediately in a hexane-rinsed stainless steel bowl, subsampled into 250 mL amber glass jars (VWR International Ltd., Victoria, BC, Canada), and frozen at -20°C until sample analysis.

In August 2007, eight surficial sediment samples (0-10 cm) were collected in Burrard Inlet using a Petit Ponar® surface grab sampler (Ponar). Water depths ranged from <1.0 m – 35.0 m (average 15.3 m). Sampling followed the July 24 Kinder Morgan pipeline rupture, as a result of which 234 000 L of crude oil were released (almost 210 000 L were reportedly recovered) [72]. Crude oil flowed into Burrard Inlet via the Burnaby sewer system, affecting approximately 1200 m of shoreline [72]. A boom was put in place to try to contain the oil entering the marine environment. Sediment samples were taken just outside the boom.

Hydrocarbon analysis
Sediment subsamples (~15 g) from ten EN sites and eight BI sites were analysed for resolved alkanes and the unresolved complex mixture (UCM), the C$_{27}$, C$_{29}$, C$_{30}$ and C$_{31}$ 17α(H),21β(H)-hopanes, and polycyclic aromatic hydrocarbons (PAHs; Appendix I) by Axys Analytical Services Ltd (Sidney, BC, Canada) using high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS). Sediments were combined with anhydrous sodium sulphate and spiked with perdeuterated surrogate standards (four alkane and 16 PAH surrogates) that covered the full range of compounds quantified [73].

Samples were Soxhlet extracted with dichloromethane and concentrated using a Kuderna-Danish apparatus. Following separation into fractions onto a silica gel column (Biosil, 10 g; 5% deactivated) using pentane (25-35 mL, alkane fraction) and then dichloromethane (30-35 mL, PAHs), samples were spiked with recovery standards (three PAH surrogates) and analysed using selective ion monitoring GC/MS using a minimum of two ions per analyte. Further method details are provided in Yunker et al. [73]. The tri- and tetracyclic terpanes, hopanes, steranes, diasteranes and biogenic hopanes were quantified in the alkane fraction relative to the reported 17α(H),21β(H)-hopanes using Axys chromatograms. The terpanes and hopanes used peak areas from $m/z$ 191 chromatograms (with $m/z$ 177 and 205 confirming ions for the hopanes plus 410 for the hopenes) and the sterane analyses used both peak heights and areas from $m/z$ 217 and 218 chromatograms.

A procedural blank was included with each batch of samples. Most aliphatic compounds in the blank for EN samples were below the limit of detection (LOD), with five detected at <1.0 ng/g. All EN hopanes were below the LOD in the procedural blank.
For the EN PAH blank, most compounds were below the LOD, with nine compounds detected at <1.0 ng/g. Similarly, most aliphatic compounds in the blank for BI samples had concentrations below the LOD, with seven detected at <2.5 ng/g. All BI hopanes were below the LOD in the blank. For the BI PAH blank, the majority of PAH were below the LOD, with 19 compounds detected at <2.0 ng/g. Recoveries from the spiked sediment samples included with each batch were generally within QA/QC criteria of 70-130%, with exceptions primarily from the most volatile constituents ($n$-C$_{12}$ – $n$-C$_{14}$ alkanes and naphthalene). Naphthalene values for all BI sites were reported by Axys Analytical as ‘non-quantifiable’ (NQ) due to interferences, and as such, naphthalene values could not be reported for BI sites.

**Total organic carbon (TOC) analysis**

Sediment total organic carbon (TOC) content was analysed at the Institute of Ocean Sciences (Sidney, BC, Canada) according to methods published previously [74]. Briefly, oven dried, homogenized sediment samples were acidified with 1M HCl and dried on a hot plate overnight. TOC was measured using a Leemens 440 Elemental Analyzer standardised against an acetanilide standard containing 71.09% C and 10.36% N. Standards were analyzed as a sample and tin cup/nickel sleeve blanks were analysed at the beginning and end of each analytical run. The standard deviation of replicate TOC measurements was TOC % = 0.03, n = 3 pairs.

**Data analysis**

Total hydrocarbon concentrations were calculated as the sum of the concentrations of compounds that were detectable in at least 70% of samples from each location. Detection limit substitutions were made for undetected compounds in cases
where at least 70% of sites had detectable values for that compound. Where less than 70% of sites had detectable concentrations of a compound, zero ng/g was substituted for compounds below the limit of detection (LOD). Detection limits at both EN and BI were generally <1.0 ng/g. All measured concentrations were corrected to the concentration measured in the lab blank.

Data are presented in ng/g dry weight for the purpose of comparison to national and provincial Marine Sediment Quality Guidelines for the Protection of Aquatic Life [75]; however, the effect of TOC on hydrocarbon concentrations in sediment is discussed.

Results and Discussion

Sea otters are nearshore marine mammals that meet their considerable dietary requirements by foraging for benthic invertebrates at the sediment-water interface. This, coupled with a reliance on the densest fur in the animal kingdom for insulation, makes them highly vulnerable to the impacts of oiling. Sediments are an important sink for hydrocarbons, and provide a route for uptake into the adjacent food web. Our assessment of ~85 hydrocarbons in sediments provides insight into the fate of these compounds in the coastal environment, and sheds light on the anthropogenic contribution to sediment hydrocarbon content and composition.

Alkane patterns and sources

Sediment samples could be classified into three categories based on the resolved alkane and unresolved complex mixture (UCM) concentrations (Table 2) and the appearance of the alkane chromatograms (m/z 57 ion traces; Figure 7).
Table 2. Resolved alkane and PAH source identification parameters for sites in sea otter habitat (Espenanza/Nuchatlitz Inlet areas; EN) and an impacted reference site (Burrard Inlet; BI) in British Columbia (see Figure 6 for site locations). All hydrocarbon concentrations are reported in ng/g dry weight.a

<table>
<thead>
<tr>
<th>Esperanza/Nuchatlitz Inlets (EN; sea otter habitat):</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>% TOC</td>
</tr>
<tr>
<td>Nuchatlitz Inlet (NI)</td>
<td>Sed07-03</td>
</tr>
<tr>
<td>Port Langford (PL)</td>
<td>Sed07-04</td>
</tr>
<tr>
<td>Louie Bay (LB)</td>
<td>Sed07-05</td>
</tr>
<tr>
<td>NE Catala Island (CI)</td>
<td>Sed07-06</td>
</tr>
<tr>
<td>Little Zeballos River (ZR)</td>
<td>Sed07-09</td>
</tr>
<tr>
<td>Saltery Bay (SB)</td>
<td>Sed07-10</td>
</tr>
<tr>
<td>S Centre Island (SC)</td>
<td>Sed07-11</td>
</tr>
<tr>
<td>E Nootka Island (EI)</td>
<td>Sed07-12</td>
</tr>
<tr>
<td>Harbour sites:</td>
<td></td>
</tr>
<tr>
<td>Zeballos fuel dock (ZD)</td>
<td>Sed07-08</td>
</tr>
<tr>
<td>West Tahsis harbour (WT)</td>
<td>Sed07-13</td>
</tr>
<tr>
<td>Burrard Inlet (BI; impacted reference site):</td>
<td></td>
</tr>
<tr>
<td>Sample</td>
<td>% TOC</td>
</tr>
<tr>
<td>Shell Oil dock (SO)</td>
<td>Sed07-14</td>
</tr>
<tr>
<td>Oil spill boom (OB)</td>
<td>Sed07-15</td>
</tr>
<tr>
<td>Indian Arm (IA)</td>
<td>Sed07-16</td>
</tr>
<tr>
<td>Shell Oil terminal (OT)</td>
<td>Sed07-17</td>
</tr>
<tr>
<td>Narrows to Moody Arm (MA)</td>
<td>Sed07-18</td>
</tr>
<tr>
<td>Reid Point marina (RP)</td>
<td>Sed07-19</td>
</tr>
<tr>
<td>Port Moody log sort (LS)</td>
<td>Sed07-20</td>
</tr>
<tr>
<td>Port Moody sawmill boom (PM)</td>
<td>Sed07-21</td>
</tr>
</tbody>
</table>

Cmax: alkane detected at highest concentration; CPI: carbon preference index (defined as \(25+27+29+31+33)/(26+28+30+32+34\)), which identifies proportions of terrestrial plant contributions versus fuel contamination; where values close to 1.0 indicate petroleum contamination and values >4.0 indicate terrestrial plant input; UCM: unresolved complex mixture. See the Figure 8 caption for definitions of parent and alkyl PAHs.
The first group encompassed all remote Esperanza Inlet/Nuchatlitz Inlet (EN) sites, where concentrations of the total resolved alkanes (n-alkanes plus isoprenoids) ranged from 40.9 – 6350 ng/g, alkane chromatograms showed primarily discrete alkane peaks with no apparent UCM, and the measured UCM, ranging from 116 – 3360 ng/g, was generally lower than concentrations of the resolved alkanes. Saltery Bay, Nuchatlitz Inlet (Figure 7a and b), and S. Centre Is. sites had the highest UCM concentrations in this group (1140, 1270 and 3360 ng/g, respectively; Table 2), but most of the UCM in these samples was made up of clusters of unresolved components around a few peaks (n-C\textsubscript{19}, n-C\textsubscript{21}, n-C\textsubscript{31}), rather than a full UCM envelope. The second group included the two EN harbour sites at the Zeballos fuel dock and the west Tahsis harbour (Figure 7c), which had generally higher resolved alkane concentrations (4870 and 9090 ng/g, respectively) with much higher UCM concentrations (26 800 and 21 400 ng) and alkane chromatograms exhibiting a prominent, petroleum-derived UCM [76]. Sites in urban/industrial Burrard Inlet (BI) constituted the third group (Figure 7d), where resolved alkane concentrations (792 – 9720 ng/g) were uniformly much lower than the UCM concentrations (6270 – 71 300 ng/g) and all alkane chromatograms displayed a prominent UCM.

At both EN and BI, total n-alkane concentrations were strongly correlated with TOC in sediment ($r^2 = 0.88$, $p < 0.0001$; $r^2 = 0.90$, $p = 0.0003$, respectively; data from Table 2), suggesting a relatively simple source regime (e.g. plant and petroleum). TOC in sediments reflects both planktonic and terrigenous inputs, but terrigenous carbon tends to be more effectively preserved [77]. Higher TOC concentrations at some sites in both
locations may reflect increased inputs of organic matter from bark debris due to log booms and saw mills, although inputs of oil, coal or coal tar are also possible.

