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Original Article

Environmental Toxicology

Concentrations of per- and polyfluoroalkyl substances in Canadian sea otters (*Enhydra lutris*) are higher near urban centers

Dana Price[1,2,*], Andrew W. Trites[2], Stephen Raverty[1,2,3], Paul Cottrell[4], Brendan Cottrell[5], Ivona Zysk[6], and Juan José Alava[1,2]

[1]Ocean Pollution Research Unit, Institute for the Oceans and Fisheries, University of British Columbia, Vancouver, British Columbia, Canada
[2]Marine Mammal Research Unit, Institute for the Oceans and Fisheries, University of British Columbia, Vancouver, British Columbia, Canada
[3]Animal Health Center, Ministry of Agriculture, Food and Fisheries, Abbotsford, British Columbia, Canada
[4]Department of Fisheries and Oceans, Marine Mammal Response Program, Vancouver, British Columbia, Canada
[5]Applied Remote Sensing Lab, Department of Geography, McGill University, Montreal, Canada
[6]SGS-AXYS Laboratories, Sydney, British Columbia, Canada

*Corresponding author: Dana Price. Email: d.price@oceans.ubc.ca

Abstract

Per- and polyfluoroalkyl substances (PFAS) are thousands of toxic synthetic chemicals that bioaccumulate and persist in the environment. They are known to cause immunotoxicity, organ damage, endocrine disruption, and reproductive impairments in wildlife such as sea otters (*Enhydra lutris*). However, there is limited information on the distribution of these chemicals across the northeastern Pacific, and baseline data are missing to assess their potential impacts on sea otters in regions such as British Columbia (BC), Canada. We analyzed liver ($n = 11$) and skeletal muscle samples ($n = 5$) from 11 deceased sea otters from coastal BC using the U.S. Environmental Protection Agency method 1633 with ultrahigh performance liquid chromatography coupled to a triple quadrupole mass spectrometer. We found 8 of the 40 tested PFAS were present in all sampled sea otters, although concentrations of each PFAS varied between individuals. Sea otter livers contained more PFAS compounds at higher total average concentrations than skeletal muscle (i.e., 8 PFAS totaling 10.38 ng/g wet wt vs. 1 PFAS totaling 0.38 ng/g wet wt). Only perfluorooctanesulfonamide (PFOSA) was identified in both liver and muscle tissues, whereas the remaining 7 PFAS were unique to the liver. The three PFAS that dominated the liver PFAS composition (perfluorononanoic acid, PFOSA, and perfluorooctanesulfonic acid) accounted for 84% of the contaminant load in the livers. Geographically, PFAS concentrations were more than three times higher on average in sea otters recovered near major cities and shipping routes. Identifying the contaminants accumulating in sea otters provides insights into the health threats confronted by recovering sea otter populations. Our study also establishes baseline PFAS contamination levels in BC sea otters, which can be used to monitor and regulate the presence of PFAS on marine environments in western Canada.

Keywords: sea otter, *Enhydra lutris*, per- and polyfluoroalkyl substances, perfluorinated compounds, British Columbia

Introduction

Perfluorinated and polyfluorinated alkyl substances (PFAS) are a synthetic class of contaminants containing toxic chemicals that can persist in the environment and bioaccumulate in organisms. Their long fluorinated carbon chain gives them hydrophobic and oleophobic properties that repel fluids. Perfluorinated and polyfluorinated alkyl substances are used in the lining of food packaging, nonstick cookware, waterproof and stainproof textiles, cosmetics, firefighting foams, electronics, and several other applications. Perfluorinated and polyfluorinated alkyl substances bioactively bind to proteins within an organism (Agency for Toxic Substances & Disease Registry, 2021; Dasu et al., 2022; Garg et al., 2020; Kurwadkar et al., 2022), allowing them to accumulate in blood, liver, and other protein-enriched tissues, where these substances can disrupt endocrine function, impair immune response, and contribute to developmental and reproductive toxicity.

Some of the thousands of different chemicals classified as PFAS are of environmental concern due to their prevalence, persistence, known toxicity, and bioaccumulative nature. Toxic effects in mammals include immunotoxicity, developmental delays, liver inflammation, endocrine disruption, carcinogenicity, and altered metabolism (Agency for Toxic Substances & Disease Registry, 2021; Arukwe & Mortensen, 2011; Fair et al., 2013; Temkin et al., 2020; Wirth et al., 2014,). Perfluorinated and polyfluorinated alkyl substances travel through water, soil, and the atmosphere, and are being found in all parts of the world, including the polar regions, far from human industrial activity (Alava et al., 2015; Joerss et al., 2020; Kelly et al., 2009; Xie & Kallenborn, 2023). Under the international regulatory risk assessment framework of the Stockholm Convention of Persistent Organic Pollutants (POPs), several PFAS are classified as new POPs because they are bioaccumulative, toxic, and persist in the environment, (United Nations Environment Programme Stockholm Convention, 2023).

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There is growing concern about the toxic effects of PFAS on marine life and the future negative impacts they will have on marine ecosystems as they continue to bioaccumulate and biomagnify in marine food webs (Fair & Houde, 2018; Houde et al., 2006; Kelly et al., 2009; 2024). Species considered highly susceptible to contamination from biomagnification via their food webs include predatory marine mammals with relatively long lifespans and high trophic level, such as sea otters (*Enhydra lutris*; Hart et al., 2009; Kannan et al., 2006; Kannan et al., 2008) and killer whales (*Orcinus orca*; Lee et al., 2023). Sea otters are exposed to PFAS mainly via consumption of prey that contain PFAS, maternal transfer and lactation, inhalation of airborne PFAS, and accidental ingestion while grooming. Living nearshore means close proximity to emissions and runoff from PFAS sources like landfills, factories, maritime traffic, airports, military bases, and pesticides usage (Borthakur et al., 2021).

