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Trace Elements in Biopsied Skin and Blubber From Living Long-Finned Pilot Whales (*Globicephala melas*) in Cape Breton, Nova Scotia

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Trace elements are present naturally in the environment, but the prevalence and distribution of many are heavily influenced by human activities and industries (e.g., Muir et al. 2002; Das et al. 2003; Zhou et al. 2007). Although some have no known involvement in biological processes, many of these elements are essential for life. However, all have the potential to be toxic above certain thresholds, with particular concern regarding cadmium (Cd), chromium (Cr), lead (Pb), and mercury (Hg) (e.g., Fraga 2005; Goldhaber 2003; O'Shea 1999). The quantification of trace elements in wildlife is often of interest with respect to the study populations themselves—in relation to exposure levels and subsequent health implications; but they are also an important means of environmental monitoring, where data from one species can inform what exposure levels are likely encountered by other species within the same environment (Badry et al. 2019; Squadrone et al. 2022). For the marine environment, marine mammals are thought to be good bioindicators due to their long lifespans and high positions in marine food webs (Delgado-Suarez et al. 2023; Wagemann et al. 1996).

The long-finned pilot whales (*Globicephala melas*) that summer in the waters off Pleasant Bay, Cape Breton, Nova Scotia, Canada have been studied and monitored since 1998 in partnership with local whale-watching ecotours (e.g., Augusto et al. 2017a, 2017b; McComb-Turbitt et al. 2019). Long-finned pilot whales consume primarily cephalopods and fish (Beasley et al. 2019; Fontaine et al. 2015; Gannon et al. 1997), and as a result, may be at risk for elevated cadmium and mercury bioaccumulation in their tissues (Bowles 1999; Bustamente et al. 1998; Monteiro et al. 2017). However, trace elements and potentially toxic metal(loids) have been rarely reported for coastal cetacean species in eastern Canada regions (Béland et al. 1993; Gaskin et al. 1979; Muir et al. 1988). Additionally, due to the protected status of the long-finned pilot whales that summer near Pleasant Bay (hereafter "pilot whales") and the relative rarity of stranded whales, it is difficult to consistently monitor trace element and contaminant concentrations in living whales.

Many published studies assessing contaminants in small cetaceans, including *Globicephala* spp., rely on opportunistic sampling of stranded whales (e.g., Aubaul et al. 2013; Bolea-Fernandez et al. 2019; Meador et al. 1993) or tissue sampling from hunted individuals (e.g., Dam and Bloch 2000; Sonne et al. 2010). Such samples are critical for providing access to the entire organism and therefore allow for comparative analyses of pollution and elemental concentrations across different tissues and organs (e.g., Aguilar et al. 1999; Aubaul et al. 2013; Palmer et al. 2024). These studies have shown, for example, that many organic pollutants are lipophilic and therefore accumulate in the blubber (Aguilar 1985), whereas non-lipophilic contaminants will accumulate in different tissues. For example, mercury tends to accumulate in muscle and brain, cadmium and arsenic in the kidneys and liver (Honda et al. 1982), and lead primarily accumulates in bone (Fujise et al. 1988). However, one downside of relying on stranded individuals is that decomposition and availability can result in inconsistent sampling. More recently, the use of remote biopsy sampling for contaminant and trace element analyses has been assessed with great success for a number of large and small cetacean species (Méndez-Fernandez et al. 2016; Savery et al. 2013; Wise et al. 2012) and has been found to be minimally injurious once the biopsy wound is healed (Giménez et al. 2011; Noren and Mocklin 2012). Such biopsy sampling provides a means to collect samples from living and free-swimming individuals, which allows for larger sample sizes of arguably more representative individuals. Moreover, biopsy samples provide a "two for one" opportunity, where one sample can be a source of two tissues: skin and blubber (Noren and Mocklin 2012). Blubber can provide key information on levels of lipophilic contaminants, and both skin and blubber levels of mercury and arsenic are correlated with levels within the internal organs, such as the liver; indicating that biopsy samples can be an informative surrogate for internal organ concentrations for at least some elements (Aubaul et al. 2013; Sun et al. 2017).