Resolved alkane patterns for all sediment samples in this study were dominated by the terrestrial plant-related higher \( n \)-alkanes \( (n-C_{23} - n-C_{33}) \) with an odd carbon predominance (CPI centred at \( C_{29} \) is 1.9 – 10.5, 6.0 – 13.6 and 3.4 – 5.2 for groups one to three, respectively; Table 2). The strong presence of plant-derived alkanes is consistent with the input of plant debris from the heavily forested coastal mountains surrounding the two study areas as a result of both natural runoff and forestry activity [77]. A number of sites at both EN and BI exhibited a markedly elevated \( n-C_{35} \) peak. Enhancement of \( n-C_{35} \) is often due to the coelution of \( n-C_{35} \) with the \( C_{40} \) isoprenoid lycopane, particularly in anoxic sediments present in oxygen minimum zones [78]. However, reanalysis of the Saltery Bay sample (Figure 7b; the sample with the most prominent \( n-C_{35} \) peak) by full scan GC/MS provided a very close match for \( n-C_{35} \) to both the authentic alkane in the calibration standard and to the NIST reference spectrum. Diagnostic ratios of \( m/z \) 183/\( (\text{mean of } 169 + 197) \) and \( m/z \) 183/182 of 0.24 and 1.63, respectively, in the sediment extract also are much closer to the ratios of 0.25 and 1.71 in the \( n-C_{35} \) standard than to the ratios of 0.45 and 2.32 published for lycopane [78]. Hence, a plant wax source for \( n-C_{35} \) appears likely, although we are not aware of a literature precedent for such an \( n-C_{35} \) enhancement.
PAH patterns and sources

Total parent and alkyl PAH concentrations at remote EN sites ranged from 4.1 – 252 and 6.6 – 359 ng/g, respectively, while concentrations at impacted harbour sites (the Zeballos fuel dock and the west Tahsis harbour) were 19 600 ng/g and 7450 ng/g, and 1640 ng/g and 1760 ng/g, respectively. Total parent and alkyl PAH concentrations at industrialized BI sites ranged from 327 – 6650 ng/g and 241 – 4840 ng/g, respectively. The sum of the 16 priority PAH designated by the US Environmental Protection Agency (Σ16 USEPA PAH) ranged from 3.7 – 238 ng/g for remote EN sites (excluding the
Zeballos fuel dock and the west Tahsis harbour sites, which had concentrations of 18 100 and 1540 ng/g respectively) and from 303 – 6220 ng/g for BI sites.

Levels at contaminated sites in both locations were similar to, and in some cases above, those detected in other parts of the world. Heavily polluted US harbour sites had total PAH concentrations ranging from 7300 – 358 000 ng/g [79], while concentrations at sites of varying degrees of development in Hong Kong ranged from 7.25 – 4420 ng/g (minus benz[a]anthracene) [27]. In Brazil, sites ranging from remote islands to busy harbours had concentrations from 6.30 – 277 ng/g [80], and concentrations at Guanabara Bay, Brazil (home to industrial parks, oil refineries, commercial ports, oil terminals, and shipyards) ranged from 79 – 487 ng/g [81].

Parent and alkyl PAH composition profiles for remote EN sites generally showed comparable concentrations of the alkyl naphthalenes, alkyl phenanthrene/anthracenes and parent PAHs, with lesser amounts of the other alkyl PAHs (Figure 8a). The relative proportion of the alkyl PAH series to the parent PAHs did vary (Figure 8b), but alkyl substituted PAHs generally dominated over the parent for the alkyl naphthalenes, fluorenes, dibenzothiophenes and phenanthrene/anthracenes. Conversely, parent PAHs dominated for the fluoranthene/pyrenes and benz[a]anthracene/chrysenes.

This profile, which is consistent with petroleum dominance for the two and three ring PAHs and combustion dominance for the four and higher ring PAHs, is typical of both present day and pre-1700 sediment core PAH profiles from BC’s west coast (Yunker et al., in preparation). Alkyl PAHs can be derived from naturally occurring seeps, as well as bitumen, coal, and mature organic matter [82,83]. At EN harbour sites, PAH profiles were dominated by pyrogenic parent PAHs and the heavier three- and four-
ring alkyl PAHs (Figure 8c; FP1 is much less prominent at the Zeballos site). The pyrogenic parent PAHs in this profile are consistent with the presence of soot or chars from fossil fuel and wood combustion, while the heavier alkyl PAHs indicate heavier or weathered petroleum.

For BI sites the PAH profile was again dominated by the parent PAHs, but the two and three ring alkyl PAH series were present in comparable proportions to the four ring alkyl PAHs (Figure 8d). Unlike EN, BI is a major harbour with a long history of intense and varied human use. Anthropogenic hydrocarbon inputs include wastewater from oil refineries, sewer overflows, stormwater outfalls, and sawmills, as well as vehicle exhaust, furnaces (coal, wood, and oil burning) and industrial emissions. This usage history was reflected in an even distribution of parent and alkyl PAHs (\( \bar{x} \pm SD = 49.9 \pm 7.4\% \) and 50.1 ± 7.4%, respectively), as seen in previous studies of urban sites [84]. The importance of pyrogenic 4- and 5-ring parent PAH at most sites reflects significant anthropogenic inputs, and confirms the findings of earlier work in BI [32,73]. These compounds are commonly detected in PAH profiles near urban/industrial areas, as they are the predominant components of gas and diesel soot and coal combustion emissions [32].
Figure 8. PAH composition profiles for the representative locations in Fig. 2, showing a) Nutchatlitz Inlet, b) Saltery Bay, c) west Tahsis harbour, and d) Burrard Inlet at the narrows to Moody Arm. Profiles, from left to right, show the alkyl naphthalenes series (N0-N4), biphenyl (Bi), fluorenes (F0-F3), dibenzothiophenes (D0-D3), benzo[b]naphthothiophenes (BNT, with 1,2-d, 2,1-d and 2,3-d isomers), phenanthrene/anthracenes (P/A0 – P/A4) plus retene (Ret), fluoranthene/pyrenes (F/P0-F/P3), benzo[a]anthracene/chrysenes (B/C0 – B/C3), followed by the parent PAH series by molecular mass for fluorene (166), phenanthrene plus anthracene (178), fluoranthene, acephenanthrylene and pyrene (202), benzo[a]anthracene plus chrysene (228), benzo[b/k]fluoranthene, benzo[a]pyrene and benzo[e]pyrene (252), indeno[7,1,2,3-cd]chrysene, indeno[1,2,3-cd]pyrene, benzo[ghi]perylene and anthanthrene (276) and dibenz[a,j]anthracene, dibenz[a,h]anthracene, pentaphene, benzo[b]chrysene and picene (278).
Additional insight into PAH sources can be provided using PAH ratios that are calculated within a given molecular mass to minimize differences in volatility, water solubility, adsorption, and other physicochemical properties [reviewed in 32]. Ratios of An/(An + Ph) for EN and BI sites were greater than the 0.10 boundary for petrogenic vs. combustion sources for all but one sediment sample (Saltery Bay at 0.07), corroborating a combustion source for the parent PAHs.

PAH ratio cross plots for Fl/(Fl + Py) vs. BaA/(BaA + Ch) and IP/(IP + BgP) provided further information that most parent PAHs in EN samples were derived from biomass or solid fuel combustion while the major source for BI sites was petroleum combustion from burning liquid fossil fuels in vehicles and furnaces (Figure 9). PAHs in sediments often have mixed sources, so it is likely that source contributions to each PAH mass are not uniform, particularly given that petroleum predominantly contains two and three ring PAHs while combustion has a dominance of four to six ring PAHs. Accordingly, Fl/(Fl + Py) showed the clearest distinction between sample types with most EN samples having a biomass combustion source, BI samples dominated by petroleum combustion and the west Tahsis harbour site (TH) dominated by petroleum. At higher masses the BaA (BaA + Ch) and IP/(IP + BgP) ratios showed most samples from EN and BI having combustion or biomass combustion sources, respectively, while the BaA (BaA + Ch) ratio showed most EN sites as being marginally within the range of having mixed sources (E Nootka Is. is the exception). The IP/(IP + BgP) ratio showed a clearer distinction between BI and EN samples in terms of petroleum combustion and biomass combustion, respectively (Figure 9).
Ratios $C_0/(C_0 + C_1)$ P/A and F/P were > 0.5 for most sediment samples, confirming a combustion source for the parent PAHs (the west Tahsis harbour being the major exception), while ratios of $1,7/(1,7 + 2,6)$-dimethylphenanthrene ranged between 0.45 and 0.70, indicating mixed combustion sources.

Saltery Bay had the lowest proportion of alkyl PAHs to the parent PAHs of any of the remote EN sites (its PAH profile has 58.0% HMW parent PAH; Figure 8b), and source indicator ratios all pointed to biomass combustion (Figure 9). Nevertheless, the bay was the site of a fish salting plant until the late 1920s [85], raising the possibility that creosote and/or coal tar may have been discharged at the site. Weathered/degraded coal tar residues are compositionally similar to combustion-derived PAHs [32,86].

While patterns revealed the presence and importance of petrogenic alkyl PAH throughout BI, source ID ratios clearly identified an overlying combustion source (primarily fossil fuel combustion) at most sites [cf. 73]. This conclusion agrees with Stout et al., who identified an “urban background” of anthropogenic sources derived from both petro- and pyrogenic nonpoint sources, including stormwater and surface runoff, direct deposition, and petroleum discharges [73,84].
Figure 9. PAH cross plots for the ratios of a) fluoranthene (Fl) to fluoranthene plus pyrene (Py) and b) benz[a]anthracene (BaA) to BaA plus chrysene (Ch) vs. indeno[1,2,3-cd]pyrene (IP) to IP plus benzo[ghi]perylene (BgP) for sediments from the Esperanza Inlet/Nuchatlitz Inlet (EN) areas and Burrard Inlet (BI).
Strong correlations were observed between perylene and retene at both EN ($r^2 = 0.94, p = 0.0001$) and BI ($r^2 = 0.82, p = 0.002$) sites. These PAHs are often associated with early diagenesis of organic matter in marine sediments, where retene originates in runoff from coniferous forests, making it likely that the organic matter has a terrestrial source, and perylene is a general indicator of diagenesis of terrigenous debris [77,87-89]. A >10% contribution of perylene to total penta-aromatics likely indicates primarily diagenetic input, while a contribution <10% points to supplementation by a pyrogenic source [90]. At non-harbour EN sites, contributions ranged from 23-42% (29 ± 13%), while at the Zeballos fuel dock and the west Tahsis harbour, contributions were 5.0 and 10% respectively. Perylene contributions to total penta-aromatic concentrations were less variable and lower at BI sites (range 7.1-15%; mean 10 ± 2.4%; $p = 0.0005$), confirming patterns and indicator ratio evidence of pyrogenic inputs at EN harbour sites and at most BI sites.

