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Trace Element Concentrations in Livers of Pacific Harbor Seals (*Phoca vitulina richardii*) from San Juan County, Washington, USA

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ABSTRACT: Approximately 5,000 Pacific harbor seals (*Phoca vitulina richardii*) reside year-round in San Juan County (SJC), Washington (US) in the center of the binational Salish Sea. We retrospectively analyzed total cadmium (Cd), copper (Cu), iron (Fe), mercury (Hg), magnesium (Mg), manganese (Mn), lead (Pb), selenium (Se), and zinc (Zn) in livers of dead stranded harbor seals (n=57) collected in SJC between 2009 and 2012 to identify age-related and regional patterns of trace element exposure. Consistent with prior studies of contaminants in pinnipeds, Hg, Cd, and Se concentrations increased with age, and Se:Hg molar ratios approached 1:1 in adult seals. Concentrations of Cd and Hg were below putative marine mammal toxicity thresholds. Mercury concentrations were comparable among Salish Sea populations. Although SJC is less urbanized with fewer industrial inputs than South Puget Sound (SPS), SJC nonpups had greater concentrations of Cd, Cu, and Zn, and pups had greater concentrations of Zn compared to SPS seals. We hypothesize these regional differences could be due to prey preference and availability or to natural geochemical processes. Reported concentrations inform future sampling protocols and can assist in tracking long-term temporal and spatial trends of trace elements in marine organisms.

Key words: Contaminants, harbor seal, heavy metals, *Phoca vitulina*, Puget Sound, Salish Sea, trace elements.

Pacific harbor seals (*Phoca vitulina richardii*) are common along the western coast of North America. Adults are nonmigratory, long-lived, have a small home range, and are generalist and opportunistic predators consuming over 60 species of fish, crustaceans, and mollusks (Zier and Gaydos 2014). Consequently, harbor seals are sentinels of essential (calcium, Ca; selenium, Se; copper, Cu; iron, Fe; magnesium, Mg; manganese, Mn; zinc, Zn) and nonessential, potentially toxic (cadmium, Cd; mercury, Hg; lead, Pb) trace elements over relatively small spatial scales. Monitoring of trace element concentrations in harbor seals is important to assess contaminant exposure risks to seals (e.g., impaired lymphoid organ and immune function; Desforges et al. 2016), other wildlife, and humans.

The inland waters of Washington, US and British Columbia, Canada are collectively known as the Salish Sea. Although anthropogenic pollution in the Salish Sea has decreased in recent decades, Cd, Cu, Hg, Pb, and Zn continue to enter the system primarily through surface runoff (Ecology and King County 2011). San Juan County (SJC), Washington is a 161,000 ha region in the central Salish Sea. Comprised of sparsely inhabited islands, SJC has little industry and few impervious surfaces (San Juan County 2011). San Juan County has a robust harbor seal population estimated at approximately 5,000 animals (Jeffries et al. 2003). We retrospectively investigated trace element concentrations in SJC harbor seals, identified age-related patterns of exposure, and compared findings to published results from seals sampled in other regions of the Salish Sea.

From 2009 through 2012, the San Juan County Marine Mammal Stranding Network analyzed trace element concentrations in liver samples from 57 dead, beach-cast harbor seals found in good postmortem condition (carcass code 2 or 3; Geraci and Lounsbury 2005). Wet tissues were digested with mixtures of nitric acid and either sulfuric or perchloric acids. Liver total Hg (measured using flameless atomic absorption spectrometry), Se (measured using fluorometric determination with diaminonaphthalene), and Ca, Cd, Cu, Fe, Mg, Mn, Pb, and Zn (measured using standard flame atomic absorption spectrometry) were reported in micrograms per gram (μg/g) wet weight (ww). All analyses were run based on quality control criteria from the National Institute of Standards and Technology. Necropsy, including gross and histologic examination as well as ancillary diagnostic testing (e.g., bacteriology, virology), was performed on all 57 animals, and no findings indicated signs of trace element toxicity (e.g., hepatic lesions; Siebert et al. 1999). Cases were classified as pups (n=47) or nonpups (n=10), with the latter category combining adults (n=9) and subadults (n=1), based on body straight length (Geraci and Lounsbury 2005).