In Pleasant Bay, remote biopsy sampling using a crossbow and biopsy darts from a boat has been conducted to collect skin and blubber samples from adult pilot whales for genetic and ecotracer analyses (Kowarski et al. 2014). Remotely collected biopsy samples of pilot whales consist of a small plug of thick dark skin plus a small layer of blubber under the skin, offering a set of paired tissues from a single sample for each biopsied individual.

The aims of this study were to assess the patterns of mercury and other trace elements in skin and blubber from collected remote biopsies from known adult pilot whales in Pleasant Bay, and compare the data with international literature values for pilot whale blubber and skin.

Sample collection ($N = 52$ samples) took place during regular research whale surveys between July 28, 2012 and August 20, 2012; just off the coast of Pleasant Bay, Nova Scotia (46° 49′ 58″ N, 60° 47′ 46″ W). Once located, adult pilot whales were identified using the number and position of mark points (i.e., nicks and internal corners of notches) on the dorsal fin (Auger-Méthé and Whitehead 2007). Once identification was confirmed, skin biopsies were collected using biopsy darts launched by a crossbow (Kowarski et al. 2014). Targeted whales already had been monitored and sex and other information tracked, so we are able to contribute to a growing database for each whale. Samples were collected under permits from Fisheries and Oceans Canada (DFO), and all sample collection protocols and procedures were approved by the Saint Mary's University Animal Care Committee, and were in accordance with the Canadian Council on Animal Care (CCAC) policies and guidelines.

The biopsy plug vials were frozen immediately upon return to shore and sent to Saint Mary's University (SMU). In a clean laboratory at SMU, the blubber and skin components of each biopsy were separated and placed in separate vials. Samples for chemical analyses were sent to the Analytical Services Unit at Queen's University (accredited by the Canadian Association for Laboratory Accreditation) for trace metal(loid) and mercury analyses.

Whale blubber samples were analyzed for both mercury and trace elements, while whale skin could only be analyzed for mercury due to very small sample masses. Dried homogenized whale skin and blubber samples were analyzed for mercury using a direct mercury CV-AAS (Cold Vapor Atomic Absorption Spectrometry). Blubber samples were acid-digested as per ASU protocols and 30 elements (Al, Sb, As, Ba, Be, Bo, Cd, Ca, Cr, Co, Cu, Fe, Pb, Mg, Mn, Mo, Ni, P, K, Se, Ag, Na, Sr, S, Tl, Sn, Ti, U, V, Zn) were analyzed via ICP-MS (Inductively Coupled Plasma Mass Spectrometry). For both instruments, blanks, duplicates and NRC-TORT-3 certified reference materials all came within specifications.

Due to the small sample masses, the following 17 elements in both tissue types did not have concentrations consistently above method detection limits (MDL) and therefore are not included in the subsequent data analyses: Al, Sb, As, Ba, Be, Bo, Ca, Co, Cu, Pb, Mn, Mo, P, S, Tl, Sn, U, and V.

All data analyses were conducted in R v 4.5.0 (R Core Team 2025), and figures were created using the *ggplot2* package (Wickham 2016). As mercury and selenium are known to be antagonistic in many vertebrates (Yang et al. 2008), Se (78.96 g/mol) and THg (200.59 g/mol) were converted to molar ratios. The raw data, as well as all code for generating the figures and statistical analyses are available at https://github.com/timothyfrasier/pilotwhale-contaminants.

Total mercury concentrations ([THg]) were consistently higher in skin than in blubber for both females and males (female skin: mean = 4874 ng/g, SD = 861.0; female blubber: mean = 2100 ng/g, SD = 1162; $t = 8.665$, df = 37.68, $p = 1.681 \times 10^{-10}$; male skin: mean = 4629 ng/g, SD = 1452; male blubber: mean = 1245 ng/g, SD = 526.9; $t = 10.98$, df = 30.00, $p = 4.921 \times 10^{-12}$) (Figure 1, Table 1). These results are consistent with those from other studies; specifically, data from four other odontocete species in the northeast Atlantic also found that mercury concentrations were higher in skin than in blubber (Table 2).