*Distributions of hopane, sterane, triterpane biomarkers*

Numerous hopane, sterane, and triterpane biomarker parameters related to the source and original depositional environment of organic matter have been developed to determine the origin, maturation and level of degradation of oils [28]. Well-defined series of the diagenetic isomers of hopane and sterane biomarkers can be found in sedimentary rock, petroleum source rocks, crude oils, coals, etc. [28]. The terpanes, hopanes, and steranes are generally absent from refined products such as gasoline but are present in crude and heavy oils, coal, motor oils, asphalt, and by-products including lubricating oils, muffler soot, and road dust [28,82,91]. Biomarkers in these contaminants enter the aquatic environment primarily via stormwater runoff [32], but can
also enter via atmospheric transport (e.g. coal dust) or direct spills. Diagenetic hopanes and steranes have saturated cyclic structures which provide few pathways for biotransformation [92] and are therefore very stable in the environment compared to the acyclic alkanes, making them useful composition indicators for petroleum contaminants [80,90].

Biogenic (bacterial) hopanes (Appendix I) generally made the largest contributions to sediment samples from most remote EN sites. Rearranged steranes and tricyclic terpanes were absent or present only in trace amounts, and concentrations of diagenetic hopanes were relatively low. These biomarker distributions generally corroborate the PAH and UCM results and suggest that anthropogenic petroleum inputs at remote EN sites are very low [82].

At EN harbour sites (the Zeballos fuel dock and west Tahsis harbour), the presence of a full suite of tricyclic terpanes, diagenetic hopanes, steranes, and diasteranes (Table 3) corroborated the alkyl PAH and UCM data, and confirmed the importance of anthropogenic petroleum inputs at these sites [28,82].

At BI sites, sediment samples were dominated by diagenetic hopanes, with substantial amounts of tricyclic terpanes, rearranged steranes, and diasteranes. Interestingly, the sample taken just outside the oil spill boom had lower concentrations of these biomarkers relative to other BI sites, suggesting that the boom was largely successful in containing the spilled oil, or that this sampling site failed to capture the dispersal of spilled oil over space and time. As at EN harbour sites, the presence of these biomarkers confirmed UCM and alkyl PAH data, and, not surprisingly, points to chronic anthropogenic petroleum inputs throughout the urbanized environment around BI.
Biomarker ratios of both maturity and source provide valuable insight into petroleum sources (Table 3). EN sites with undetectable biomarker concentrations included Louie Bay, NE Catala Island, and Little Zeballos River. At other remote EN sites, while biomarkers were detected, signal noise was relatively high due to low hydrocarbon concentrations, resulting in decreased precision in ratio values.

Most samples at both EN and BI sites had sterane maturity parameter ratios at or below levels signifying thermal equilibrium [28,82]. The $\beta\beta/($ $\beta\beta + \alpha\alpha)$ ratio values for the C$_{29}$ steranes and the 20S/(20S + 20R) ratio values for the 29$\alpha$ steranes were mostly below the equilibrium values of 0.7 and 0.55 respectively [28,93], while the 22S/(22S+22R) values for the 17$\alpha$(H),21$\beta$(H)-homohopanes (C$_{31}\alpha\beta$) were generally very close to equilibrium values of ca. 0.55-0.62 [28,93]. Because samples were collected in surface sediment, maturity values lower than equilibrium may represent some additional input of biogenic material (e.g. $\alpha\alpha\alpha$ steranes from sterols). Maturity ratios (22S/(22S+22R), $\beta\beta/($ $\beta\beta + \alpha\alpha)$, and 20S/(20S+20R)) among all EN sites were similar to those observed at BI.

Source indicator ratio values for two of the major diagenetic hopanes (29$\alpha\beta$ and 30$\alpha\beta$) were similar between locations, with slightly more variability at remote EN sites (0.29-0.45) than EN harbour or BI sites (0.36-0.41). Similarly, diasterane to regular sterane ratio (27d$\beta$S/(27d$\beta$S + 27$\alpha\alpha$R)) values at remote EN sites (0.49-0.65) were also within the range of values observed at both EN harbour and BI sites (0.39-0.69). In contrast, proportions of tri- and tetracyclic terpanes to the major hopanes ($\SigmaTT/($ $\SigmaTT + 27\alpha + 29\alpha\beta + 30\alpha\beta$)) at remote EN sites (0.17-0.32) were generally below the range of values observed at EN harbour and BI sites (0.26-0.43). Overall, the lack of significant
differences between biomarker content and patterns between BI sites and EN harbour sites indicates that petroleum discharges at both sites were associated with anthropogenic activity and that the petroleum likely has a common source.
Table 3. Harbour sites in the Esperanza/Nuchatlitz Inlet (EN) area and Burrard Inlet (BI), the impacted reference site, had similar biomarker ratio values, strongly suggesting that the two locations share a common, anthropogenic petroleum source.

<table>
<thead>
<tr>
<th>Location</th>
<th>Biomarker Maturity</th>
<th>22S/(22S+22R) 31αβ hopane</th>
<th>ββ/(ββ + αα) 20R+20S C29 sterane</th>
<th>20S/(20S + 20R) 29ααα sterane</th>
<th>Ts/ (Ts + Tm)</th>
<th>29αβ/(29αβ + 30αβ)</th>
<th>ΣTT/(ΣTT + 27α + 29αβ + 30αβ)</th>
<th>27dβS/(27dβS + 27ααR)</th>
<th>27ββ:28ββ:29ββ</th>
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<tr>
<td><strong>Esperanza/Nuchatlitz Inlets (EN; sea otter habitat)</strong></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Remote sites:</td>
<td></td>
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<tr>
<td>Nutchatlitz Inlet (NI)</td>
<td>0.51</td>
<td>0.46</td>
<td>0.41</td>
<td>0.28</td>
<td>0.45</td>
<td>0.17</td>
<td>0.49</td>
<td>0.27 : 0.36 : 0.37</td>
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<tr>
<td>Port Langford (PL)</td>
<td>0.54</td>
<td>0.45</td>
<td>0.35</td>
<td>0.29</td>
<td>0.34</td>
<td>0.19</td>
<td>0.49</td>
<td>0.19 : 0.44 : 0.37</td>
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<tr>
<td>Saltery Bay (SB)</td>
<td>0.47</td>
<td>0.46</td>
<td>0.44</td>
<td>0.46</td>
<td>0.31</td>
<td>0.29</td>
<td>0.54</td>
<td>0.26 : 0.42 : 0.32</td>
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<tr>
<td>S. Centre Island (SC)</td>
<td>0.61</td>
<td>0.49</td>
<td>0.37</td>
<td>0.56</td>
<td>0.33</td>
<td>0.27</td>
<td>0.61</td>
<td>0.28 : 0.34 : 0.38</td>
<td></td>
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<tr>
<td>E Nootka Island (EI)</td>
<td>0.66</td>
<td>0.54</td>
<td>0.50</td>
<td>-a</td>
<td>0.29</td>
<td>0.32</td>
<td>0.65</td>
<td>0.25 : 0.37 : 0.38</td>
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<td><strong>Harbour sites:</strong></td>
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<tr>
<td>Zeballos fuel dock (ZD)</td>
<td>0.61</td>
<td>0.54</td>
<td>0.42</td>
<td>0.36</td>
<td>0.36</td>
<td>0.31</td>
<td>0.58</td>
<td>0.26 : 0.39 : 0.35</td>
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<tr>
<td>W Tahsis Harbour (WT)</td>
<td>0.62</td>
<td>0.41</td>
<td>0.56</td>
<td>0.52</td>
<td>0.40</td>
<td>0.31</td>
<td>0.63</td>
<td>0.32 : 0.31 : 0.37</td>
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<td><strong>Burrard Inlet (BI; impacted reference site)</strong></td>
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<tr>
<td>Shell Oil dock (SO)</td>
<td>0.56</td>
<td>0.48</td>
<td>0.34</td>
<td>0.40</td>
<td>0.37</td>
<td>0.38</td>
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<td>Oil spill boom (OB)</td>
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<td>0.52</td>
<td>0.40</td>
<td>0.50</td>
<td>0.40</td>
<td>0.37</td>
<td>0.60</td>
<td>0.28 : 0.35 : 0.37</td>
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<tr>
<td>Indian Arm (IA)</td>
<td>0.58</td>
<td>0.51</td>
<td>0.53</td>
<td>0.42</td>
<td>0.38</td>
<td>0.27</td>
<td>0.68</td>
<td>0.28 : 0.35 : 0.38</td>
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<tr>
<td>Shell Oil terminal (OT)</td>
<td>0.58</td>
<td>0.48</td>
<td>0.44</td>
<td>0.40</td>
<td>0.39</td>
<td>0.26</td>
<td>0.69</td>
<td>0.29 : 0.33 : 0.37</td>
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<tr>
<td>Narrows to Moody Arm (MA)</td>
<td>0.58</td>
<td>0.53</td>
<td>0.45</td>
<td>0.52</td>
<td>0.39</td>
<td>0.43</td>
<td>0.63</td>
<td>0.27 : 0.36 : 0.37</td>
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<tr>
<td>Reed Pt Marina (RP)</td>
<td>0.58</td>
<td>0.51</td>
<td>0.51</td>
<td>0.40</td>
<td>0.39</td>
<td>0.41</td>
<td>0.65</td>
<td>0.33 : 0.42 : 0.24</td>
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<tr>
<td>Port Moody logsort (LS)</td>
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<td>0.54</td>
<td>0.44</td>
<td>0.55</td>
<td>0.39</td>
<td>0.37</td>
<td>0.39</td>
<td>0.29 : 0.33 : 0.38</td>
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<tr>
<td>Port Moody sawmill boom (PM)</td>
<td>0.59</td>
<td>0.51</td>
<td>0.48</td>
<td>0.42</td>
<td>0.41</td>
<td>0.33</td>
<td>0.67</td>
<td>0.29 : 0.31 : 0.39</td>
<td></td>
</tr>
</tbody>
</table>

*a* - not detectable

See Appendix I for a list of all terpanes, hopanes, steranes, and diasteranes measured. 22S/(22S+22R) = ratio of the S isomer at the C-22 position in the C21 17α(H),21β(H)-homohopane to the sum of the S and R isomers; ββ/(ββ + αα) = ratio of the ββ configuration at the C-14 and C-17 positions in the 20S and 20R C29 regular steranes to the sum of the ββ and αα configurations; 20S/(20S + 20R) = ratio of the S isomer at the C-20 position in the C29 5α(H),14α(H),17α(H)-steranes to the sum of the S and R isomers; Ts/(Ts + Tm) = ratio of C27 hopanes 18α(H)-trisnorhopane (Ts) to the sum of Ts and 17α(H)-trisnorhopane (Tm); 29αβ/(29αβ + 30αβ) = ratio of C29 17α(H),21β(H)-norhopane to the sum of C29 and C30 17α(H),21β(H)-hopanes; ΣTT/(ΣTT + 27α + 29αβ + 30αβ) = ratio of the tricyclic terpanes (TT) to the sum of TT and the C27, C29 and C30 17α(H),21β(H)-hopanes; 27dβS/(27dβS + 27ααR) = ratio of the S isomer of the C27 13β(H),17α(H) diasterane (27dβS) to the sum of 27dβS and the R isomer of the C27 5α(H),14α(H),17α(H)-sterane; 27ββ:28ββ:29ββ = relative abundances of the C27, C28, and C29 steranes.
Risk evaluation

Most research into the health effects of hydrocarbons has focused on PAHs. Effects in invertebrates include DNA damage [94] and behavioural effects [95], while effects in fish include malformations, genetic damage, mortality (including decreased survival in salmon upon entry to the marine environment), decreased size, reduced swimming ability, yolk sac edema, and premature hatching [96-98]. In mammals, observed effects have included anemia and increased oxygen consumption [99], growth retardation and immunotoxicity [100], reproductive effects [101], neurotoxicity [102], mutagenesis [reviewed in 103], and carcinogenesis [reviewed in 67].