Statistical analyses were performed in R v3.3.2 (R Core Team 2016). We used an alpha level of 0.05 for all analyses. The Shapiro-Wilk test and Bartlett's test were used to evaluate data for normality and homoscedasticity. Concentrations of Mn were normally distributed. Concentrations of Ca, Cu, and Zn were transformed using log10 (x+1). We performed a one-way analysis of variance using straight body length as a continuous variable. To evaluate elements with distributions that were neither normal nor log-normal (Cd, Fe, Hg, Mg, Se), we used a generalized linear model (GLM) with straight body length as a continuous variable. Lead was omitted from analyses because concentrations were below detectable limits (2 μg/g). We used the nonparametric Kruskal-Wallis rank sum test to identify significant differences due to age class. We converted Se and Hg liver concentrations from mg/g to nmol/g to calculate Se:Hg molar ratios. Because all 10 nonpup liver samples were from females, sex could not be assessed as a potential factor affecting trace element exposure.

Separating harbor seals into pups and nonpups, we used a Gaussian GLM to compare Cd, Cu, Hg, Se, and Zn concentrations to seals from South Puget Sound (SPS; n=28) and the Strait of Juan de Fuca (SJF; n=13) that were sampled in prior studies (Calambokidis et al. 1991; Akmajian et al. 2014; Fig. 1). Cadmium was omitted from analyses for pups because all concentrations were below detectable limits (0.2 μg/g). To facilitate comparison of results, we converted dry weights reported in prior studies to wet weights assuming 75% moisture (Das et al. 2003). We reported the median (±SE) and range of liver trace element concentrations by age class for harbor seals in the Salish Sea (SJC, SPS, SJF) and in Central and Northern California (Brookens et al. 2007) as an outgroup for comparison (Table 1).

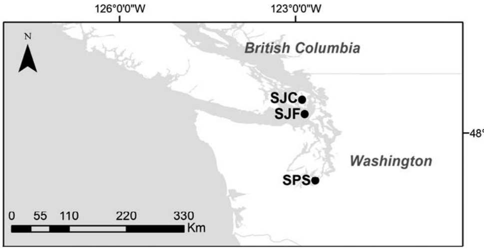

FIGURE 1. Locations of dead, beach-cast harbor seals (*Phoca vitulina richardii*) sampled for liver trace element analysis. Harbor seals used in this study were collected in San Juan County (SJC; n=57). Liver trace element concentrations in SJC harbor seals were compared to those of harbor seals from South Puget Sound (SPS; n=28) and the Strait of Juan de Fuca (SJF; n=13) using published data (Calambokidis et al. 1991; Akmajian et al. 2014).

Concentrations of Cd, Hg, and Se significantly increased with seal length (GLM, $P<0.002$; Fig. 2) and age class (Kruskal-Wallis test, df=4, $P<0.001$). Nonpups (n=10) had an average Se:Hg molar ratio of 1.35 (SD=0.32), excluding three outliers (5, 406, 30). In nonpups, concentrations of Cd (P=0.033; Fig. 3A), Cu (P=0.005; Fig. 3B), and Zn (P=0.023; Fig. 3E) were significantly greater in SJC than in SPS. In pups, Zn was also greater in SJC than in SPS (P=0.044; Fig. 4D), and Cu was comparable across all regions (Fig. 4A). Concentrations of Hg in nonpups were comparable among all locations (Fig. 3C). In pups, Hg concentrations were lower in SJC than in SJF (P=0.005; Fig. 4B). Concentrations of Se in pups were greater in SJC than in SPS ($P<0.001$; Fig. 4C).

As observed in harbor seals sampled in prior studies (Akmajian et al. 2014; Noël et al.