Sea otters consume large amounts (~25% of their body wt each day) of protein-rich seafood in nearshore habitats that are susceptible to PFAS exposure and bioaccumulation (Kurwadkar et al., 2022; Riedman & Estes, 1990; Tansel, 2024). Sea otters also have high site fidelity, with home ranges of 1–50 km^2 (Garshelis & Garshelis, 1984; Laidre et al., 2009; Loughlin, 1980). They do not migrate or travel great distances and are therefore useful indicators for region-specific analysis of contaminant accumulation from food sources often harvested and consumed by humans. In British Columbia (BC), sea otters were entirely extirpated by the fur trade that occurred from the mid-1700s to the early 1900s and only returned to BC after 89 individuals were relocated from Alaska in 1969–1972 (Bodkin, 2015). Since then, their population has grown to more than 8,000 individuals as of 2017, but they have yet to recolonize most of their historic habitat (Committee on the Status of Endangered Wildlife in Canada & Committee on the Status of Endangered Wildlife in Canada, 2022; Nichol et al., 2020).

Perfluorinated and polyfluorinated alkyl substance concentrations have been measured in sea otters across most of their range from California to Russia. The first measured concentrations in dead California sea otters (1993–1994) detected 8.9 ng/g wet weight perfluorooctanesulfonic acid (PFOS; Kannan et al., 2001). Higher concentrations of PFOS and perfluorooctanoic acid (PFOA) were subsequently correlated with a greater probability of California sea otters dying from infectious and noninfectious diseases, indicating a significant health risk to the species (Kannan et al., 2006). Ongoing surveillance and tissue analysis showed that PFOS liver concentrations increased in California sea otters from 1992–1998 and then decreased following 3M's voluntary phase out of its use after 2000 (Kannan et al., 2006). A similar decrease in PFOS was found in Alaska (1992–2007). However, the same was not true for perfluorononanoic acid (PFNA), which was not phased out of production and increased over time in Alaskan sea otters (Hart et al., 2009). Perfluorinated and polyfluorinated alkyl substance concentrations appear to be highest in Washington and lowest in Russia (Kannan et al., 2008), although those relative values have likely changed with time.

The presence and concentrations of PFAS have been reported for sea otters living in California, Washington, Alaska, and Russia. They have been shown to vary across their geographical range in the northeastern Pacific (Hart et al., 2009; Kannan et al., 2001, 2006, 2008) and significantly affect the health and mortality rates of sea otters (Kannan et al., 2006). Although high PFAS concentrations were observed in liver and skeletal muscles tissues collected from necropsied killer whales from the northeastern Pacific (Lee et al., 2023), no data exist on the presence of PFAS for the recovering population of sea otters in BC, Canada. This baseline information is critical to assess sea otter exposure and susceptibility to contaminants and the potential impact of PFAS on their health and population growth.

On the west coast of Canada, the sea otter exerts top-down control on benthic invertebrates (Committee on the Status of Endangered Wildlife in Canada & Committee on the Status of Endangered Wildlife in Canada, 2022; Ford, 2014; Larson et al., 2015), and is a traditionally, culturally, ecologically, and economically significant species for coastal Indigenous communities and the eco-tourism industry in BC (MacDuffee et al., 2016; Salomon et al., 2015). It is considered a species of "Special Concern" in Canada due in part to anthropogenic stressors that include marine pollution (Alava, 2019; Committee on the Status of Endangered Wildlife in Canada & Committee on the Status of Endangered Wildlife in Canada, 2022; Fisheries & Oceans Canada, 2013, Harris et al., 2011). However, the extent to which sea otters in BC are carrying PFAS and the threat that these compounds pose to their health is unknown. Assessing contaminant exposure is important for determining the potential impact on the long-term health of sea otters.

The goal of our research was to understand the accumulated exposure to and chemical composition of these toxic substances and fill in the geographical gap of PFAS research in British Columbia by assessing total concentrations in sea otters, the variety of unique PFAS, and potential relationships between PFAS contamination and pathology. Based on existing literature, we expected concentrations of PFAS accumulation to differ by age, sex, location, and year of death. We analyzed tissue samples (liver and skeletal muscle) from dead stranded sea otters recovered along the BC coastline between 2016–2021 to provide baseline PFAS concentrations in sea otters in BC and insights into how these contaminants may affect sea otter health. We also compared the relative presence of different PFAS in sea otters across the North Pacific and present baseline data to monitor the effectiveness of PFAS restrictions and assess how they are reflected in the environment and whether stricter regulations should be implemented.

Methods

Sample collection

Tissue samples were collected during necropsies of 11 sea otters found dead along the BC coastline (Figure 1) under permit from the Department of Fisheries and Oceans Canada (DFO XMMS 2 2021). Information recorded for each individual sea otter included the location where they died, approximate age, cause of death, sex, and carcass condition (Table 1). Because precise age data was not available, we grouped sea otters into age classes (i.e., juveniles, subadults, and adults) based on characteristics that included dentition, evidence of reproduction (females), and body size. We also assessed carcass condition using a five-point code that categorized decomposition from 1 (alive) to 2 (freshly dead), 3 (moderate decomposition), 4 (advanced decomposition), or 5 (skeletonized/desiccated; Geraci & Lounsbury, 2005; Raverty et al., 2018).

A total of 16 samples were collected from the 11 deceased sea otters that included their livers ($n = 11$) and skeletal muscle ($n = 5$). Tissue samples were wrapped in aluminum foil to prevent contact with the airtight bags they were stored in and kept frozen at –20 °C until further laboratory analysis.

Tissue analysis

Tissue samples were sent to SGS AXYS laboratories (Sidney, British Columbia) for PFAS chemical analysis. All samples were

Figure 1. Geographical location of approximate coastal sites where 11 dead (stranded) sea otters (*Enhydra lutris*) used in this study were collected in British Columbia, Canada. For spatial analysis, sea otters located north of Vancouver Island were classified as being in the northern group and those located on the western or southern side of Vancouver Island were classified as the southern group.

Table 1. Information on each sea otter (*Enhydra lutris*) sampled in British Columbia, including animal ID, year of recovery, the tissues analyzed, location, age, sex, and carcass condition scored fresh (1) to autolyzed/mummified (5).