Mercury concentrations in the blubber of female pilot whales were significantly higher than for those for males (see raw means and SDs above, $t = 3.187$, df = 28.23, $p = 0.003495$), and although values were also higher in skin samples from females than for males, this difference was not statistically significant (Figure 1, Table 1). There was a positive relationship between mercury concentrations in the skin and the blubber for both sexes (Figure 2); however, this relationship was only statistically significant for males (estimated slope for males = 1.34, 95% CI = 0.194–2.49, $p = 0.032$). The different slopes of these relationships suggest distinctive patterns for each sex.

In comparison with other published values, mercury concentrations found in pilot whale **skin** tissues from Pleasant Bay are similar to those found for stranded pilot whales from the Kerguelen Islands (Table 2), and for striped (*Stenella coeruleoalba*) and bottlenose dolphins (*Tursiops truncatus*) from the northeastern Atlantic (Table 2). Mercury concentrations in pilot whale skin samples tended to be higher than those for common dolphins (*Delphinus delphis*) and harbor porpoises (*Phocoena phocoena*), also from the NE Atlantic (Table 2). In contrast, the mercury concentrations found in pilot whale blubber tissues from Pleasant Bay were higher by an order of magnitude than those reported for other pilot whales and for other small cetaceans (Table 2). This finding is consistent with other studies

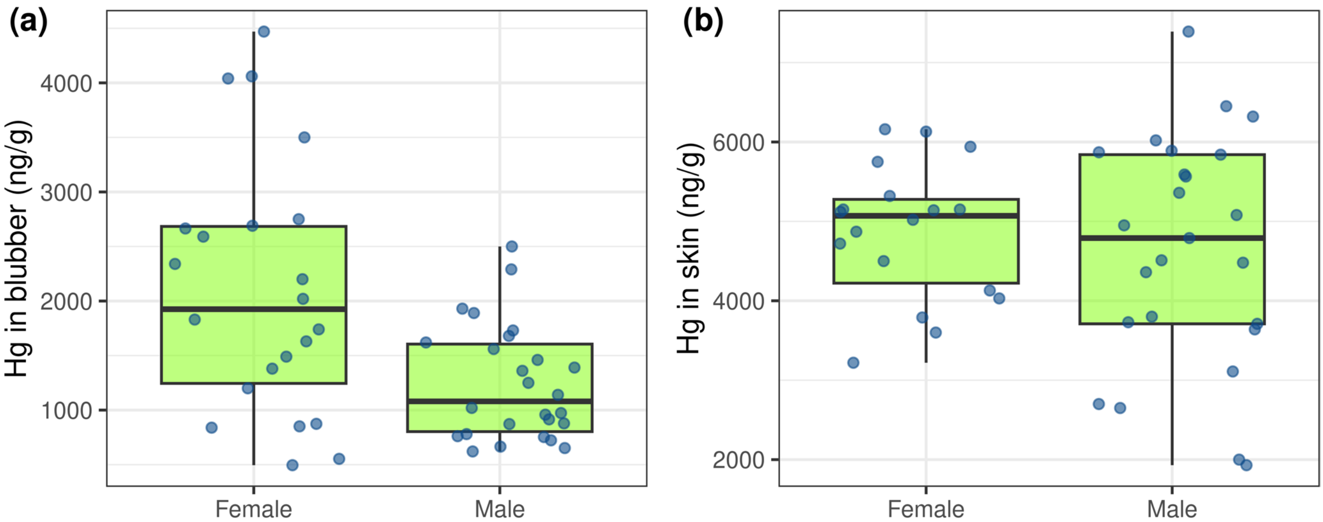

FIGURE 1 | Mercury (Hg) concentrations (ng/g) in male and female long-finned pilot whales off Pleasant Bay, Nova Scotia, Canada. (a) concentrations in blubber, (b) concentrations in skin. Note the different scales of the *y*-axis among the panels.