The Canadian Council of Ministers of the Environment (CCME) provides both Interim Sediment Quality Guidelines (ISQGs) for the protection of aquatic life and Probable Effects Levels (PELs) for 13 PAHs (Table 4). Of the EN sites (sea otter habitat), concentrations at the Zeballos fuel dock exceeded all ISQGs and eight PELs (Ph, An, Fl, Py, BaA, Ch, BaP, and DahA), while concentrations at the west Tahsis harbour exceeded nine ISQGs.

Concentrations were also compared to the five PAH marine sediment quality guidelines for the protection of aquatic life published by the BC Ministry of Environment (MOE; Table 4). Concentrations at the two EN harbour sites exceeded three of these guidelines (Na, Ch, and BaP) and Port Langford and East Nootka Island samples both exceeded the guideline for naphthalene.

Concentrations at five of eight BI sites (OB, OT, MA, RP, PM) exceeded all CCME ISQGs. Concentrations measured near the Port Moody log sort exceeded eight ISQGs (Ayl, Aen, F, Ph, Fl, Py, DA, and N1), concentrations at the Shell oil dock
exceeded two (Aen and N1), and concentrations in Indian Arm exceeded one (Ayl). No BI sites exceeded CCME PELs. Of the five BC MOE guidelines, only the guideline for Ch was exceeded, and at several sites (OB, OT, MA, RP, PM). Naphthalene concentrations for BI sites were not quantifiable.

While it is instructive to first evaluate the toxicity of sediments based on individual PAHs, PAHs in the environment exist as mixtures [104,105]. For this reason, sediment quality guidelines (SQGs) have been developed for PAH mixtures based on a consensus of SQGs derived from laboratory, field, and theoretical studies [104]. The guidelines include a threshold effects concentration (TEC) of 290 000 ng/g (range: 119 000 - 461 000), a median effects concentration (MEC) of 1 800 000 ng/g (range: 682 000 – 2 854 000), and an extreme effects concentration (EEC) of 10 000 000 ng/g. None of the sites sampled at either location exceeded these mixtures guidelines; however, the Zeballos fuel dock sample fell within the 95% confidence interval for the threshold effect concentration. The mixture includes 13 parent PAHs but no alkyl PAHs, an omission which may substantially underestimate the toxicity of sediment-associated PAH [105,106].
Table 4. Hydrocarbon concentrations in sediment samples taken in sea otter habitat in the Esperanza/Nuchatllitz Inlet (EN; n = 10) areas and in an impacted reference site (Burrard Inlet; BI; n = 8) exceeded both national and provincial sediment quality guidelines for the protection of aquatic life. All values are reported in ng/g dry wt unless otherwise noted.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>CCME ISQG</th>
<th>% exceedance</th>
<th>CCME PEL</th>
<th>% exceedance</th>
<th>BC MoE Guideline</th>
<th>% exceedance</th>
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<tbody>
<tr>
<td></td>
<td>EN</td>
<td>BI</td>
<td>EN</td>
<td>BI</td>
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<td>BI</td>
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<tr>
<td>Naphthalene</td>
<td>Na</td>
<td>34.6</td>
<td>20</td>
<td>NQ</td>
<td>391</td>
<td>0</td>
</tr>
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<td>2-Methylnaphthalene</td>
<td>Nl</td>
<td>20.0</td>
<td>10</td>
<td>88</td>
<td>201</td>
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<tr>
<td>Acenaphthylene</td>
<td>Ayl</td>
<td>5.87</td>
<td>20</td>
<td>88</td>
<td>88.9</td>
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<tr>
<td>Acenaphthen</td>
<td>Aen</td>
<td>6.71</td>
<td>20</td>
<td>88</td>
<td>128</td>
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</tr>
<tr>
<td>Fluorene</td>
<td>F</td>
<td>21.2</td>
<td>10</td>
<td>75</td>
<td>144</td>
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</tr>
<tr>
<td>Phenanthrene</td>
<td>Ph</td>
<td>86.7</td>
<td>10</td>
<td>75</td>
<td>544</td>
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<tr>
<td>Anthracene</td>
<td>An</td>
<td>46.9</td>
<td>10</td>
<td>63</td>
<td>245</td>
<td>10</td>
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<tr>
<td>Fluoranthene</td>
<td>Fl</td>
<td>113</td>
<td>20</td>
<td>75</td>
<td>1494</td>
<td>0</td>
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<tr>
<td>Pyrene</td>
<td>Py</td>
<td>153</td>
<td>20</td>
<td>75</td>
<td>1398</td>
<td>0</td>
</tr>
<tr>
<td>Benz[a]anthracene</td>
<td>BaA</td>
<td>74.8</td>
<td>20</td>
<td>63</td>
<td>693</td>
<td>10</td>
</tr>
<tr>
<td>Chrysene</td>
<td>Ch</td>
<td>108</td>
<td>20</td>
<td>63</td>
<td>846</td>
<td>200</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>BaP</td>
<td>88.8</td>
<td>20</td>
<td>63</td>
<td>763</td>
<td>60</td>
</tr>
<tr>
<td>Dibenz[a,h]anthracene</td>
<td>DA</td>
<td>6.22</td>
<td>20</td>
<td>75</td>
<td>135</td>
<td>10</td>
</tr>
</tbody>
</table>

With sediments acting as a potential source of hydrocarbons to adjacent food webs, resource management will need to distinguish between the natural background and the anthropogenic overlay of hydrocarbons. While SQGs suggest that current levels of some PAHs already represent a threat to aquatic biota at some sites in BC, these guidelines exist only for 13 individual PAHs. Effective risk evaluation would be greatly improved by further insight into the mechanisms of hydrocarbon toxicity and the consideration of complex mixtures.
CHAPTER 3: HYDROCARBON CONCENTRATIONS AND PATTERNS IN
BRITISH COLUMBIA SEA OTTERS (*Enhydra lutris*) AND THEIR PREY

This chapter has been submitted under the following citation:


Environmental Toxicology and Chemistry.
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 [54]. 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 [54], and the British Columbia (BC) population was extirpated by 1929 [107]. Following the reintroduction of 89 individuals between 1969 and 1972, the BC population has grown to an approximate 4 700 otters today [8]. The majority of these (~ 4 000) inhabit the west coast of Vancouver Island (WCVI), and a smaller population (~700) exists adjacent to the central mainland coast [8]. While there are no data for the province’s pre-fur trade sea otter abundance, current numbers are much lower than the estimated carrying capacity of >50 000 otters [108].

Oil is considered the primary threat to BC sea otters due to the relatively small size of the population, geographical constraints to their distribution (sea otters likely occupy only about 30% of their original range), the proximity of the population to shipping lanes [4], and their life history characteristics. The extreme vulnerability of sea otters to oil exposure was demonstrated by the 1989 *Exxon Valdez* oil spill (EVOS) in Alaska, where an estimated 4 000 sea otters (95% CI = 1904 – 11 157) died [19]. Unlike other marine mammals, sea otters do not have a blubber layer, and rely instead on the thickest fur coat in the animal kingdom (approximately 100 000 hairs/cm²) to provide insulation [13]. Fouling destroys the fur’s insulative capacity, so sea otters spend a great deal of time grooming (~3 hours per day; 51). This renders them vulnerable to the
ingestion of oil during spill events. In addition, very high metabolic rates require that sea otters eat approximately 25% of their body weight per day [13], which can lead to potentially important contaminant exposures through the consumption of large amounts of prey. Sea otters also spend almost their entire lives at sea and often concentrate (‘raft’) in protected bays.

Sea otters preferentially consume nearshore benthic invertebrates, which are generally inefficient metabolisers of hydrocarbons [23] and may therefore act as hydrocarbon reservoirs. A variety of techniques have been used to document sea otter foraging behaviour, including attached time depth recorders [TDRs; 109], direct observations [e.g. 56], and stable isotope analysis [110]. Results of these studies suggest that, on average, sea otters are generalists and consume prey from at least seven phyla [13], but that individual otters are specialists [56].

Oil is made up of thousands of organic compounds, the most abundant of which are the hydrocarbons, which represent more than 75% of oil by weight [21]. Hydrocarbons are ubiquitous in the marine environment, originating from both natural and anthropogenic sources [21]. Natural sources of pyrogenic (combustion-derived) hydrocarbons include forest and grass fires, while anthropogenic sources include vehicular and industrial emissions. Petrogenic (petroleum-derived) hydrocarbons originate from natural sources including oil seeps and coal and shale deposits, while anthropogenic sources include oil spills and leakage [21]. Due to the hydrophobic nature of hydrocarbons, sediments are the primary repository in the marine environment [22], making sediment-biota partitioning an important route for hydrocarbon uptake into marine food webs [23].
One class of hydrocarbons, the polycyclic aromatic hydrocarbons (PAHs), has been detected in remote environments, including Arctic ice and snow and deep sea sediments [111]. Many PAHs are toxic, with effects ranging from reduced swimming ability to genotoxicity and increased mortality in invertebrates [94,95], fish [96,98], birds [112,113], and mammals [99,100].

While catastrophic oil spills represent a well documented threat to sea otters, little is known about dietary exposures to ambient hydrocarbons, the primary constituent of oil. An understanding of hydrocarbon source, transport and fate functions in the BC marine environment is important in the face of looming port development, increasing tanker traffic, and potential offshore oil and gas exploration and development. 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.

**Methods**

**Sea otter captures**

Sea otters (*Enhydra lutris*) were live-captured in September 2003 on the central mainland coast of British Columbia (BC) near Bella Bella (BB) and in September 2004 in the entrance area of Esperanza/Nuchatlitz Inlets (EN) on the west coast of Vancouver Island (WCVI; Figure 1). Permits were obtained from the Department of Fisheries and Oceans Pacific Region Animal Care Committee (ACC) and section 73 of the Species at Risk Act (SARA) for scientific research (permit #03-011 for 2003 and #04-017 for 2004). 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 weighed and administered fentanyl (compounded at Macdonald’s Prescriptions, Vancouver, Canada) and diazepam (Valium, Sabex Inc., Quebec, Canada) 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 and whisker samples and 60-100 mL of whole blood were collected from each animal. 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; Vacutainer®, Becton, Deckinson and Company, Franklin Lakes, USA) and placed in a cooler (4°C). Blood samples were transferred to a -80°C freezer at the Institute of Ocean Sciences (IOS; Sidney, Canada) until analysis at Axys Analytical Services, Ltd (Sidney, Canada). Upon completion of sampling, the animal was returned to the box and 0.44 mg/kg body weight naltrexone (compounded at Macdonald’s Prescriptions, Vancouver, Canada) 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 Esperanza/Nuchatlitz Inlet areas (Figure 1). Prey species representing various combinations of habitat use (intertidal, subtidal) and feeding ecologies (filter feeder, grazer, scavenger/predator) were collected in order to encompass possible variations in hydrocarbon concentration and patterns likely to be encountered in sea otter prey.