ID	Year of death	Tissue	Location	Age class	Sex	Carcass condition code
A	2016	Liver	Hakai, Calvert Island	Adult	M	3.5
B	2020	Liver + skeletal muscle	Port Hardy	Adult	M	4
C	2021	Liver	Malcolm Island	Subadult	F	3
D	2021	Liver	Malcolm Island	Adult	M	3.5
E	2020	Liver	Nootka Island	Subadult	M	3.5
F	2019	Liver	Tofino, Chesterman Beach	Neonate	M	3.5
G	2020	Liver + skeletal muscle	Tofino, Cox Bay	Subadult	M	4
H	2020	Liver + skeletal muscle	Tofino, Chesterman Beach	Adult	M	3
I	2021	Liver + skeletal muscle	Ucluelet, Terrace Beach	Adult	M	3.5
J	2017	Liver	Cape Beale	Pup	M	3
K	2020	Liver + skeletal muscle	Port Renfrew	Adult	M	4

tested for 40 PFAS (see online supplementary material Table S1) using the U.S. Environmental Protection Agency (USEPA) method 1633 (U. S. Environmental Protection Agency, 2024). Tissues were homogenized and spiked with known concentrations of isotopically labeled PFAS to serve as labeled extracted internal standards, accounting for any losses during analytical workup and matrix effects. The tissue samples then underwent three liquid extractions with a graded series of potassium hydroxide dissolved in methanol (16-hr incubation), acetonitrile (30-min sonication), and potassium hydroxide in methanol (addition and vortex), centrifuging and colleting the supernatant at each stage.

After purifying with carbon and methanol evaporation, the sample extracts were diluted and adjusted in pH to 6.5 with either formic acid or ammonium hydroxide. The extracts underwent a solid-phase extraction (SPE) cleanup using preconditioned Waters Oasis 150 mg WAX SPE cartridges. Final sample extracts were instrumentally analyzed using ultrahigh performance liquid chromatography coupled to a triple quadrupole mass spectrometer run with unit mass resolution in multiple reaction monitoring mode. Perfluorinated and polyfluorinated alkyl substances were quantified using isotope dilution, and the area of the quantification ion were compared with that of the isotopically labelled standards (U. S. Environmental Protection Agency, 2024).

Quality assurance/quality control

The PFAS analysis followed the quality control (QC) acceptance criteria of SGS AXYS Analytical Services. Accuracy was assessed with two ongoing precision and recovery samples which were clean reference tissue samples spiked with known amounts of both native and isotopically labelled PFAS. Sensitivity and laboratory background were evaluated using a procedural blank sample, consisting of a clean reference tissue sample spiked with isotopically labelled PFAS only. All batch QC samples were processed with the sea otter tissue samples using the same methodology. All other QC measures, including but not limited to instrument blanks, were followed as per USEPA method 1633.

Data analysis

Any measurement that did not meet the limit of quantification or was flagged with risk of estimation below detectable limits

was removed and considered a nondetection, with a value of zero to avoid false positives. If a procedural blank detected the presence of any PFAS, we blank-corrected data by subtracting the quantity measured to account for any potential laboratory background contamination.

Statistical analysis was done in RStudio (Ver. 2022.02.3 + 492). The Shapiro-Wilkes test was applied to determine data normalcy and the F-test was used to assess relative variances. The small sample size and predominantly nonparametric data limited the types of analyses that could be performed. When comparing two sets of data, we used a Welch's *t*-test or standard *t*-test for normally distributed data depending on variance. Similarly, we used a Welch's analysis of variance (ANOVA) to compare multiple groups of nonparametric data of unequal variance. Linear regressions were used to assess whether there were significant trends in time series.

Results

Perfluorinated and polyfluorinated alkyl substances were present in all 11 livers that were sampled and in two of the five sampled skeletal muscles. We detected eight of the 40 PFAS assessed, of which all eight were in the livers and one (perfluorooctanesulfonamide [PFOSA]) was also in the skeletal muscles (Figure 2, Table 2). The three most prevalent PFAS in the livers were PFNA, PFOSA, and PFOS (Figure 3). We found no correlations between the concentrations of PFOSA in the livers and skeletal muscles. Overall, livers accumulated significantly higher total concentrations of PFAS (i.e., two orders of magnitude higher) than skeletal muscles (mean: 10.38 ng/g wet wt vs. mean: 0.38 ng/g wet wt; $p = .003$, Wilcox test).

Total concentrations of PFAS in livers differed significantly between sea otter habitats ($p = 0.014$, *t*-test). Specifically, sea otters at the northern end of Vancouver Island and further north had lower concentrations (3.73 ng/g wet wt) of the PFAS measured in sea otters recovered from the western and southern parts of Vancouver Island (14.17 ng/g wet wt). Unfortunately, insufficient numbers of animals were sampled to regionally compare PFAS concentrations in skeletal muscles. Per- and polyfluoroalkyl substance chemical proportions were fairly similar across locations, averaging 30% PFNA, 30% PFOSA, 22% PFOS, 6% perfluoroundecanoic acid (PFUnA), 5% perfluorodecanoic acid (PFDA), 4% PFOA, 2% perfluorotridecanoic acid, and 1% perfluorobutanoic acid in the southwest side of Vancouver Island and 48% PFNA, 26% PFOS, 17% PFOSA, 5% PFDA, and 4% PFUnA to the north (Figure 4). In terms of other factors that might have influenced total PFAS accumulations in sea otter livers, no significant relationships were found with age (Kruskal-Wallis, $p = 0.022$), sex,