TABLE 1 | LFPW biopsy average concentrations ±standard deviations (minimum–maximum values) for each potentially toxic (top three rows) and essential (bottom three rows) trace elements for female and male.

	[THg] skin	[THg] blubber[a]	[Cd]	[Se]	[Ag]	[Sr]
F	4.87 ± 0.86	2.12 ± 1.61	0.07 ± 0.02	4.13 ± 1.82	2.18 ± 5.12	2.39 ± 0.55
(*N* = 24)	(3.22–6.16)	(0.50–4.47)	(0.03–0.12)	(2.3–8.2)	(0.03–23)	(1.2–3.1)
M	4.63 ± 1.45	1.25 ± 0.53	0.05 ± 0.03	2.96 ± 1.23	10.17 ± 37.94	2.74 ± 0.76
(*N* = 28)	(1.93–7.39)	(0.62–2.50)	(0.03–0.17)	(1.5–5.7)	(0.02–190)	(1.3–5)

	[Cr][a]	[Fe]	[Mg]	[Ni]	[K]	[Na]
F	23.86 ± 23.83	142.71 ± 144.5	539.55 ± 250	12.72 ± 17.71	1440.95 ± 480.03	8590.91 ± 4948.58
(*N* = 24)	(1.3–73)	(17–600)	(270–1500)	(0.69–80)	(730–2500)	(3900–26,000)
M	12.14 ± 10.17	85.24 ± 62.5	508.08 ± 129.65	25.73 ± 39.28	1449.62 ± 409	7111.54 ± 1595.58
(*N* = 28)	(1.3–36)	(14–230)	(260–830)	(0.99–160)	(590–2500)	(3400–9800)

Note: Skin (THg only) and blubber values are listed (µg/g dry weight).
[a]Indicates significant differences between sexes (see text for details).

showing that mercury levels can be particularly high in some tissues from cetaceans inhabiting the western North Atlantic (e.g., Reif et al. 2015), supporting concerns regarding exposure in this area.

Mercury and selenium levels are often correlated in marine organisms (Shultz and Ito 1979; Julshamn et al. 1987), and selenium is known to have an antagonistic effect on mercury toxicity (Yang et al. 2008). Similar to other studies, we found a positive correlation between mercury and selenium concentrations in blubber (Figure S2). This relationship did not appear to be an artifact resulting from overall differences in elemental concentrations across individuals, because selenium was the only element that stood out in a model comparing the concentrations of all measured elements to that of mercury (Figure S3). The average concentrations of mercury and selenium in the blubber of these pilot whales were 1.64 and 3.54 µg/g, respectively, resulting in a molar ratio of 5.56 Se:Hg. These values are higher than levels found in long-finned pilot whales in the Faroe Islands (mean values in the blubber of 0.7 and 0.12 µg/g, respectively, Julshamn et al. 1987) but mercury concentration is higher, and selenium concentration lower, than in long-finned pilot whales in New Zealand (mean values in the blubber of 0.96 and 9.27 µg/g, respectively, Lischka et al. 2021) and blubber tissues from short-finned pilot whales (Se:Hg molar ratio of 4:33). This is in contrast with a global study of sperm whale skin biopsies which averaged a molar ratio of 59:1 (Savery et al. 2013), and indicates that further investigation into Se and Hg ecotoxicology and chemistry in cetaceans is needed before inferences about ecotoxicology can be made (e.g., Zampetti and Brandt 2023).

However, inferring possible toxicity of mercury based on selenium concentrations is not possible because the chemical form of both selenium and mercury is an important consideration plus the detoxification mechanisms may be tissue- and taxon-specific (Dang and Wang 2011; Campbell et al. 2005). As a result, seafood and toxicity guidelines still rely on mercury concentrations alone. Given the linear relationships found between

TABLE 2 | Mercury literature values for small odontocete cetacean blubber and skin.