Black turban snails (*Tegula funebralis*; intertidal grazer) and California 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 (*Panopea abrupta*; subtidal 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 (Nuchatlitz). Clams were immediately shipped in sea water to IOS. Finally, a total of ten red rock crabs (*Cancer productus*; subtidal scavenger/predator) were collected in water depths ranging from four to ten metres (49°48.901N; 126°59.467W and 49°49.72N; 127°58.26W). Traps were baited with herring in bait jars and set for at least 12 hours. All species were depurated for at least 18 hours in order to void gastrointestinal contents and then frozen at -80°C until homogenization. The soft tissue of all individuals of each prey type was homogenized to create a single sample for analysis. Homogenate was stored at -80°C until analysis at Axys Analytical Services Ltd. Hereafter, black turban snails will be referred to as ‘snails’, California mussels as ‘mussels’, geoduck clams as ‘clams’, and red rock crabs as ‘crabs’.

*Tissue hydrocarbon analysis*
Whole blood samples from 29 sea otters and homogenized soft tissue from four prey species were analyzed for alkanes and polycyclic aromatic hydrocarbons (PAHs) by Axys Analytical Services Ltd. using high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS). Blood samples were spiked with a suite of perdeuterated surrogate standards (4 alkane and 16 PAH surrogates) and extracted by shaking with a mixture of ethanol, hexane, and saturated ammonium sulphate solution. The hexane extract was then backwashed by shaking with water and dried over anhydrous sodium sulphate.

To extract prey samples, subsamples of 10-30 g were combined with 100 mL of methanol and spiked with a suite of perdeuterated surrogate standards (4 alkane and 16 PAH surrogates). A solution of potassium hydroxide was added and the mixture was boiled under reflux for one hour. Ultra pure water was added and boiling continued for another 1.5 hours. Samples were extracted with pentane, and the extract was then washed with ultra pure water and dried over anhydrous sodium sulphate.

Extracted samples were then loaded onto a silica gel column and eluted into two fractions using pentane (containing the alkanes) followed by dichloromethane (containing the PAHs). Samples were then concentrated, spiked with recovery standards, and analyzed using selective ion monitoring GC/MS.

**Stable isotope analysis**

Stable isotope analysis was conducted on sea otters and their prey to provide elementary insight into the feeding ecology of this generalist species. 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 hours. Subsamples of whole prey homogenate were freeze-dried at -50°C for at least 48 hours 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 (Victoria, Canada) using a Fisons NA 1500 Elemental Analyser-Isotope Ratio Mass-Selective (Milan, Italy) interfaced to a FinniganMAT 252 Isotope Ratio Mass Spectrometer (Bremen, Germany). 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:

\[
δX = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000
\]

(1)

where δX represents the heavier isotope (e.g. \(^{15}\)N or \(^{13}\)C), \(R_{\text{sample}}\) is the raw ratio of heavy to light isotope in the sample, and \(R_{\text{standard}}\) is the raw ratio of heavy to light isotope in an internationally recognized standard (Peedee Belemnite (PBD) for carbon and atmospheric nitrogen for nitrogen [114]). 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. Where 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; sub-adult sea otters were defined as ‘juveniles’ (0-3 years; [115]).
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 substitutions were made for undetected compounds in cases where at least 70% of animals had detectable values for that compound. Where fewer than 70% of the otters had detectable concentrations of a compound, zero ng/g was substituted for compounds below the limit of detection (LOD). In the case of invertebrates, detection limit substitutions were made for all undetected compounds to ensure patterns resulting from differences in habitat preference or feeding ecology were conserved. Detection limits for both EN and BB otters were generally <1.0 ng/g. All measured concentrations were corrected to the concentration measured in the lab blank.

All data is presented as mean +/- standard deviation (\( \bar{x} \pm SD \)), and all statistical tests are considered significant at \( p < 0.05 \).

**Biota-sediment accumulation factor (BSAF) calculations**

BSAFs for prey species were calculated according to the equation:

\[
BSAF = \frac{(C_b / L)}{(C_{sed} / TOC)}
\]  

(3)

where \( C_b \) = contaminant concentration in biota (ng/g), \( C_{sed} \) = contaminant concentration in sediment (ng/g), \( L \) = % lipid fraction of biota tissue, and \( TOC \) = % total organic carbon (OC) content in sediment. Average \( \Sigma PAH \) concentrations in sediments from seven remote sites in the EN area (Figure 10) that are reported elsewhere (Chapter 2) were used for our BSAF calculations. Assuming chemical equilibrium, and that OC and lipid 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 [105]. Barriers to equilibrium may include the degree to which biota
are exposed to the sediment, and sediment properties including TOC content [116]. Assumptions made when calculating BSAFs are that the organism possesses minimal capability to metabolize the compound in question, that sorption and desorption kinetics are rapid relative to uptake kinetics so that PAHs are bioavailable, and that organic carbon is the only sorptive phase present in sediment [117].

*Biomagnification factor (BMF) calculations*

BMFs, used to measure changes in tissue contaminant concentrations with increasing trophic level, were calculated according to the equation:

\[ BMF = \frac{C_{\text{pred}} / L}{C_{\text{prey}} / L} \]  

(4)

where \( C_{\text{pred}} = \) contaminant concentration in the predator (ng/g), \( C_{\text{prey}} = \) contaminant concentration in the prey (ng/g), and \( L = \% \) lipid fraction 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 comprised 100% of the diet, while the fifth used a “food basket” approach, with each prey item contributing equal proportion (25%) to the diet. BMFs were calculated based on contaminant concentrations observed in the average EN sea otter (excluding unweaned juveniles).

*Results and Discussion*

While sea otters are highly vulnerable to the acute effects of oiling, their considerable dietary requirements (~25% of body weight per day; [13]) also underscore the potential for prey to deliver a significant hydrocarbon dose even when prey hydrocarbon concentrations are low. Despite this, little is known about exposure,
accumulation, and metabolism related to dietary uptake in sea otters. Hydrocarbons were measured in 45 sea otters that were live-captured from two regions of British Columbia, comprising both sexes and two age categories (juvenile and adult; Table 5). In addition, four putative sea otter prey species were collected from the EN area on the West Coast of Vancouver Island (Table 5).

*Stable isotopes of carbon and nitrogen*

Carbon and nitrogen ratios did not reveal any major differences in trophic level or feeding ecology between the two sampling locations, although EN juveniles had higher δ¹⁵N values than males (Table 5; p = 0.04). We found no relationship between hydrocarbon concentrations and C or N values for any of the age, sex or location groups (results not shown), perhaps reflecting rapid metabolic elimination of many compounds. Despite the lack of detailed knowledge of prey preferences for our sea otters, trophic level estimated by stable isotope data provided some support for the choice of these species for the five diet scenarios (four prey species plus an equally weighted “food basket”) (Table 5).
Table 5. Sea otter (*Enhydra lutris*) samples were collected in 2003 near Bella Bella and in 2004 in the Esperanza/Nuchatlitz Inlet (EN) areas. Marine invertebrates were collected in the EN areas in July and August 2007 and August 2008.

<table>
<thead>
<tr>
<th></th>
<th>Body weight (kg; $\bar{X} \pm SD$)</th>
<th>matrix sampled</th>
<th>% lipid ($\bar{X} \pm SD$)</th>
<th>$\delta^{15}$N ($\bar{X} \pm SD$)</th>
<th>$\delta^{13}$C ($\bar{X} \pm SD$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>a. Sea otters (<em>Enhydra lutris</em>)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bella Bella</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>juveniles ($n = 6$)</td>
<td>20.4 ± 3.3</td>
<td>blood</td>
<td>0.12 ± 0.034</td>
<td>13.6 ± 0.641</td>
<td>-12.6 ± 0.880</td>
</tr>
<tr>
<td>adult males ($n = 5$)</td>
<td>37.5 ± 0.72</td>
<td>blood</td>
<td>0.14 ± 0.053</td>
<td>13.2 ± 0.314</td>
<td>-12.2 ± 0.174</td>
</tr>
<tr>
<td>adult female ($n = 1$)</td>
<td>23.8</td>
<td>blood</td>
<td>0.18</td>
<td>13.1</td>
<td>-13.7</td>
</tr>
<tr>
<td>Esperanza/Nuchatlitz Inlets</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>juveniles ($n = 5$)</td>
<td>17.6 ± 6.5</td>
<td>blood</td>
<td>0.21 ± 0.029</td>
<td>14.9 ± 1.09</td>
<td>-12.7 ± 1.15</td>
</tr>
<tr>
<td>adult males ($n = 6$)</td>
<td>31.6 ± 6.4</td>
<td>blood</td>
<td>0.25 ± 0.10</td>
<td>13.3 ± 1.12</td>
<td>-12.9 ± 1.33</td>
</tr>
<tr>
<td>adult females ($n = 6$)</td>
<td>25.5 ± 1.4</td>
<td>blood</td>
<td>0.33 ± 0.22</td>
<td>13.9 ± 0.614</td>
<td>-12.3 ± 0.641</td>
</tr>
<tr>
<td><strong>b. Prey Samples</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Geoduck clam (<em>Panopea abrupta</em>; composite of 6 individuals)</td>
<td>588 ± 215</td>
<td>whole soft tissue</td>
<td>0.78</td>
<td>9.95</td>
<td>-14.8</td>
</tr>
<tr>
<td>California mussel (<em>Mytilus californianus</em>; composite of 6 individuals)</td>
<td>69.7 ± 21.5</td>
<td>whole soft tissue</td>
<td>2.00</td>
<td>10.2</td>
<td>-17.2</td>
</tr>
<tr>
<td>Turban snail (<em>Tegula funebralis</em>; composite of 24 individuals)</td>
<td>2.25 ± 0.55</td>
<td>whole soft tissue</td>
<td>2.92</td>
<td>10.1</td>
<td>-13.8</td>
</tr>
<tr>
<td>Red rock crab (<em>Cancer productus</em>; composite of 10 individuals)</td>
<td>8.92 ± 2.28</td>
<td>hepatopancreas</td>
<td>9.70</td>
<td>10.9</td>
<td>-19.4</td>
</tr>
</tbody>
</table>

*The ‘juvenile’ age class ranges in age from unweaned pups (< 1 year) to sub-adults (up to 3 years).

b *The ‘adult’ age class encompasses those otters > 3 years.*
Sea otter prey

Alkanes

Alkane concentrations measured in this study were generally lower than those reported in a limited number of previous studies \[e.g. 118; Table 6\]. However, 100% of the 32 compounds measured were detected in both sea otters and all sampled prey species, underlining the ubiquity of alkanes in the marine environment.