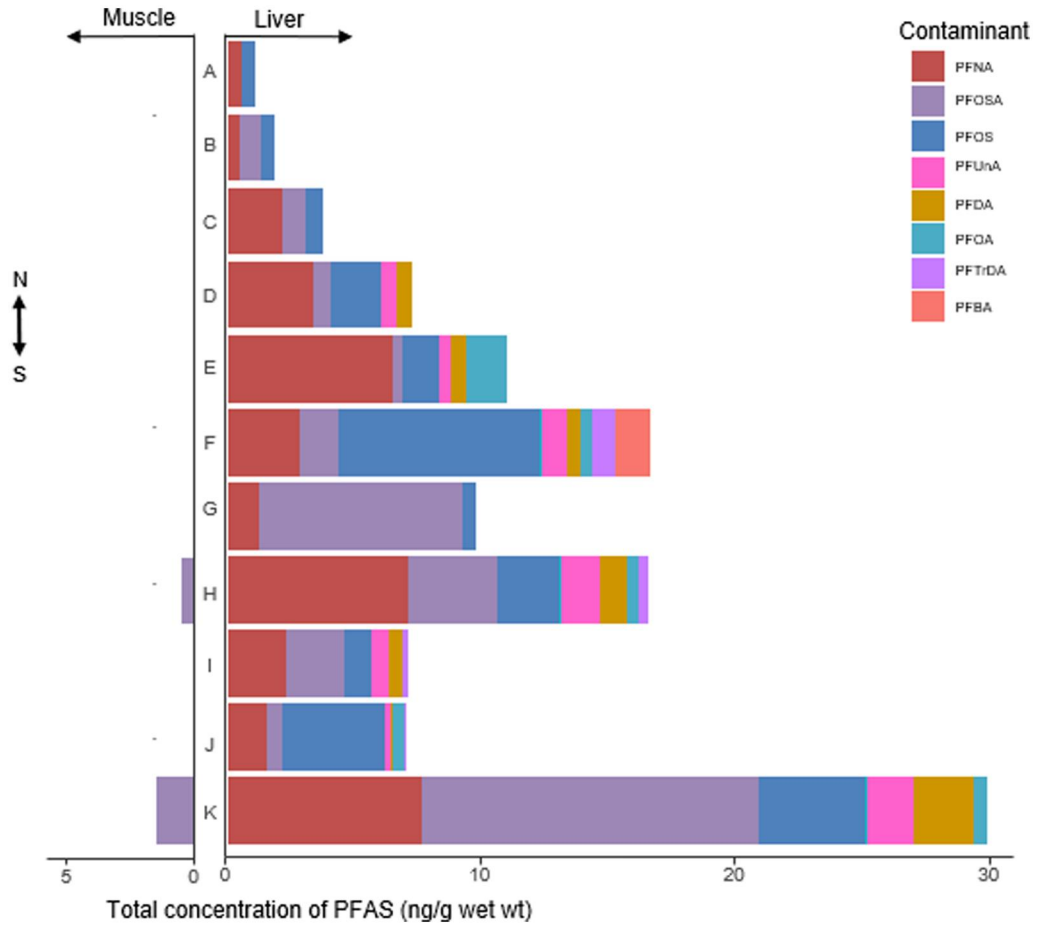

Figure 2. Per-/polyfluoroalkyl substances (PFAS) found in the liver (right) and skeletal muscle (left) of 11 deceased sea otters. Each animal is identified by a letter that reflects where it was recovered in coastal British Columbia, Canada (Figure 1). Greater numbers of PFAS were detected in livers (8) than in muscles (1) with only perfluorooctanesulfonamide being present in both tissues. Significantly greater concentrations occurred in liver (average 10.4 ng/g wet wt) compared with skeletal muscle (0.4 ng/g wet wt). Note that muscle samples were not taken or analyzed for six of the 11 otters as indicated with an asterisk. PFNA = perfluorononanoic acid; PFOSA = perfluorooctanesulfonamide; PFOS = perfluorooctane sulfonate; PFUnA = perfluoroundecanoic acid; PFDA = perfluorodecanoic acid; PFOA = perfluorooctanoic acid; PFTrDA = perfluorotridecanoic acid; and PFBA = perfluorobutanoic acid.

Table 2. Concentrations of per-/polyfluoroalkyl substance (PFAS) for each tissue type analyzed.

PFAS	Liver ($n = 11$; ng/g wet wt)			Skeletal muscle ($n = 5$; ng/g wet wt)		
	Mean ± SD	Med	Min–Max	Mean ± SD	Med.	Min-Max
PFNA	3.41 ± 2.61	2.45	0.70–7.82	–	–	–
PFOSA	2.93 ± 4.12	0.89	0–13.29	0.38 ± 0.62	0	0-1.43
PFOS	2.33 ± 2.30	1.49	0.47–7.96	–	–	–
PFUnA	0.59 ± 0.66	0.38	0–1.87	–	–	–
PFDA	0.53 ± 0.71	0.53	0–2.36	–	–	–
PFOA	0.32 ± 0.47	0	0–1.53	–	–	–
PFTrDA	0.14 ± 0.28	0	0–0.91	–	–	–
PFBA	0.13 ± 0.42	0	0–1.38	–	–	–
∑PFAS	10.38			0.38		

Note. Perfluorononanoic acid (PFNA), perfluorooctanesulfonamide (PFOSA), perfluorooctane sulfonate (PFOS), perfluoroundecanoic acid (PFUnA), perfluorodecanoic acid (PFDA), perfluorooctanoic acid (PFOA), perfluorotridecanoic acid (PFTrDA), and perfluorobutanoic acid (PFBA) in livers ($n = 11$) and PFOSA in skeletal muscle ($n = 5$). The total PFAS value includes all detected PFAS (PFNA, PFOSA, PFOS, PFDA, PFUnA, PFOA, PFTrDA, and PFBA). Concentrations are measured in nanograms per gram of wet weight.