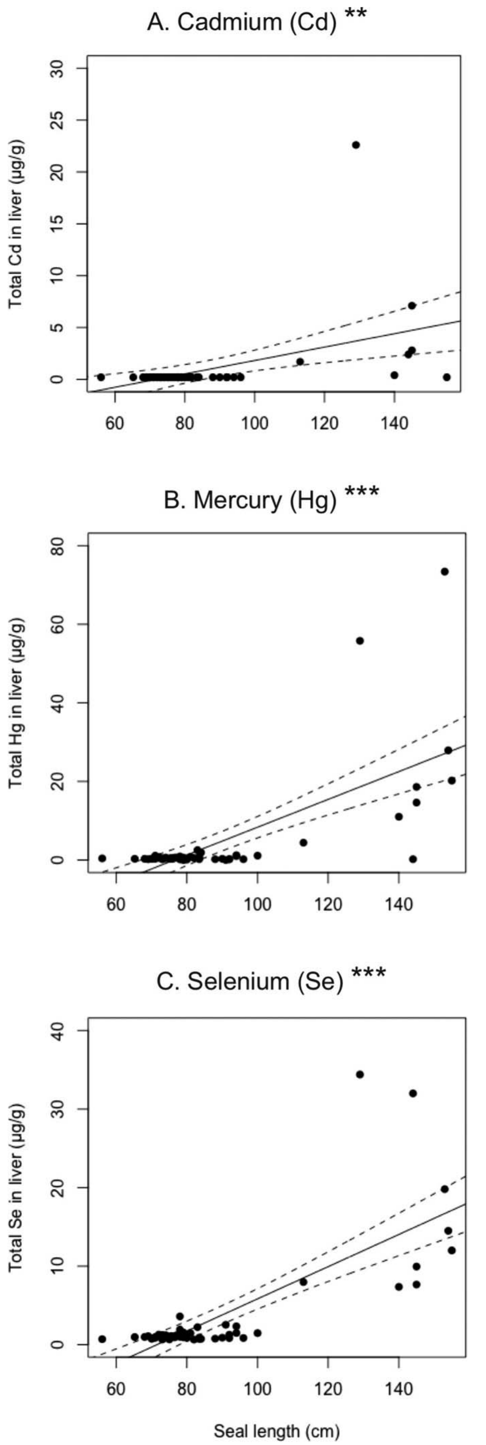

FIGURE 2. Wet weight (ww) concentrations (µg/g ww) of liver total cadmium (A), mercury (B), and selenium (C) by straight body length (cm) of dead, beach-cast harbor seals (*Phoca vitulina richardii*) from San Juan County, Washington, USA. Cadmium concentrations that were under the detection limit of 0.2 µg/g ww are plotted as 0.2 µg/g ww. Dashed lines are 95% confidence bands. Asterisks indicate the statistical significance of generalized linear models evaluating the relationship between liver trace element concentrations and harbor seal length, the proxy for age in this study. **=$P<0.01$ (0.003); ***=$P<0.001$.

2016), seals in SJC exhibited age-accumulation of Cd, Hg, and Se (Fig. 2). Other elements are regulated to a greater degree via excretion and absorption and do not accumulate with prey preference during a marine mammal's lifetime (Law 1996). In contrast to prior studies where older animals displayed lower concentrations of Cu and Zn (Akmajian et al. 2014), we found that concentrations of these elements did not differ by seal length, the proxy for seal age in this study, perhaps due to a lack of statistical power.

Mean liver concentrations of Cd, Cu, and Zn in SJC seals were within or below liver tissue ranges reported elsewhere (Europe; Law et al. 1991; Kakuschke et al. 2012). Consistent with these findings, Cu and Zn contamination is not considered a high-priority concern in the Salish Sea (Ecology and King County 2011). Median Cd concentrations in SJC harbor seal livers were well below 20 µg/g ww, the putative lower limit for renal dysfunction in marine mammals (Law 1996). Compared to SPS seals, nonpups in SJC had greater concentrations of Cd, Cu, and Zn (Fig. 3A, B, E), and SJC pups had greater concentrations of Zn (Fig. 4D). Because SJC is rural, less populated, and less industrialized, we do not suspect it has more land-based, anthropogenic sources of Cd, Cu, or Zn (e.g., pesticides, plumbing, roofing materials) than SPS has. We hypothesize that regional differences might be due to prey preference and availability or natural geochemical processes.