Site (year) method of collection	Source	Tissue (N)	[Hg]
Grand Beach, Nfld, Canada (1980) Stranded	Muir et al. 1988	LFPW Blubber (14)	0.15 ± 0.14 (0.008–0.48)
Point Leamington, Nfld, Canada (1980) Stranded	Muir et al. 1988	LFPW Blubber (26)	0.13 ± 0.17 (0.001–0.17)
Faroe Islands (1977) Hunted	Julshamn et al. 1987	LFPW Blubber (9)	0.70 ± 0.28 (MeHg: 0.17 ± 0.10, 25% MeHg)
Kerguelen Islands (2010) Stranded	Fontaine et al. 2015	LFPW Skin (F = 15, M = 11)	F: 4.0 ± 1.9 (0.9–6.8) M: 3.5 ± 2.7 (0.8–7.6)
Kerguelen Islands (2006) Stranded	Fontaine et al. 2015	LFPW Skin (F = 37, M = 21)	F: 5.1 ± 2.2 (0.5–8.1) M: 3.7 ± 2.8 (0.5–8.8)
Corsica, Italy, Mediterranean Sea (1993) Stranded	Frodello et al. 2000	LFPW Skin (1)	25 ± 1.0
Northeast Atlantic:	Aubaul et al. 2013	CODO Blubber (79)	0.3 ± 0.3 (0.01–1.5)
Portugal to France (2001–2008)		CODO Skin (79)	1.7 ± 0.7 (0.2–3.5)
Stranded		HAPO Blubber (17)	0.3 ± 0.3 (0.05–1.2)
		HAPO Skin (17)	1.6 ± 0.7 (0.8–3.3)
		STSO Blubber (19)	0.7 ± 0.9 (0.04–2.9)
		STSO Skin (19)	3.0 ± 1.7 (1.03–6.8)
		BODO Blubber (16)	0.8 ± 0.7 (0.1–2.5)
		BODO Skin (16)	5.7 ± 2.9 (2.2–14.4)

Note: Units are standardized to µg/g. Site, year, and method of sampling are indicated, along with source, species, tissue type, and sample number (*N*). Abbreviations: BODO, bottlenose dolphin; CODO, common dolphin; HAPO, harbor porpoise; LFPW, long fin pilot whales; STDO, striped dolphin.

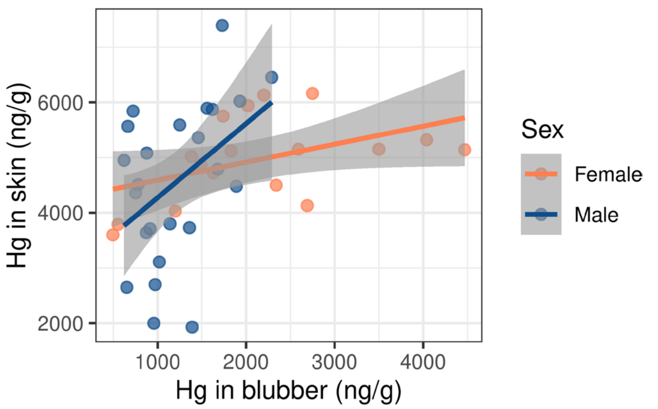

FIGURE 2 | Relationship between mercury (Hg) concentrations in skin versus blubber for male and female long-finned pilot whales.

mercury levels in skin, blubber, kidney, and liver, Aubaul et al. (2013) suggest that a mercury concentration of 6.0 µg/g dry weight (dw) in skin roughly corresponds to the reported toxic threshold of 300 µg/g dw in liver. Five of the 48 individuals for which we had blubber concentrations (~10%, two females and three males) had skin mercury concentrations above this suggested threshold (Figure 1).

Chromium is another element for which exposure is a concern: high levels in humans can lead to lung cancer and reduced fertility (Tsuneta et al. 1980; Al-Hamood et al. 1998). Previous work has found alarmingly high levels in different whale species around the world, including species that inhabit the Gulf of Maine and the waters along the east coasts of the US and Canada (Wise Sr. et al. 2008, 2009; Wise et al. 2015; Wise Jr. et al. 2019). Contrary to many other elements—such as mercury, where exposure is

TABLE 3 | Trace element literature values for small odontocete cetacean blubber and skin.