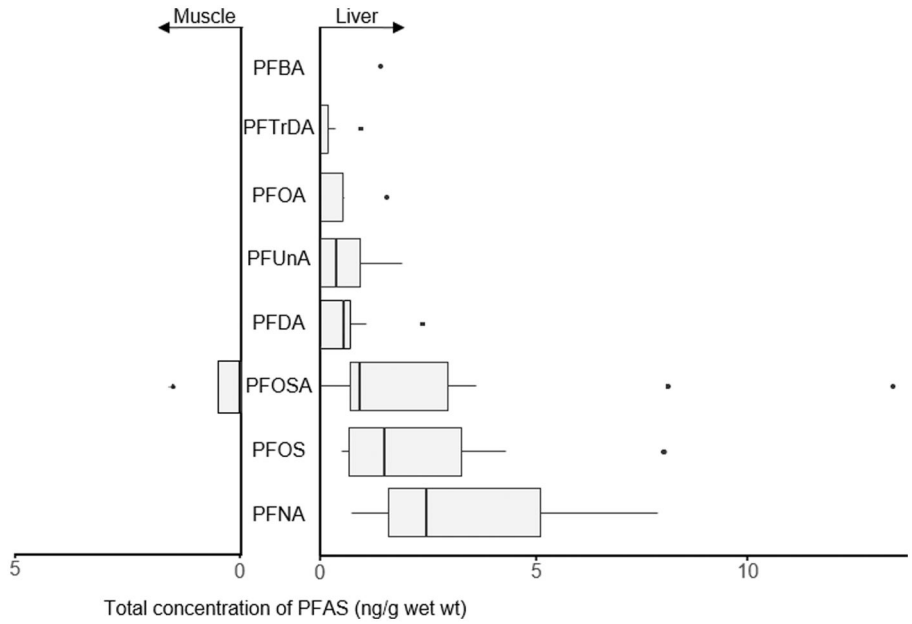

Figure 3. Distributions of 8 per-/polyfluoroalkyl substances (PFAS) identified in British Columbia, Canada sea otters in liver (right) and skeletal muscle (right) tissues. Perfluorononanoic acid (PFNA), perfluorooctane sulfonate (PFOS), and perfluorooctanesulfonamide (PFOSA) dominated in liver tissue, whereas PFOSA comprised all of the PFAS load of the skeletal muscle tissues. PFUnA = perfluoroundecanoic acid; PFDA = perfluorodecanoic acid; PFOA = perfluorooctanoic acid; PFTrDA = perfluorotridecanoic acid; and PFBA = perfluorobutanoic acid.

carcass condition at time of sample recovery (ANOVA, $p = 0.087$), and year of sample recovery (linear regression, $p = 0.025$; Figure 5). Note that only one female fell within the range of comparable males for the assessment of sex effect, and that sample size was insufficient to assess whether there was a relationship between PFAS concentrations and cause of death.

Discussion

We assessed the concentrations and distributions of PFAS in sea otters from BC to understand how this group of persistent and bioaccumulative contaminants accumulate in different tissues (liver and muscle) and how the results compare with PFAS levels in other geographic regions along the northeastern Pacific rim. Our findings provide baseline values for future monitoring, management and conservation efforts in British Columbia while also offering insights into the effects of sex, age, year, tissue type, and geographic location on PFAS accumulation.

In terms of preferential tissue bioaccumulation, the total concentration of PFAS present in sea otter livers was more than 25 times higher than in muscles. This difference is most likely due to the different proteins of the two tissues and PFAS binding sorption capacity to specific protein-rich tissues. The unique hydrophobic and oleophobic properties of PFAS preferentially bind to proteins such as those found in high concentrations in liver tissues (Pérez et al., 2013; Pizzurro et al., 2019). They interact with a number of proteins including liver fatty-acid binding proteins, albumin and other transport proteins, peroxisome proliferator-activated receptors, and the membranes of cells and mitochondria (Forsthuber et al., 2020; Woodcroft et al., 2010; Zhao et al., 2023). However, of the eight PFAS detected in the sea otter livers, only PFOSA was also present in the skeletal muscles.

Sea otters had lower concentrations of total PFAS in both liver and skeletal muscle tissues (liver: 10.38 ng/g wet wt, muscle: 0.38 ng/g wet wt) compared with concentrations in killer whales (685.0 vs. 161.4 ng/g wet wt; Lee et al., 2023) in BC. Sea otters and

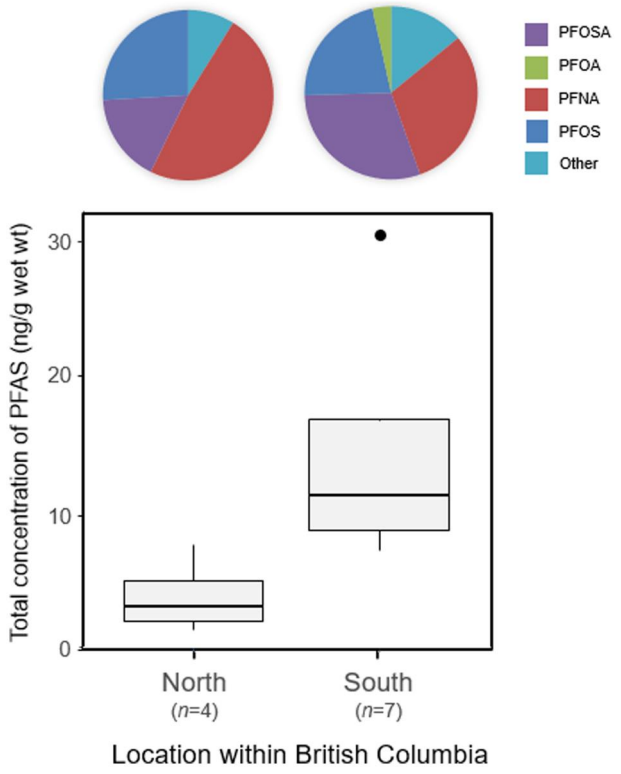

Figure 4. Chemical composition (pie charts) and total concentration of per-/polyfluoroalkyl substances (PFAS; box plots) measured in liver tissues recovered from stranded sea otters north and south of Vancouver Island, BC, Canada (as shown in Figure 1). Average PFAS total concentrations were significantly higher (14.17 ng/g wet wt) in the southern group than the northern group (3.73 ng/g wet wt). The internal line across the middle of the box identifies the median sample concentration values; the ends of the box are the 25% and 75% quartiles; and the whisker bars are the minimum and maximum concentration values. PFNA = perfluorononanoic acid; PFOSA = perfluorooctanesulfonamide; PFOS = perfluorooctane sulfonate; PFOA perfluorooctanoic acid.