Although the four invertebrate species we sampled represented ecological niches, only two distinct alkane patterns were observed. Patterns in subtidal species (clam and crab) were dominated by alkanes in the \(n\text{-C}_{15} – n\text{-C}_{21}\) range. Conversely, the intertidal species (mussels and snails) exhibited a peak in the \(n\text{-C}_{27} – n\text{-C}_{31}\) range in addition to important contributions by \(n\text{-C}_{15}\) and \(n\text{-C}_{17}\) (Table 6). This pattern difference may have reflected a greater influence of terrestrial plant-derived alkanes in the intertidal species. Marine invertebrates do not appear to metabolize \(n\)-alkanes \[reviewed in 119\], indicating the potential for these species to act as alkane reservoirs.
Table 6. Resolved alkane (n = 32) and polycyclic aromatic hydrocarbon (PAH; n = 43) concentrations were measured in four sea otter prey species, with alkane patterns exhibiting a peak in the \(n-C_{15} - n-C_{17}\) range for all species, and a second peak in the \(n-C_{27} - n-C_{31}\) range for California mussels and turban snails. PAH patterns were dominated by low molecular weight (LMW) alkylated PAHs in all species.

<table>
<thead>
<tr>
<th></th>
<th>Geoduck clam</th>
<th>California mussel</th>
<th>Turban snail</th>
<th>Red rock crab</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\Sigma) alkanes (ng/g ww)(^a)</td>
<td>171</td>
<td>290</td>
<td>510</td>
<td>513</td>
</tr>
<tr>
<td>Top five alkanes (% contribution to total)</td>
<td>(n-C_{17}) (34.6)</td>
<td>(n-C_{15}) (18.2)</td>
<td>(n-C_{17}) (13.6)</td>
<td>Pristane (29.6)</td>
</tr>
<tr>
<td>Pristane (13.2)</td>
<td>(n-C_{15}) (9.26)</td>
<td>(n-C_{29}) (9.94)</td>
<td>(n-C_{29}) (9.94)</td>
<td>(n-C_{17}) (14.3)</td>
</tr>
<tr>
<td>(n-C_{16}) (9.40)</td>
<td>(n-C_{17}) (7.94)</td>
<td>(n-C_{28}) (8.21)</td>
<td>(n-C_{16}) (5.25)</td>
<td></td>
</tr>
<tr>
<td>(n-C_{21}) (3.32)</td>
<td>(n-C_{27}) (7.74)</td>
<td>(n-C_{31}) (7.68)</td>
<td>(n-C_{27}) (4.53)</td>
<td></td>
</tr>
<tr>
<td>% contribution of top five alkanes</td>
<td>77.3</td>
<td>51.2</td>
<td>48.7</td>
<td>59.0</td>
</tr>
<tr>
<td>(\Sigma) PAH (ng/g ww)(^b)</td>
<td>40.2</td>
<td>36.2</td>
<td>61.5</td>
<td>73.0</td>
</tr>
<tr>
<td>Top five PAHs (% contribution to total)</td>
<td>F3 (42.2)</td>
<td>F3 (32.6)</td>
<td>F3 (23.7)</td>
<td>N2 (44.8)</td>
</tr>
<tr>
<td>F2 (26.9)</td>
<td>N1 (13.8)</td>
<td>F2 (17.6)</td>
<td>F2 (13.1)</td>
<td></td>
</tr>
<tr>
<td>F1 (5.24)</td>
<td>F1 (12.2)</td>
<td>N2 (16.1)</td>
<td>F1 (6.31)</td>
<td></td>
</tr>
<tr>
<td>N3 (2.53)</td>
<td>N3 (7.29)</td>
<td>N3 (4.08)</td>
<td>PA4 (1.54)</td>
<td></td>
</tr>
<tr>
<td>% contribution of top five</td>
<td>82.8</td>
<td>79.7</td>
<td>79.2</td>
<td>92.5</td>
</tr>
</tbody>
</table>

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

\(^b\) PAHs include biphenyl (Bi), naphthalene (Na), acenaphthylene (Ayl), acenaphthene (Aen), fluorene (F), phenanthrene (Ph), anthracene (An), fluoranthene (Fl), pyrene (Py), benz[a]anthracene (BaA), chrysene (Ch), benzo[b]fluoranthene (BbfK), benzo[a]pyrene (BaP), perylene (Per), dibenz[a,h]anthracene (DahA), picene (P1), indeno[1,2,3-cd]pyrene (IP), benzo[g,h,i]perylene (BghiP), anthanthrene (AA), dibenzothiophene (D), retene (Ret), \(C_{1}-C_{4}\) naphthalenes (\(N_{1}-N_{4}\)), \(C_{1}-C_{3}\) fluorenes (\(F_{1}-F_{3}\)), \(C_{1}-C_{3}\) dibenzothiophenes (\(D_{1}-D_{3}\)), \(C_{1}-C_{4}\) phenanthrene/anthracenes (PA1-PA4), and \(C_{1}-C_{3}\) fluoranthene/pyrenes (FP1-FP3). Compounds designated priority pollutants by the US EPA include Na, Ayl, Aen, F, Ph, An, Fl, Py, BaA, Ch, BbfK, BaP, DahA, IP, and BghiP.
PAHs

Different feeding ecologies also appeared to contribute to differences in total PAH concentrations in the four invertebrate species (Table 6). For example, turban snails, which are grazers, had higher PAH concentrations than filter-feeding mussels on both a wet weight and lipid weight basis. Grazers generally exhibit relatively high contaminant concentrations as a consequence of preferential feeding on organic films that can concentrate contaminants [31]. Conversely, increased metabolic capacity relative to the other species studied likely influenced PAH concentrations in crab [reviewed in 34]: only 54.2% of measured compounds were detected, compared with 93.8-95.8% of measured compounds detected in clam, snails, and mussels. Total PAH concentrations in our invertebrates were relatively low compared with those reported elsewhere [reviewed in 23].

While feeding mode can drive compositional differences in PAHs in marine invertebrates [31,34], patterns among our study species were similar (Figure 10). Patterns were dominated by LMW alkyl PAHs, particularly C1-C4 naphthalenes and C1-C3 fluorenes, with the top five PAHs accounting for 79% - 93% of total concentrations (Table 6). The predominance of LMW PAHs, coupled with low concentrations of HMW PAHs that are typically sediment-associated [34], points to water as an important PAH exposure route [120] in our invertebrates.
Figure 10. The percent contribution of individual PAHs to total PAH concentrations revealed an overarching petrogenic signature (2- and 3-ring alkyl PAHs) in sediments from remote sites in sea otter habitat; pattern similarities (predominance of 2- and 3-ring PAHs) across marine invertebrate prey species regardless of their feeding ecology; and patterns in both EN (black bars) and BB (gray bars) sea otters similar to those in prey, likely reflecting dietary uptake.

**Biota-sediment accumulation factors (BSAFs)**

Biota-sediment accumulation factors (BSAFs) provided insight into the movement of PAHs between sediments and sea otter prey. Sediment PAH concentrations from the EN study area (Chapter 2) were used to derive a range of BSAFs between 0.0 – 2.5 for invertebrates. Crab had the lowest BSAFs, generally about one order of magnitude lower than clams, mussels, and snails, providing additional support for the notion that crab have a greater capacity to metabolize PAHs [34]. Only two BSAFs >1.0
were observed; both of these were in clam. In clam, snail, and crab, BSAFs for alkyl PAHs were higher than those of parent compounds (results not shown; p = 0.02, 0.02, and 0.01, respectively). Calculated BSAFs for PAHs were in general agreement with those reported for other marine invertebrates [23,121].

BSAFs lower than unity may occur for several reasons. Black carbon in sediment can sequester/adsorb PAHs, lowering their bioavailability [122]. Alternatively, PAHs in invertebrates may have been driven by exposure via the water column, such that BSAFs may not fully explain PAH accumulation. Whereas dietary uptake (i.e. via sediment) would distribute PAHs to tissue compartments from which elimination is relatively slow, uptake from water allows for rapid diffusion of PAHs over the gills [34].

Average BSAFs exhibited a quadratic relationship with the octanol-water partitioning coefficient (log $K_{ow}$) for parent PAHs in all species except crab (Figure 11a), an exception likely explained by the heightened metabolic capacity of crustaceans. As the other species (clams, mussels, snails) studied here are generally thought to have a limited ability to metabolise PAHs, metabolism is not likely to explain BSAF patterns. Instead, the pattern likely reflects the importance of water as an exposure route, wherein bioavailability decreases with decreasing solubility in water, while some of the HMW PAHs are taken up via dietary uptake in the log $K_{ow}$ range ~6.5-7.0.

Conversely, within alkyl PAH groups (e.g. F1-F3), increasing BSAFs were observed with increasing alkylation, suggesting bioaccumulation (Figure 11b). Since increasing log $K_{ow}$ with increasing alkyl groups would be expected to reduce bioavailability, this relationship likely reflects decreased depuration and/or excretion. In previous work, alkyl PAHs have been observed to bioaccumulate to a greater degree than
parent compounds [38]. While somewhat similar patterns of uptake have been reported for invertebrates exposed via the water column \textit{i.e.} BAFs; 38], this to our knowledge is the first report of a relationship with log $K_{ow}$ for sediment:invertebrates \textit{i.e.} BSAFs).
Figure 11. Biota-sediment accumulation factors (BSAFs), the ratio of lipid-corrected PAH concentrations in invertebrates (geoduck clams, California mussels, and turban snails) to organic carbon (OC)-corrected PAH concentrations in sediment, exhibited (a) a quadratic relationship with increasing log $K_{ow}$ values for parent PAHs. Interestingly, alkyl PAHs within groups (b) exhibited increasing BSAFs with increasing log $K_{ow}$ values, suggesting that the addition of alkyl groups led to greater retention.

Closed circles: napthalenes; open circles: fluorenes; closed triangles: dibenzothiophenes; open triangles: phenanthrene/anthracenes; closed squares: flouranthene/pyrenes; open squares: benz[a]anthracenes/chrysenes.
Sea otters

For both practical and ethical reasons, a non-invasive sampling technique was used to examine hydrocarbon concentrations and patterns in blood samples from free-ranging sea otters. In southern sea lions, PAH concentrations in blood had generally higher concentrations than other tissues [123]. Concentrations of PAHs in the blood of yellow-legged gulls were in the range of concentrations measured in other seabird tissues [liver, muscle; 124], suggesting that blood was an effective proxy for PAH concentrations in other tissues.