Site (year)	Source	Tissue (N)	[Cu]	[Zn]	[As]	[Pb]	[Cd]	[Se]
Arysen Region, Chile (2016) Stranded	Garcia-Cegarra et al. 2020	LFPW Blubber (5)	2.19 ± 1.26 (0.33–3.15)	63.29 ± 51.64 (11.90–149.21)	~	~	~	~
Grand Beach, Nfld, Canada (1980) Stranded	Muir et al. 1988	LFPW Blubber (14)	0.09 ± 0.04 (0.02–0.18)	1.54 ± 0.63 (0.62–3.04)	0.44 ± 0.43 (0.03–1.33)	0.01 ± 0.01 (0.0004–0.03)	0.03 ± 0.02 (0.001–0.07)	0.49 ± 0.37 (0.16–1.62)
Pt Leamington, Nfld, Canada (1980) Stranded	Muir et al. 1988	LFPW Blubber (26)	0.09 ± 0.03 (0.03–0.16)	1.53 ± 0.49 (0.85–2.2.45)	0.25 ± 0.21 (0.03–0.94)	0.01 ± 0.01 (0.001–0.019)	0.03 ± 0.03 (0.001–0.17)	0.59 ± 0.26 (0.15–1.42)
Faroe Islands (1977) Hunted	Julshamn et al. 1987	LFPW Blubber (9)	1.1 ± 0.8	2.1 ± 1.6	10 ± 5		0.8 ± 0.4	0.12 ± 0.08

Site (year)	Source	Tissue (N)	[Cu]	[Zn]	[Fe]	[Ni]	[Mn]
Northeast Atlantic	Aubaul et al. 2013	CODO Blubber (79)	2.4 ± 5.4 (0.6–47.9)	85.5 ± 79.4 (16.5–407)	64.9 ± 48.2 (12.8–234)	1.8 ± 3.6 (0.30–22.1)	0.7 ± 0.5 (0.5–1.6)
Portugal to France (2001–2008)		CODO Skin (79)	9.0 ± 4.8 (3.3–35.2)	2672 ± 1272 (1189–9429)	86.5 ± 113 (8.4–777)	0.5 ± 0.1 (0.2–0.7)	1.01 ± 0.8 (0.5–4.4)
Stranded		HAPO Blubber (17)	1.8 ± 0.9 (0.6–3.8)	87.6 ± 72.9 (10.9–279)	51.2 ± 29.9 (14.8–119.1)	1.9 ± 1.9 (0.3–6.5)	0.8 ± 0.4 (0.6–1.3)
		HAPO Skin (17)	6.8 ± 3.7 (1.8–15)	2330 ± 1273 (82.4–5139)	172 ± 294 (12.1–972)	0.8 ± 0.7 (0.4–2.3)	1.2 ± 0.6 (0.6–3)
		STSO Blubber (19)	2.4 ± 1.1 (0.6–5.3)	142 ± 151 (29.7–681)	54.1 ± 37.7 (10.6–145.8)	10.9 ± 24.4 (0.3–91.5)	1.2 ± 0.7 (0.6–2.1)
		STSO Skin (19)	7.8 ± 4.2 (2.4–18.5)	2104 ± 1151 (1009–4379)	6.01 ± 13	1 ± 0.9 (0.3–3.2)	1 ± 0.5 (0.6–2.3)
		BODO Blubber (16)	3.1 ± 1.9 (0.6–7.9)	210 ± 186 (20.1–726)	176 ± 138 (25.8–502)	6.01 ± 13 (0.3–51.3)	2.2 ± 3.2 (0.6–10)
		BODO Skin (16)	8.5 ± 2.8 (6.2–18.1)	2213 ± 842 (1345–4808)	206 ± 173 (22–514)	0.6 ± 0.3 (0.2–0.9)	1.9 ± 1.6 (0.5–5.8)