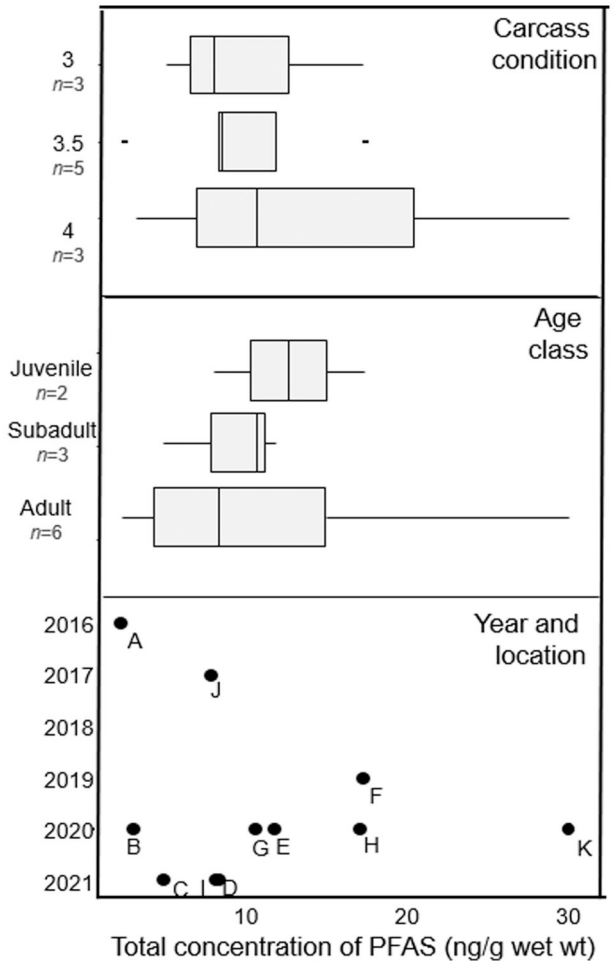

Figure 5. Analysis of factors that may influence the total per-/polyfluoroalkyl substance (PFAS) concentration in sea otter liver tissues in British Columbia, Canada. Top panel: Carcass condition (CC) code at the time of sample collection of carcasses deemed to be moderately decomposed (CC3, $n = 3$) and advanced decomposition (CC4, $n = 3$) or halfway in between (CC3.5, $n = 5$). No relationship was found between total concentration of PFAS and the condition of the carcasses at the time of analysis. Middle panel: Total concentration of PFAS in liver tissues by age class: juvenile ($n = 2$), subadult ($n = 3$), and adult sea otters ($n = 6$). Age classes were based on body size and development at the point of death. There was no significant relationship between the age class of sea otter at their point of death and the total PFAS load in their liver tissue. The internal line across the middle of the box identifies the median sample concentration values; the ends of the box are the 25% and 75% quartiles; and the whisker bars are the minimum and maximum concentration values. Bottom panel: Total concentration of all PFAS in the liver tissues of 11 sea otter over time (2016–2021). Each point represents an individual sea otter identified by letter corresponding to where it was collected (Figure 1). No significant trends were observed.

killer whales also accumulated different types and proportions of PFAS. In sea otters, the dominant compounds were PFNA, followed by PFOSA and PFOS, whereas killer whales had higher concentrations of 7:3 fluorotelomer carboxylic acid (7:3 FTCA), PFOS, and PFOSA. Notably, the most abundant PFAS in killer whales (7:3 FTCA), was entirely absent from sea otter tissues. These differences likely reflect ecological distinctions between otters and killer whales in their trophic levels, diets, metabolisms, lifespan, and home ranges. Additional insights into the large-scale impacts of PFAS bioaccumulation and biomagnification in regional food webs would come from measuring concentrations of PFAS in prey species such as harbor seals, Chinook salmon for killer whales, and macrobenthic organisms, including sea urchins, crustaceans, and shellfish, for sea otters.

At present, PFAS concentrations appear to be below toxic thresholds established in other mammalian species. Although no toxicity studies have been conducted specifically on sea otters—and PFAS metabolism and elimination rates vary across mammals—the no-effect concentration levels for rats are considerably higher than the concentrations we detected (i.e., approximately 1,500 ng/g for PFOS, and 25 ng/g for PFOA). However, it is important to recognize that these values do not account for potential mixing effects from multiple PFAS compounds or co-exposure to other contaminants (Goodrum et al., 2021).

Age and sex effects

Another factor that can influence PFAS accumulation in sea otters is reproductive status (age and sex; Kannan et al., 2006). In our case, we only had a single female among our sample cohort (a dead subadult sea otter C), which limits fully exploring sex differences. Adult females can offload PFAS to offspring in-utero and via milk (Cai et al., 2020; Grønnestad et al., 2017), which increases concentrations in offspring and reduces them in mothers. As such, adult females can have much lower concentrations compared with adult males (Grønnestad et al., 2017). Sea otter C in our study had not yet reproduced at the time of her death and presumably had not offloaded any of her accumulated PFAS. This likely explains why sea

otter C had PFAS contamination levels that were comparable with the male sea otters in the area where she was recovered.

The majority of male sea otters we examined were sexually mature (i.e., >3–6 years) and were classified as adults at their time of death ($n = 6$; Schneider, 1978; Jameson & Johnson, 1993). In addition, we analyzed three subadults (~1–3 years old) that had separated from their mothers but had not yet reached reproduction age. Two sea otters were <1 year of age (a pup and a neonate that died at birth). Although we had low statistical power and found no significant difference in contaminant concentration between the age categories we compared, it is worth noting that the neonate had the third highest total concentration of PFAS and the highest concentrations of PFOS, suggesting transplacental transfer from the mother (Cai et al., 2020; Grønnestad et al., 2017).

Although PFAS are considered chemicals that are sustained indefinitely in the environment through biotransformation and excretion over time, they are relatively less persistent in some organisms, depending on the species' metabolic and enzymatic capacity to breakdown these substances. The half-life of PFAS varies greatly by species (Russell et al., 2013) and may be shorter in sea otters compared with other marine mammals due to their high metabolic rates (Kannan et al., 2001).