Mercury contamination is a high-priority concern in the Salish Sea, and advisories to limit human consumption of fish due to Hg concentrations have been issued in SJC (Washington State Department of Health 2015). Despite these concerns, liver total Hg

TABLE 1. Mean±SE and range of total trace element concentrations in liver (µg/g wet weight) of harbor seal (*Phoca vitulina richardii*) pups and nonpups from San Juan County (SJC), South Puget Sound (SPS), the Strait of Juan de Fuca (SJF), and Central/Northern California (CNC), USA. In SJC, lead and cadmium (in pups only) concentrations were below the detection limits of 2 µg/g and 0.2 µg/g, respectively.

	Trace elements (µg/g wet weight)											
	Calcium	Cadmium	Copper	Iron	Mercury	Magnesium	Manganese	Lead	Selenium	Zinc	Year	Reference
Adults												
SJC (n=10)	66±7.9 (42–103) (n=7)	5.3±3.0 (0.2–22.6)	28.0±7.7 (10–94)	304±92.4 (99–942)	22.6±7.6 (0–73.4)	188±20.8 (122–363)	2.7±0.34 (1.5–4.4)	<2	14.8±3.4 (2.4–34.4)	64.7±12.1 (29–134)	2009–12	Our study
SPS (n=14)		0.51±0.10 (0.115–1.25)	8.7±1.1 (1.0–13.2)		27.3±9.0 (0.21–129)			0.064±0.07 (0.02–0.31)	6.1±2.64 (0.13–40.7)	37.4±5.63 (5.2–71.5)	2004–07	Akmajian et al. 2014
CNC (n=12)					62.9±15.1 (13.6–162.8)			0.027±0.006[a] (0.007–0.08)	23.8±5.3 (4.65–57.9)		2006	Brookens et al. 2007
Pups												
SJC (n=47)	67.7±3.92 (33–198) (n=41)	<0.2 (n=41)	18.3±2.27 (4–79) (n=46)	326±96.7 (24–3,920) (n=46)	0.49±0.06 (0–2.5)	207±17.5 (124–762)	2.5±0.15 (0.3–6.2)	<2	1.17±0.08 (0.64–3.6)	74.9±6.8 (24–295)	2009–12	Our study
SPS (n=7)			14.6±5.7 (1.6–40.2)		0.41±0.07 (0.17–0.62)				0.6±0.07 (0.17–0.75)	71.6±17.4 (19.2–116)	2004–07	Akmajian et al. 2014
SPS (n=7)		0.003±0.000 (0.002–0.006)	6.64±2.85 (4.14–12.4)		0.55±0.05 (0.34–0.85)			0.029±0.002 (0.021–0.042)	0.66±0.03 (0.4–1.05)	74.6±5.2 (34.3–117)	1990	Calambokidis et al. 1991
SJF (n=8)			17.9±5.4 (5–42.2)		1.15±0.54 (0.3–4.75)				0.95±0.06 (0.72–1.3)	101±17.3 (51.5–186)	2004–07	Akmajian et al. 2014
SJF (n=5)		0.002±0.000 (0.002–0.003)	15.2±6.6 (5.0–26.8)		0.95±0.32 (0.4–2.2)			0.039±0.003 (0.029–0.048)	0.9±0.07 (0.66–1.05)	18.7±3.2 (8.5–31.5)	1990	Calambokidis et al. 1991
CNC (n=28)					1.44±0.25 (0.15–7.00)			0.029±0.007 (<0.007–0.17)	0.74±0.04 (0.04–1.29)		2006	Brookens et al. 2007

[a] Mean and SE calculated without 62.06 µg/g wet weight outlier.