Note: Units are standardized to µg/g. Site, year, and method of sampling are indicated, along with source, species, tissue type and sample number (*N*).
Abbreviations: BODO, bottlenose dolphin; CODO, common dolphin; HAPO, harbor porpoise; LFPW, long fin pilot whales; STDO, striped dolphin.

likely highest through food consumption—chromium exposure in whales is most likely through inhalation, and therefore representative of air exposure (e.g., Wise Jr. et al. 2019). We found significantly higher levels of chromium in the blubber of female versus male pilot whales (females: mean = 23.86 μg/g, SD = 23.83, males: mean = 12.14 μg/g, SD = 10.17, t = 2.15, df = 27.4, p = 0.0408). Average concentrations for both sexes are similar to those found in the skin of other whales inhabiting the North Atlantic (e.g., fin whales, *Balaenoptera physalis*, average = 10.07 μg/g, range = 1.71–19.6 μg/g, Wise et al. 2015; humpback whales, *Megaptera novaeangliae*, average = 12.39 μg/g, range of averages by year = 2.20–18.98 μg/g, Wise Jr. et al. 2019), which are generally higher than in whales sampled in other oceans, and are markedly higher than levels known to cause health issues in humans (e.g., Wise Sr. et al. 2008; Wise Jr. et al. 2019). Thus, these data add to those indicating possible elevated exposure to chromium via the air in the western North Atlantic, and raise potential health concerns for the organisms that inhabit this region.

For other elements, we found higher concentrations of cadmium (Cd), chromium (Cr), and selenium (Se) in females than in males (Table 1, Figure S1a,b); however, only the differences in chromium levels were statistically significant (females: mean = 23.86 μg/g, SD = 23.83, males: mean = 12.14 μg/g, SD = 10.17, t = 2.15, df = 27.4, p = 0.0408). We found no significant difference between sexes for the other elements (Table 1, Figure S1a,b). Published values for cadmium concentrations in pilot whale blubber from Newfoundland (0.03 μg/g, Table 3) are consistent with those from Pleasant Bay (0.07 μg/g, Table 1). Conversely, Faroe Islands pilot whales had blubber cadmium concentrations that are at least an order of magnitude higher than those for Pleasant Bay (0.8 μg/g, Table 3).

Our metal(loid) results for the Pleasant Bay whales indicate that the individuals have values consistent with similar species found within the western North Atlantic and elsewhere. In addition to this initial assessment, these data will provide an important baseline against which future studies can compare to assess and monitor trends in elemental concentrations over time. Moreover, these data should be useful for future comparative studies across species.

Author Contributions

Linda M. Campbell: conceptualization, formal analysis, funding acquisition, investigation, methodology, project administration, validation, visualization, writing – original draft. **Joana F. Augusto:** data curation, funding acquisition, investigation, project administration. **Timothy R. Frasier:** data curation, formal analysis, funding acquisition, investigation, project administration, visualization, writing – original draft.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in GitHub at https://github.com/timothyfrasier/pilotwhale-contaminants.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1a:** Elemental concentrations in the blubber of male and female long-finned pilot whales off Pleasant Bay, Nova Scotia. Included are concentrations of cadmium (Cd), chromium (Cr), iron (Fe), magnesium (Mg), nickel (Ne), and potassium (K). **Figure S1b:** Elemental concentrations in the blubber of male and female long-finned pilot whales off Pleasant Bay, Nova Scotia. Included are concentrations of selenium (Se), silver (Ag)—at the log scale, sodium (Na), and strontium (Sr). **Figure S2:** Relationship between mercury and selenium concentration in blubber. Estimated slope is 0.29, SE = 0.088, $p = 0.0025$. **Figure S3:** Plot of estimated coefficients, and associated uncertainty, from the model with mercury concentration as the predicted variable and all other elemental concentrations as predictor variables. To account for the large differences in scales of some elements, prior to running the model a z-transformation was performed. This is obtained, for each element, by subtracting the mean from each value and dividing by the standard deviation. The resulting data for each element have a mean of zero and a standard deviation of one.