Annual and regional comparisons

Historic concentrations of some PFAS, such as PFOS, PFOA, and PFNA, have increased and decreased over time in sea otters in parallel with their increasing application or mandated restricted use (Hart et al., 2009). In our sampled sea otters, we did not detect a significant change in concentrations of the three dominant PFAS over the stranding time interval. This lack of variation in concentrations over time does not indicate that there has been no change but likely reflects the small numbers of sampled otters and the unevenness of samples collected across time and space. Only three of the 11 sampled sea otters were recovered from 2016–2019, and five were from 2020 alone. Thus, we were limited in our ability to detect changes in concentrations over time (Figure 5).

Sea otters are a good indicator species to monitor regional contaminant accumulation in marine-coastal ecosystems because they consume large quantities of macrobenthic invertebrates (Alava, 2019; Hale et al., 2019; Harris et al., 2011) and have small home ranges relative to other marine mammals (e.g., 1–50 km for sea otters compared with nearly 20,000 km migrations of grey whales; Hoelzel et al., 2021). As such, they provide insights into the local distribution of PFAS compounds in the coastal waters of BC. Our comparison of contaminant loads of BC sea otters on the north side of Vancouver Island (a large island that makes up most of the current range of sea otters in BC) and mainland northern BC with those found on the western and southern sides of Vancouver Island suggest the proximity to urban and industrialized settings is a determinate factor in contaminant levels.

Most of BC's population is concentrated in the southern coastal regions (Environmental Reporting BC, 2018). Consequently, the sea otters using the northern portion of their range are in more remote areas located further away from major urban and industrial areas. As a result, these more remote sea otters are less exposed to human activities and release of PFAS into their environment. This is consistent with the sea otters living away from human centers having lower concentrations of PFAS compared with those that lived in the south of their range within Canada. Although the amounts of contaminants differ by region, it is noteworthy that the proportional composition of PFAS in the liver and skeletal muscle were consistent throughout our study area.

Comparing our results to those of previous assessments of sea otter exposure to PFAS in other regions of the northeastern Pacific Ocean revealed distinct patterns of accumulation (Figure 6). Published accounts suggest that throughout the western seaboard of North America, the further away sea otters are from dense human populations and industrial activity, the lower their total tissue concentrations of PFAS. This is consistent with sea otters from California having the highest concentration of PFAS, whereas those in Alaska exhibit the lowest (Hart et al., 2009; Kannan et al., 2006). However, it is important to note that liver samples from California were collected prior to regulation of PFOS and PFOA, whereas samples from BC and Alaska were obtained after stricter regulations had been implemented. This temporal difference aligns with shifts in the relative proportions of PFAS across the different studies—where the earlier samples from the late 1990s contained higher levels of PFOS and PFOA but very little PFNA, whereas the more recently collected samples including those from our study showed PFNA to be the predominant compound.

Although PFOSA has its own toxic effects in animals, it can also act as a precursor to other PFAS (primarily PFOS) through biodegradation and enzymatic oxidation in the liver, resulting in a more stable and toxic product (Zhao et al., 2018). Perfluorooctanesulfonamide may also exhibit distinct accumulation behavior, with a higher affinity for lipids compared with other PFAS. As such, measured PFOS concentrations may reflect both direct environmental PFOS and bio-transformed PFOSA. It is also important to note that our study screened 40 PFAS and detected eight, whereas the previous studies in Alaska and California focused on fewer PFAS that were of highest concern at the time (Hart et al., 2009; Kannan et al., 2006).

Health risks and conservation consequences

We found no associations between the concentrations of total PFAS, PFOS, or PFOA and the cause of death or comorbidities involving disease ($n = 5$), parasite infection ($n = 2$), and emaciation ($n = 3$). Although associations have been reported between disease-caused deaths of sea otters and the concentrations of PFOS and PFOA in livers, the recorded causes of death were associated with concentrations 40 times higher for PFOS and more than 150 times higher for PFOA than in the BC sea otter livers (i.e., reported deaths occurred at PFOA concentrations of 68.9 ng/g wet wt and PFOS concentrations of 95.0 ng/g wet wt; Kannan et al., 2006). However, having hepatic concentrations of PFAS in BC sea otters below lethal toxic effect concentrations does not discount the possibility of associated health issues. Toxic effects of PFAS accumulations can manifest themselves in many ways (Agency for Toxic Substances & Disease Registry, 2021; Fair et al., 2013; Ma et al., 2023). In addition, there are likely other contaminants present in sea otters that could exacerbate the toxic effects of PFAS.

Sea otters are susceptible to a number of infectious pathogens, including protozoal parasites. This makes the immunosuppression effect of PFAS a particular concern for sea otter recovery. Per- and polyfluoroalkyl substances can disrupt the regulation of cytokines which mediate inflammatory responses to infections (Nian et al., 2022) and are essential to overcome *Toxoplasma gondii*, which threaten sea otters near freshwater urban and agriculture runoffs (Burgess et al., 2018; Miller et al., 2002; Sana et al., 2022).

Per- and polyfluoroalkyl substances also interfere with animal metabolism, which is a major health concern for sea otters that lack blubber and rely on dense insulating fur and metabolically generated heat to survive the cold waters of the North Pacific. Elevated levels of PFAS concentrations in mammals have been linked to metabolic disruption, including decreased resting metabolic rates (Liu et al., 2018), insulin resistance (Zheng et al., 2024),