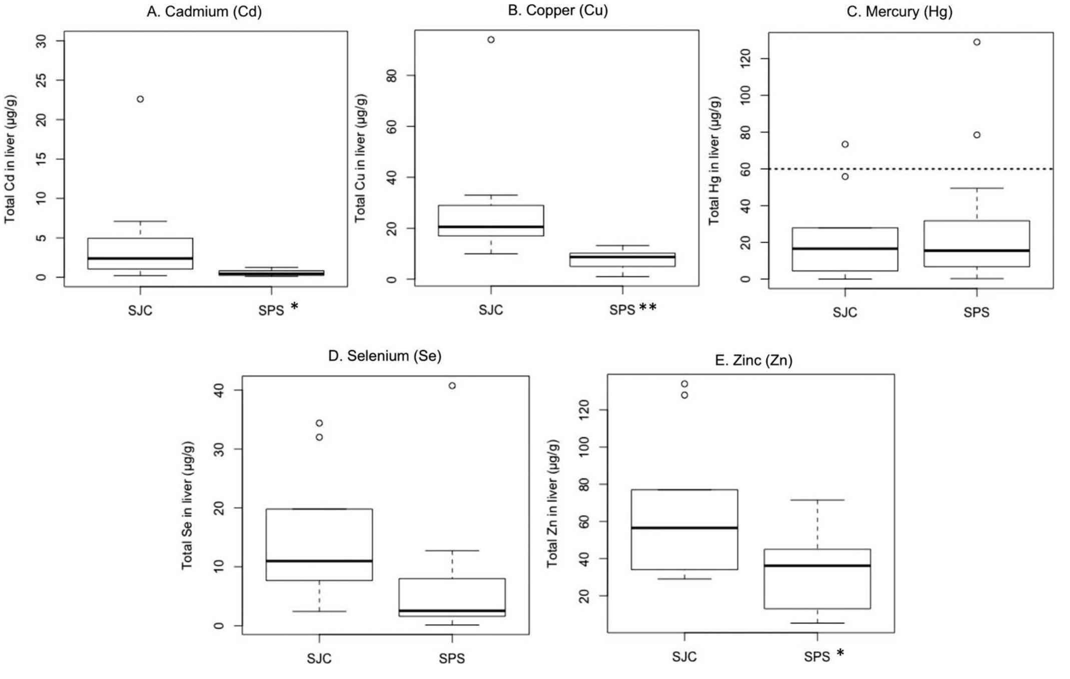

FIGURE 3. Medians and quartiles of trace element wet weight (ww) concentrations in livers (µg/g ww) of harbor seal (*Phoca vitulina richardii*) nonpups (adults and subadults) by sampling location. SJC=San Juan County; SPS=South Puget Sound (Akmajian et al. 2014). Asterisks indicate the statistical significance of generalized linear models comparing SJC to SPS. *=$P<0.05$ (0.033 for Cd; 0.023 for Zn); **=$P<0.01$ (0.005). The dotted line in C indicates published toxicity thresholds for Hg (60 µg/g ww).

concentrations in SJC pups and nonpups were well below the putative toxicity threshold for marine mammals (60 µg/g ww; Arctic Monitoring and Assessment Programme 1998) with the exception of one adult seal with an Hg concentration of 74 µg/g ww. Although this seal had no hepatic lesions indicative of Hg intoxication, the possibility of immunotoxic effects cannot be discounted (Das et al. 2003). To examine the significance of this finding, it is necessary to consider the various forms of Hg and its interactions with other elements and endogenous proteins. The bulk of a marine mammal's Hg burden comes from absorption of the organic, more-toxic methylmercury (MeHg). Marine mammals have evolved various processes for protection against mercury toxicity, including MeHg demethylation in the liver (Ikemoto et al. 2004), production of metallothioneins to sequester harmful elements (Das et al. 2000), and formation of inert mercury selenide (Khan and Wang 2009). Selenium's protective abilities are hypothesized to be sufficient when the Se:Hg molar ratio exceeds one (Peterson et al. 2009). The Se:Hg molar ratio declines as a seal ages and accumulates inorganic Hg in the liver. All SJC nonpups displayed Se:Hg molar ratios above or near 1:1, and all pups had molar ratios well above 1:1 due to negligible Hg concentrations.