Alkanes

Alkane concentrations in both EN and BB sea otter populations were generally lower than those reported previously [125,126; Table 7]. Within sites, BB males had higher total alkane concentrations than BB juveniles (p = 0.05), while 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), while 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. Further, the absence of trophic level ($\delta^{15}N$) differences between sites ruled out higher trophic position as the reason for higher alkane concentrations in BB otters. Thus, it is likely that higher total alkane concentrations in BB males were due to differences in local alkane levels in prey and/or to differences in diet. The BB area habitat has not been occupied for as long as the EN habitat [127], and as such a different prey assemblage may be available to the BB otters. A greater number
of compounds were detected in BB males than EN males (76.9 ± 2.8% vs 62.5 ± 9.8%, respectively; p = 0.002), suggesting a greater degree of exposure.

Despite these inter-site differences in total alkane concentrations in sea otters, patterns were similar. Alkane patterns in all age and sex classes exhibited a parabolic shape with a peak at \( n-C_{26} – n-C_{27} \), likely resulting from a combination of preferential metabolism and/or excretion of shorter chain alkanes and restricted uptake of longer chain alkanes. This pattern has been observed previously in sea otters, as well as in other vertebrates [126,128]. Alkanes appear to be readily metabolized by vertebrates [119,129], and may also be excreted unchanged via feces [129]. The lack of a similar parabolic shape in invertebrates further underscores the role that metabolism plays as the primary driver of alkane patterns in sea otters.

The lack of toxicological information on alkanes constrains our interpretation of the low concentrations observed in sea otters. 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; results not shown), suggests a limited health risk for this class of compounds. These compounds are readily used in lipid synthesis pathways, and/or undergo rapid elimination in vertebrates [130-132].

**PAHs**

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 – 29.2 ng/g ww in BB otters and from 1.2 – 13.1 ng/g ww in EN otters (Table 7), with 31.1 ± 15.7% of the 43 compounds sought detected in the otters. Concentrations were lower
than those reported previously for both sea otters and other marine mammals [123,125,133,134], but different matrices, analytical approaches, and reporting formats make comparisons difficult. In addition, most studies report only the 16 PAHs designated by the United States Environmental Protection Agency (USEPA) as priority pollutants ($\Sigma 16$ USEPA PAH). In our study, the mean $\Sigma 16$ USEPA PAH was $1.93 \pm 3.22$ ng/g ww in BB otters and $1.72 \pm 2.93$ ng/g ww in EN otters, accounting for just $25.8 \pm 13.7\%$ and $26.4 \pm 31.1\%$ of $\Sigma$PAH concentrations, respectively.

The absence of relationships between total PAH concentrations and age, sex, and trophic position ($\delta^{15}\text{N}$) is consistent with the dominant role of metabolism in driving PAH concentrations [121,135]. PAH half-lives in marine invertebrates are on the order of days to weeks [120], and are thought to be shorter in sea otters [133]. Since 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 [126].

Low molecular weight PAHs dominated PAH profiles in the sea otters, with F1-F3 and PA1-PA4 representing major constituents (average % contributions to $\Sigma$PAH: 48.7 and 17.3% respectively; Figure 10). Similarities in pattern between sea otters and their prey point to dietary uptake as an important driver of PAH patterns. Interestingly, BB males exhibited a higher contribution of the pyrogenic HMW 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.
Table 7. Resolved alkane (n = 32) and polycyclic aromatic hydrocarbon (PAH; n = 43) concentrations were measured in sea otters from the Bella Bella (BB) area and the Esperanza Inlet/Nuchatlitz Inlet (EN) areas on British Columbia’s west coast. Alkane patterns were generally dominated by compounds in the \( n-C_{25} \) – \( n-C_{29} \) range, while PAH patterns, like those in prey species, were generally dominated by low molecular weight (LMW) alkylated PAHs.

<table>
<thead>
<tr>
<th></th>
<th>BB juveniles</th>
<th>BB males</th>
<th>BB female</th>
<th>EN juveniles</th>
<th>EN males</th>
<th>EN females</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \Sigma ) alkanes ng/g ww average ± SD (range)( ^{a} )</td>
<td>713 ± 283 (162 – 972)</td>
<td>2010 ± 1310 (114 – 3460)</td>
<td>15.4</td>
<td>488 ± 346 (43.9 – 899)</td>
<td>293 ± 113 (164 – 495)</td>
<td>541 ± 375 (249 – 1280)</td>
</tr>
<tr>
<td>Top 5 alkanes (% contribution to total)</td>
<td>( n-C_{27} ) (16.3)</td>
<td>( n-C_{26} ) (16.2)</td>
<td>( n-C_{15} ) (12.1)</td>
<td>( n-C_{27} ) (16.1)</td>
<td>( n-C_{26} ) (16.6)</td>
<td>( n-C_{26} ) (15.5)</td>
</tr>
<tr>
<td></td>
<td>( n-C_{26} ) (15.6)</td>
<td>( n-C_{27} ) (16.1)</td>
<td>( n-C_{14} ) (8.3)</td>
<td>( n-C_{26} ) (15.6)</td>
<td>( n-C_{28} ) (15.2)</td>
<td>( n-C_{26} ) (16.4)</td>
</tr>
<tr>
<td></td>
<td>( n-C_{28} ) (14.5)</td>
<td>( n-C_{28} ) (13.4)</td>
<td>( n-C_{36} ) (8.1)</td>
<td>( n-C_{28} ) (15.3)</td>
<td>( n-C_{27} ) (14.1)</td>
<td>( n-C_{28} ) (15.0)</td>
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<tr>
<td></td>
<td>( n-C_{25} ) (12.0)</td>
<td>( n-C_{25} ) (13.0)</td>
<td>( n-C_{35} ) (6.9)</td>
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<td></td>
<td>( n-C_{29} ) (11.2)</td>
<td>( n-C_{29} ) (11.2)</td>
<td>( n-C_{13} ) (7.7)</td>
<td>( n-C_{29} ) (11.2)</td>
<td>( n-C_{29} ) (11.2)</td>
<td>( n-C_{29} ) (11.2)</td>
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<tr>
<td>% contribution of top five</td>
<td>69.5</td>
<td>68.3</td>
<td>42.1</td>
<td>68.6</td>
<td>64.4</td>
<td>70.4</td>
</tr>
<tr>
<td>( \Sigma )PAH ng/g ww average ± SD (range)( ^{a} )</td>
<td>4.26 ± 7.21 (0.14 – 18.5)</td>
<td>10.5 ± 12.4 (1.07 – 29.7)</td>
<td>0.45</td>
<td>3.20 ± 2.01 (1.23 – 6.01)</td>
<td>4.27 ± 1.36 (2.52 – 6.14)</td>
<td>5.67 ± 3.65 (1.67 – 13.1)</td>
</tr>
<tr>
<td>Top 5 PAH (% contribution to total)</td>
<td>F2 (39.3)</td>
<td>F2 (32.0)</td>
<td>F2 (62.2)</td>
<td>F2 (28.3)</td>
<td>N (27.0)</td>
<td>N (23.6)</td>
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<tr>
<td></td>
<td>Na (17.8)</td>
<td>N2 (12.0)</td>
<td>Ph (21.8)</td>
<td>F3 (21.4)</td>
<td>F2 (14.8)</td>
<td>F2 (16.1)</td>
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<tr>
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<td>Ph (10.4)</td>
<td>N (9.0)</td>
<td>N1 (10.2)</td>
<td>N (17.8)</td>
<td>F3 (9.2)</td>
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<tr>
<td></td>
<td>N1 (9.9)</td>
<td>PA4 (7.2)</td>
<td>N2 (4.4)</td>
<td>N4 (4.6)</td>
<td>PA4 (8.8)</td>
<td>PA4 (11.4)</td>
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<tr>
<td></td>
<td>Fl (4.9)</td>
<td>N1 (5.8)</td>
<td>Py (1.4)</td>
<td>N2 (3.4)</td>
<td>N1 (8.3)</td>
<td>D2 (7.1)</td>
</tr>
<tr>
<td>% contribution of top five</td>
<td>82.2</td>
<td>66.1</td>
<td>100</td>
<td>75.5</td>
<td>68.1</td>
<td>71.5</td>
</tr>
</tbody>
</table>

\(^{a}\) See Table 6 footnotes for a list of resolved alkanes and PAHs measured in this study.
*Predator : prey patterns and biomagnification factors (BMFs)*

We limited our food web study to one of the two sea otter study areas, namely the EN area on the WCVI. While PAH patterns in all prey and sea otter samples were dominated by LMW alkyl PAHs, a closer examination revealed important changes between prey and predator. For the most part, % contribution of parent PAHs was constant between prey and predator, with the exception of naphthalene, which increased from 3% in prey to 8% in otters. However, the majority of alkyl PAH groups increased in % contribution from prey to predator (D1-D3: 2.5% to 7.4%, PA1-PA4: 3.1% to 17.3%, FP1-FP3: 2.4% to 4.2%, and BC1-BC3: 0.14% to 1.1%), indicating some retention of these compounds.

BMFs, calculated here to characterize the movement of individual PAHs between prey and sea otters, were <1.0 in most cases (Figure 12). This likely reflected metabolism, but may also reflect low gut assimilation efficiency relative to recalcitrant organochlorine contaminants such as polychlorinated biphenyls (PCBs) in the same log K\textsubscript{ow} range [136].

However, an average of 14 (± 2.7) of the 35 PAHs used in BMF calculations biomagnified (BMF > 1.0) under the various diet scenarios. PAHs that biomagnified under all five scenarios included Na, Bi, D2, D3, and PA3, while FP1, FP3, and BC3 biomagnified in four of the scenarios. Interestingly, both molecular weights (MW; 128 - ~270) and log K\textsubscript{ow} values (3.33 – 7.44) for parent and alkyl PAHs fell in the range of those reported for bioaccumulative PCBs (MW range ~223 – 464; log K\textsubscript{ow} range ~5.3 – 9.14 [136]). However, the biodilution observed for most PAHs underscores the metabolic vulnerability of these compounds compared to PCBs, while the more complex
structures of the alkyl PAHs may explain their heightened retention in sea otters compared to parent PAHs.

The apparent biomagnification of many alkyl PAHs in our study (Figure 12) has not been previously reported in mammals. A complete suite of PAHs is rarely measured, and there exists a prevailing view that that most parent PAHs undergo ‘biodilution’ and do not biomagnify [121,135,136]. Alkyl PAHs are less water soluble, less volatile, and have higher log $K_{ow}$ values than their respective parent PAHs, and may therefore persist longer in environmental matrices and bioaccumulate in invertebrates to a greater extent [39].