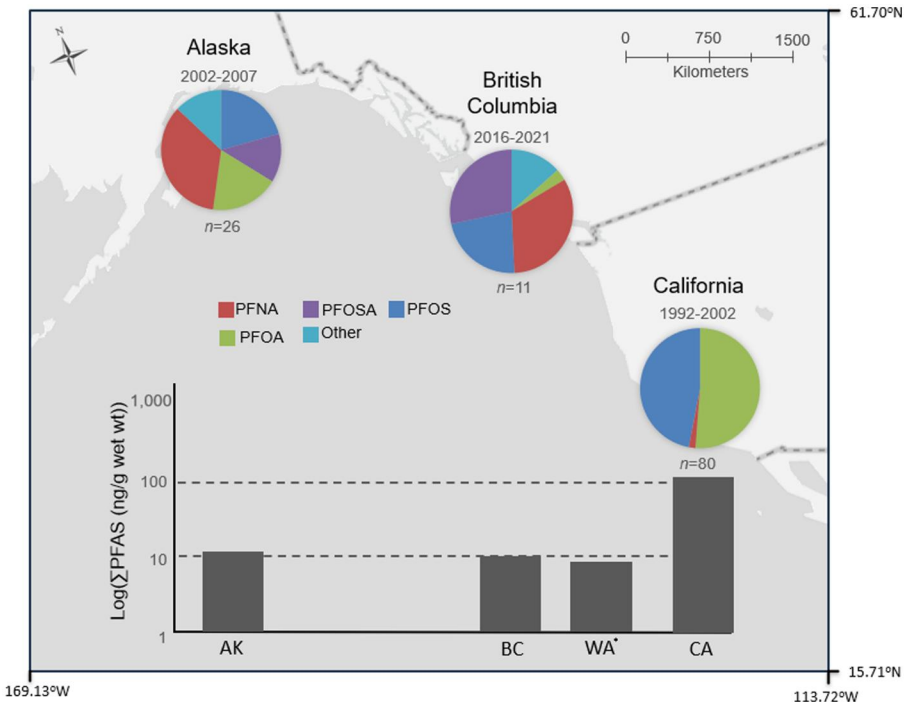

Figure 6. Comparison of per-/polyfluoroalkyl substance (PFAS) composition and concentrations of 11 sea otters in British Columbia (BC, Canada; our study) with other regions of North America: AK, $n = 16$ (Hart et al., 2009), WA, $n = 6$, 1995–1998 (Kannan et al., 2008), and CA, $n = 80$ (Kannan et al., 2006). Data from Alaskan sea otters are an average of the most recent 3 years of sample collection (2005–2007). Californian sea otters had much higher concentrations of PFAS (~117.1 ng/g wet wt) compared with Alaska (13.1 ng/g wet wt), British Columbia (10.4 ng/g wet wt), and Washington (8.7 ng/g wet wt). *Composition of PFAS sampled in Washington were not reported. PFNA = perfluorononanoic acid, PFOSA = perfluorooctanesulfonamide; PFOS = perfluorooctane sulfonate; PFOA = perfluorooctanoic acid.

impaired liver function (Zhang et al., 2023), enteric dysbiosis/imbalanced gut flora (Wang et al., 2020), and decreased intestinal absorption (Li et al., 2022). Given the connection between hypothermia and caloric deficits and hypoglycemia, sea otters must maintain an adequate metabolism to ensure homeostasis (Lahner et al., 2018). Equally concerning for recovering populations are the associated effects of PFAS on fetal mortality and birth defects (Lam et al., 2014; Liew et al., 2020).

The environmental persistence, bioaccumulation, and toxicity of many PFAS has resulted in stricter regulations on their production and application. However, regulations vary for each PFAS and country, and despite being produced since 1938 and being recognized as toxic chemicals in 1970, restrictions were not implemented until the 2000s (Gaber et al., 2023; Seymour & Kirshenbaum, 1986). A milestone in PFAS regulation came when 3M, a major producer and user of PFOS worked with the USEPA to voluntarily phase out production and use of PFOS (Weppner, 2000).

In Canada, a suite of PFAS, including PFOS, PFOA, and long-chain perfluorocarboxylic acids (LC-PFCAs) as well as derivative salts and precursors were added to the Canadian Schedule 1 List of Toxic Substances in 2006 (PFOS) and 2012 (PFOA and LC-PFCAs) to manage the toxic risks to the environment associated with these substances (Government of Canada, 2022). Since then, some additional international regulations have been placed on PFAS beginning with PFOS being classified as a POP and restricted by the Stockholm Convention in 2009 to Annex B. This was followed by the addition of PFOA in 2019 and more recently by perfluorohexane sulfonic acid in 2022 under Annex A for elimination (United Nations Environment Programme Stockholm Convention, 2023). Despite these regulatory measures, most PFAS remain unrestricted and are increasingly detected in animal tissues.

The continued production and use of PFAS highlights the need to further study and monitor the presence of these compounds in the environment and assess their potential health consequences in animals (Lam et al., 2016). It also highlights the importance of public education to encourage companies to voluntarily phase out the use of unrestricted PFAS that threaten vulnerable wildlife. Given the thousands of individual PFAS chemicals in current use, regulating them one by one will be a slow process unless broader, class-based approaches are adopted (Cousins et al., 2019; Kwiatkowski et al., 2020).

Conclusion

This is the first study to document composition and concentration levels as well as regional patterns of PFAS contamination in sea otters in BC. This keystone species can serve as an indicator of contamination of local nearshore environments. We identified at least eight PFAS compounds that have entered the food chain and have accumulated in sea otter tissues. Although the long-term consequences of our findings are not yet known, the data should serve as a valuable refence point for future investigations in BC and across the northeastern Pacific to aid in recovery efforts for sea otters. Moreover, the results from our study advance the understanding of the impact of another set of man-made chemicals on marine life through broader environmental modeling to inform risk management, policy, and species conservation with exposure to marine environmental pollutants in Canada.

Supplementary material

Supplementary material is available online at *Environmental Toxicology and Chemistry*.

Data availability

Publicly available at https://github.com/DanaPrice/BCseaotterPFAS.

Author contributions

Dana Price (Formal analysis, Visualization, Writing—original draft, Writing—review & editing), Andrew W. Trites (Supervision, Visualization, Writing—review & editing), Stephen A. Raverty (Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Writing—review & editing), Paul Cottrell (Data curation, Investigation, Writing—review & editing), Brendan Cottrell (Data curation, Investigation, Writing—review & editing), Ivona Zysk (Investigation, Methodology, Writing—review & editing), and Juan José Alava (Conceptualization, Funding acquisition, Supervision, Visualization, Writing—review & editing)

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Conflicts of interest

None declared.

Ethics statement

No animal handling ethics were involved because animals were found deceased prior to any interference. Sample collection was exclusive to deceased individuals and done under DFO XMMS 2 2021 from Fisheries and Oceans Canada.

Disclaimer

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