As fetuses and neonates, phocids acquire Hg via transplacental and lactational transfer of MeHg (Habran et al. 2013). Perinates and pups are hypothesized to have underdeveloped pathways to demethylate MeHg or slow mercury selenide formation kinetics, accounting for their lower concentrations of inorganic Hg (Ewald et al. 2019). We cannot rule out the possibility that SJC harbor seal pups had elevated MeHg concentrations because we did not measure them. However, pups from SJC had greater Se concentrations than pups elsewhere in the Salish Sea (Fig. 4C), which might confer protection to the toxic effects of MeHg.

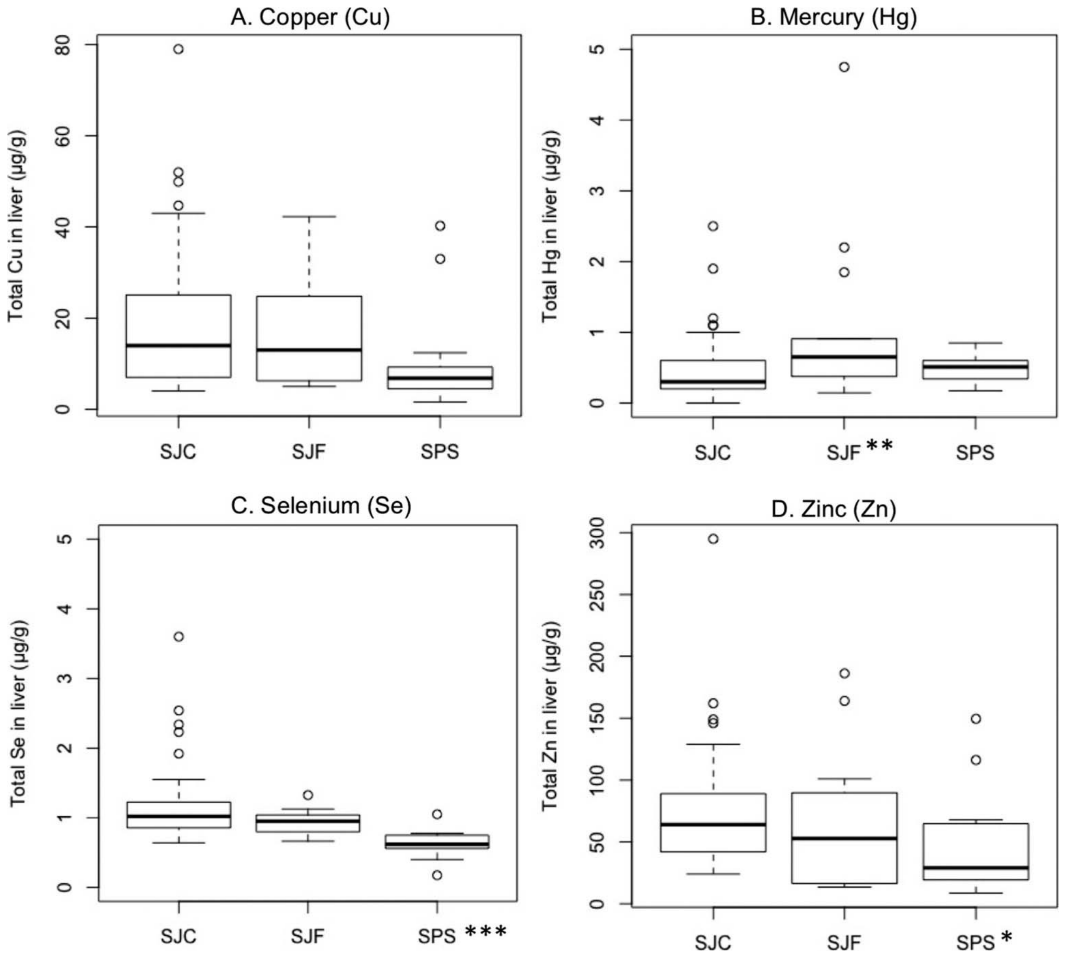

FIGURE 4. Medians and quartiles of trace element wet weight (ww) concentrations in livers (μg/g ww) of harbor seal (*Phoca vitulina richardii*) pups by sampling location. SJC=San Juan County; SJF=Strait of Juan de Fuca (Calambokidis et al. 1991; Akmajian et al. 2014); SPS=South Puget Sound (Calambokidis et al. 1991; Akmajian et al. 2014). Asterisks indicate the statistical significance of generalized linear models comparing SJC to SJF and SPS. *=$P<0.05$ (0.044); **=$P<0.01$ (0.005); ***=$P<0.001$.