**Figure 12.** Biomagnification factors (on a log scale) indicated that while sea otters appeared to metabolise and/or excrete the majority of individual PAHs (a value of zero (i.e. log(1)) indicates that concentrations in sea otters and their prey were the same), some PAHs, particularly 3- and 4-ring alkyl PAHs, biomagnified to some extent. Gray bars: parent PAHs; black bars: alkyl PAHs.
Risk

We estimated daily dietary intakes by adult sea otters of $\sum$PAHs using prey concentrations and an assumed daily consumption of 25% body weight (average otter weight $28.6 \pm 5.5$ kg). Intakes were $374 \pm 124 \mu g \text{ww/day}$ (or $17400 \pm 13400 \mu g \text{lw/day}$), corresponding to $13.1 \pm 4.34 \mu g/\text{kg body weight/day}$. No comparable data are available for marine mammals, but average daily intakes for humans (based on eight HMW parent PAHs) have been estimated to range from 1-5 $\mu g/\text{day}$ [137-139], or $0.014 – 0.071 \mu g/\text{kg body weight/day}$ based on an average body weight of 70 kg [138]. We estimated the PAH body burden in sea otters using the % lipid and % body weight of blood, kidney, and liver, to be $5120 \pm 3600 \mu g$ for the average male and $3710 \pm 2560 \mu g$ for the average female. Parent PAHs accounted for $11.1 \pm 6.7\%$ and $16.5 \pm 10.0\%$ of the burden in males and females, respectively, while alkyl PAHs comprised $88.9 \pm 6.7\%$ and $83.5 \pm 10.0\%$ respectively.

Alkyl PAHs, in addition to being more persistent and bioaccumulative than parent PAHs, can also be more toxic [38,40,41]. Alkyl PAHs represented the primary contributors to toxicity in EVOS-impacted sediment [40]. Furthermore, toxicity appears to increase with increasing alkyl substitution [21]. Interestingly, while a narcosis model underestimated the toxicity of PAH mixtures to early life stages of fish, a model based on the observed toxicity of alkyl phenanthrenes more accurately predicted both lethal and sublethal effects [140]. Thus, risk assessments focusing primarily on subsets of parent PAHs have likely underestimated risk in aquatic organisms [39].

In addition, studies typically characterize risk on the basis of exposure (dose) and/or accumulation (residue). However, the loss of parent PAHs (i.e. BMFs generally <
1.0) underscores the likely transformation of parent PAHs into highly reactive intermediates. The high body burdens of PAH metabolites compared to parent compounds in vertebrates exposed to complex PAH mixtures [e.g. 141] are a concern because these metabolites are generally more toxic [41,47]. Alkyl PAH metabolites may be particularly toxic: toxicity to rainbow trout (*Oncorhynchus mykiss*) larvae was greatest when retene (C4 phenanthrene) metabolites were the predominant form in tissues [142].

While the vulnerability of sea otters to acute oil exposure has been clearly documented [58], our study provides a first examination of chronic exposures to natural and anthropogenic hydrocarbons in their diet. High resolution analysis of a broad suite of PAHs (n = 43) enabled a comparative evaluation of parent and alkyl PAHs, and demonstrated the apparent retention and biomagnification of the latter. The biomagnification of alkyl PAHs is of concern since these ubiquitous compounds are considered more toxic than their parent compounds. Further insight into the divergent source, transport, and effect functions of parent and alkyl PAHs of natural and anthropogenic origin will help guide mitigation measures aimed at recovery of sea otters in British Columbia and elsewhere.
CHAPTER 4: CONCLUSIONS
As a SARA species of ‘special concern’, the recovering sea otter population in
British Columbia face many anthropogenic threats, including illegal harvesting,
ettlement, ship strikes, disease, and contaminant exposure. However, as a
consequence of their relatively low numbers, limited distribution, and unique life history
characteristics, the primary identifiable threat to the survival of this recovering population
is oil pollution [4].

The extreme vulnerability of sea otters to oil was demonstrated following the
1989 Exxon Valdez oil spill (EVOS) in Alaska, which was estimated to have killed over
4000 otters [19]. Symptoms observed in oiled otters included pulmonary and
subcutaneous emphysema, gastrointestinal hemorrhaging, and gastric erosions, while
many otters succumbed to hypothermia before developing any of these symptoms [58].
The EVOS prompted several studies examining parameters associated with acute, direct
oil exposure, including biomarker induction, immune responses, and physiological and
behavioural changes, generally using either mink or river otters as proxies [e.g.
99,143,144,145].

While the vulnerability of sea otters to acute oil exposure has been established,
this study provides a first examination of chronic exposure to both natural and
anthropogenic hydrocarbons in their diet. Despite the considerable dietary requirements
of sea otters [1], little is known about exposure to or accumulation and metabolism of
hydrocarbons, the primary constituent of oil, in this species.

Hydrocarbons, ubiquitous in the marine environment as a result of multiple
natural and anthropogenic sources, are considered as persistent organic pollutants (POPs)
although they differ from other POPs in important ways. These include the efficiency
with which they can be metabolized by vertebrates and the fact that, unlike many other POPs, they have not been legislated and/or banned [31].

One group of hydrocarbons in particular, the polycyclic aromatic hydrocarbons (PAHs), are of concern from a health perspective. Low molecular weight (LMW) two- and three-ring PAHs are acutely toxic via narcotic action, while phase I metabolites of high molecular weight (HMW) four-, five-, and six-ring PAHs can be carcinogenic or mutagenic.

We identified for the first time the probable sources, concentrations, and patterns of a full suite of hydrocarbons in the coastal habitat of BC sea otters. At remote sites, relatively low concentrations in sediments were observed, and alkane patterns were dominated by terrestrial plant-derived inputs. PAH patterns were dominated by LMW petrogenic compounds, with smaller inputs of HMW pyrogenic PAHs from biomass combustion. Low concentrations and the predominance of LMW PAHs indicated that hydrocarbons at these sites may have had a natural source.

Conversely, sediment concentrations at the two small harbour sites in sea otter habitat exceeded those measured in urban/industrial Burrard Inlet (BI), an impacted reference site on BC’s south coast. PAH patterns at these two sites reflected weathered petroleum inputs and fossil fuel combustion. Interestingly, similar hopane and sterane biomarker ratios strongly suggested that the two locations (EN harbour sites and BI) shared a petroleum source and that the petroleum was anthropogenic in origin.

PAH concentrations measured in sediment at harbour sites in sea otter habitat exceeded sediment quality guidelines for the protection of aquatic life set forth by the Canadian Council of Ministers of the Environment and by the BC Ministry of
Environment (MoE). This is the first recognition of PAH ‘hotspots’ in sea otter habitat on the west coast of Vancouver Island.

This study also revealed the apparent retention and biomagnification of alkyl PAHs in a sea otter food web. In sea otter prey species, biota-sediment accumulation factors (BSAFs) for parent PAHs generally decreased with increasing octanol-water partitioning coefficients (log $K_{ow}$), while those for alkyl PAHs increased, indicative of increasing retention of these compounds in the tissues of marine invertebrates.

Despite general similarities in PAH pattern between sea otters and their prey, some 3- and 4-ring alkyl PAHs demonstrated an increased percent contribution to total PAH concentrations in sea otters, indicating some preferential retention of these compounds. This finding was supported by calculated biomagnification factors (BMFs). While BMFs for parent PAHs were generally $<<1$, consistent with metabolic elimination, some higher alkylated 3- and 4-ring PAHs demonstrated BMFs $>1$, indicating some degree of biomagnification. This biomagnification of alkyl PAHs was underscored by the predominance of alkyl PAHs in the estimated total PAH body burdens for adult sea otters, which accounted for 90 ± 7% and 84 ± 10% in males and females, respectively.

This finding underlines an important gap in the literature: the few reports of the movement of PAHs through marine food webs examine a relatively small PAH suite (generally the 16 PAHs designated as priority pollutants by the US EPA), which does not include alkyl PAHs. Thus, the general consensus that PAHs undergo trophic dilution as a result of efficient metabolism by vertebrates [121,135,136], while appearing to be likely for parent PAHs, may not apply to alkyl PAHs, likely due to a combination of their
increased molecular weight and log $K_{\text{ow}}$ values in relation to their respective parent compounds [37,38,41] and the presence of alkyl groups, which may hinder metabolism.

This work provides a preliminary understanding of the sources, transport, and fate in marine food webs of hydrocarbons on the west coast of BC. However, further study is required to form a more comprehensive picture. For example, tissue concentrations of PAHs in marine invertebrates inhabiting the two EN harbour sites would provide some understanding of the bioavailability of the high concentrations of hydrocarbons measured there. Also useful would be paired sediment/invertebrate samples, which would allow a more detailed understanding of the fate of specific hydrocarbon assemblages.

Measurement of hydrocarbon levels in bulk and interstitial water (again, paired with invertebrate samples) would allow for testing our hypothesis that water column exposure is an important driver of PAH patterns in marine invertebrates. Finally, because dietary uptake is likely the current primary hydrocarbon exposure route for sea otters, and because this species exhibits strong individual dietary specialization [56], more insight into the feeding ecology of otters in various habitat locations would be extremely useful.

On a broader scale, a more complete understanding of PAH mixtures is required (ie whether compounds exhibit additive, synergistic, or antagonistic toxicity). Parent PAHs are effectively metabolized by vertebrates (making metabolite burdens a significant concern), but not all PAH toxicity is mediated via the AhR, and as such commonly used methods (e.g. trophic equivalency factors) may not be useful paradigms for determining risk.

This study provides a first examination of hydrocarbon concentrations and patterns in sediments from sea otter habitat in British Columbia. Further, a preliminary
understanding of the uptake of these compounds into benthic foodwebs was attained, using four invertebrate sea otter prey species. Finally, we were able to demonstrate that sea otters face chronic dietary exposure to hydrocarbons, and that as a consequence of their considerable dietary requirements, daily exposure is high even when prey concentrations are low. However, in the absence of toxicity guidelines for alkyl PAHs and complex mixtures, the consequences for sea otter health are difficult to assess.


Ref Type: Thesis/Dissertation


APPENDIX I
This study measured concentrations of resolved alkanes, tri- and tetracyclic terpane, hopane and sterane biomarkers, and parent and alkyl polycyclic aromatic hydrocarbons. PAHs denoted with a * comprise the 16 PAHs designated as ‘priority pollutants’ by the US Environmental Protection Agency (∑ 16 USEPA PAH: benzo[b]fluoranthene and benzo[k]fluoranthene are listed separately and only dibenz[a,h]anthracene is listed).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Abbreviation</th>
<th>Log $K_{ow}$</th>
<th>Molecular weight</th>
<th># of rings (PAHs only)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resolved alkanes</td>
<td>$n$-C$<em>{12} - n$-C$</em>{36}$</td>
<td></td>
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Parent polycyclic aromatic hydrocarbons (PAHs)\textsuperscript{a,b}

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* measured in sediment samples

\textsuperscript{b} measured in marine invertebrate whole tissue and sea otter blood samples
APPENDIX II
Molecular structures for the main parent PAHs analyzed in this study.

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<tr>
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