Our findings, in agreement with necropsy reports that did not find signs of trace element toxicity in any seal in this study, suggest that trace element concentrations are not currently a major health concern for harbor seals in San Juan County, Washington. However, because ocean acidification can alter metal speciation and biological availability in the ocean (Stockdale et al. 2016), it is possible that trace metal concentrations in harbor seals could change in the future. Based on our findings, further surveillance for trace elements in harbor seals in SJC should focus on adults and include a larger sample size with a more representative sex ratio. It is important to note that seals sampled in this study were found stranded and might not be representative of the living seal population. To assess the entire seal population distribution in SJC, future sampling would require the analysis of trace elements in blood or hair from live seals. Our findings highlight the value of stranded harbor seals as a sentinel species for detecting differences in trace element concentrations between various geographic regions. Regional variations in seal diet or natural geochemical processes could explain the unexpected differences found between SJC and more industrialized study areas within the same ecosystem.

All samples were collected under permits from the National Marine Fisheries Service Marine Mammal Health and Stranding Response Program permit 18786. This study was conducted while E. Ashley was an intern through the National Oceanic and Atmospheric Administration's Ernest F. Hollings Undergraduate Scholarship Program. Harbor seal stranding response and necropsies were performed using funding from the John H. Prescott Marine Mammal Rescue Assistance Grant with in-kind support from The Whale Museum and the SeaDoc Society, a program of the Karen C. Drayer Wildlife Health Center, University of California, Davis School of Veterinary Medicine. We thank the numerous San Juan County Marine Mammal Stranding Network volunteers who helped collect carcasses and assisted with necropsies. We also thank R. Amos for data entry help and A. Akmajian, R. Davis, T. Brookens, R. Davis, D. Montecino-Latorre, B. Poppenga, J. West, and two anonymous reviewers for comments that improved this article.

LITERATURE CITED

Akmajian A, Calambokidis J, Huggins J, Lambourn D. 2014. Age, region, and temporal patterns of trace elements measured in stranded harbor seals (*Phoca vitulina richardii*) from Washington inland waters. *Northwest Nat* 95:83–91.

Arctic Monitoring and Assessment Programme. 1998. Heavy metals. In: *AMAP assessment report: Arctic pollution issues.* Arctic Monitoring and Assessment Programme, Oslo, Norway, pp. 373–453.

Brookens TJ, Harvey JT, O'Hara TM. 2007. Trace element concentrations in the Pacific harbor seal (*Phoca vitulina richardii*) in central and northern California. *Sci Total Environ* 372:676–692.

Calambokidis J, Steiger GH, Lowenstine LJ, Becker DS. 1991. Chemical contamination of harbor seal pups in Puget Sound. *Puget Sound Estuary Program. PTI Environmental Services, Report 910/9-91-032*. US Environmental Protection Agency, Seattle, Washington, 43 pp.

Das K, Debacker V, Bouquegneau JM. 2000. Metallothioneins in marine mammals. *Cell Mol Biol* 46:283–294.

Das K, Debacker V, Pillet S, Bouquegneau J. 2003. Heavy metals in marine mammals. In: *Toxicology of marine mammals*, Vos JG, Bossart G, Fourier M, O'Shea T, editors. Taylor & Francis, New York, New York, pp. 135–167.

Desforges JP, Sonne C, Levin M, Siebert U, De Guise S, Dietz R. 2016. Immunotoxic effects of environmental pollutants in marine mammals. *Environ Int* 86:126–139.

Ecology and King County. 2011. *Control of toxic chemicals in Puget Sound: Assessment of selected toxic chemicals in the Puget Sound Basin, 2007–2011*. Washington State Department of Ecology, Olympia, Washington and King County Department of Natural Resources, Seattle, Washington. https://fortress.wa.gov/ecy/publications/documents/1103055.pdf. Accessed May 2018.

Ewald JD, Kirk JL, Li M, Sunderland EM. 2019. Organ-specific differences in mercury speciation and accumulation across ringed seal (*Phoca hispida*) life stages. *Sci Total Environ* 650:2013–2020.

Geraci JR, Lounsbury VJ, editors. 2005. *Marine mammals ashore: A field guide for strandings*. National Aquarium in Baltimore, Baltimore, Maryland, 371 pp.

Habran S, Pomeroy PP, Debier C, Das K. 2013. Changes in trace elements during lactation in a marine top predator, the grey seal. *Aquat Toxicol* 126:455–466.

Ikemoto T, Kunito T, Tanaka H, Baba N, Miyazaki N, Tanabe S. 2004. Detoxification mechanism of heavy metals in marine mammals and seabirds: Interaction of selenium with mercury, silver, copper, zinc, and cadmium in liver. *Arch Environ Contam Toxicol* 47: 402–413.

Jeffries S, Huber H, Calambokidis J, Laake J. 2003. Trends and status of harbor seals in Washington State: 1978–1999. *J Wildl Manag* 67:207–218.

Kakuschke A, Gandrass J, Luzardo OP, Boada LD, Zaccaroni A, Griesel S, Grebe M, Pröfrock D, Erbsloeh HB, Valentine-Thon E, et al. 2012. Postmortem health and pollution investigations on harbor seals (*Phoca vitulina*) of the islands Helgoland and Sylt. *ISRN Zool* 2012:106259.

Khan MA, Wang F. 2009. Mercury-selenium compounds and their toxicological significance: Toward a molecular understanding of the mercury-selenium antagonism. *Environ Toxicol Chem* 28:1567–1577.

Law RJ. 1996. Metals in marine mammals. In: *Environmental contaminants in wildlife: Interpreting tissue concentrations*, Beyer WN, Heinz G, Redmon-Norwood A, editors. Lewis Publishers, Boca Raton, Florida, pp. 357–376.

Law RJ, Fileman CF, Hopkins AD, Baker JR, Harwood J, Jackson DB, Kennedy S, Martin AR, Morris RJ. 1991. Concentrations of trace metals in the livers of marine mammals (seals, porpoises and dolphins) from waters around the British Isles. *Mar Pollut Bull* 22:183–191.

Noël M, Jeffries S, Lambourn DM, Telmer K, Macdonald R, Ross PS. 2016. Mercury accumulation in harbour seals from the northeastern Pacific Ocean: The role of transplacental transfer, lactation, age and location. *Arch Environ Contam Toxicol* 70:56–66.

Peterson SA, Ralston NVC, Whanger PD, Oldfield JE, Mosher WD. 2009. Selenium and mercury interactions with emphasis on fish tissue. *Environ Bioindicat* 4:318–334.

R Development Core Team. 2016. *R: A language and environment for statistical computing*. R Foundation, Vienna, Austria. http://www.R-project.org. Accessed May 2018.

San Juan County. 2011. *Best available science synthesis. Chapter 1: Introduction and overview*. https://www.sanjuanco.com/DocumentCenter/View/13085/BAS-Synthesis. Accessed July 2019.

Siebert UC, Joiris L, Holsbeek L, Benke H, Failing K, Frese K, Petzinger E. 1999. Potential relation between mercury concentrations and necropsy findings in cetaceans from German waters of the North and Baltic Seas. *Mar Pollut Bull* 38:285–295.

Stockdale A, Tipping E, Lofts S, Mortimer RJG. 2016. Effect of ocean acidification on organic and inorganic speciation of trace metals. *Environ Sci Technol* 50: 1906–1913.

Washington State Department of Health. 2015. *Puget Sound fish consumption advice guide: Washington State Department of Health Publication 334-098*. https://www.doh.wa.gov/Portals/1/Documents/Pubs/334-098.pdf. Accessed July 2019.

Zier J, Gaydos JK. 2014. *Harbor seal species profile, Encyclopedia of Puget Sound*. https://www.eopugetsound.org/articles/harbor-seal-species-profile. Accessed May 2018.

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