Metal tissue levels in Steller sea lion (*Eumetopias jubatus*) pups

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ABSTRACT

The endangered Western population of the Steller sea lion declined for three decades for uncertain reasons. We present baseline data of metal concentrations in pups as a first step towards investigating the potential threat of developmental exposures to contaminants. Seven metals were investigated: arsenic, cadmium, silver, aluminum, mercury, lead and vanadium. Vanadium was detected in only a single blubber sample. Mercury appears to be the most toxicologically significant metal with concentrations in the liver well above the current action level for mercury in fish. The concentrations of aluminum, arsenic, silver, cadmium and lead were present in one-fourth to two-thirds of all samples and were at either comparable or below concentrations previously reported. Neither gender nor region had a significant effect on metal burdens. Future work should consider metal concentrations in juveniles and adults and toxicological studies need to be performed to begin to assess the toxicity of these metals.

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1. Introduction

The Eastern and Western populations of the Steller sea lion (*Eumetopias jubatus*) are genetically distinct and geographically separate populations delineated at 145° W longitude (Bickham et al., 1996). The Western population has declined since the late 1970s and is listed under the United States Endangered Species Act as endangered (Trites and Larkin, 1996; Loughlin, 1998). The Eastern population has increased slightly and is listed as threatened. The reason(s) for the decline of the Western population remain uncertain (Trites and Larkin, 1996; Trites and Donnelly, 2003). Factors potentially contributing to the decline include malnutrition, disease, predation by killer whales, climate changes, exposure to toxic substances, entanglement in marine debris and incidental and intentional take by humans (Loughlin, 1998; Trites and Donnelly, 2003). It has also been proposed that the decline of the Steller sea lion population is linked to the inability of pups to survive and become part of the breeding population (Chumbley et al., 1997; York, 1994; Rea et al., 1998). Metals are a class of toxic substances that are prevalent in the marine environment (Barron et al., 2003) and are known to significantly disrupt all of the major organ systems in humans and terrestrial mammals (Goyer and Clarkson, 2001) and, therefore, might be a contributing factor in the decline of this species, however, Steller sea lion exposure to metals is not well understood.

Previous studies have shown that Steller sea lions are exposed to metals (Ando-Mizobata, 2006; Hamanaka et al., 1982; Sydeman and Jarman, 1998; Saeki et al., 1999, 2001; Beckmen et al., 2002). One study focused on silver in juvenile and adult Steller sea lion liver tissue and found silver loads positively correlated with vanadium, selenium and mercury concentrations; however, the exact concentrations were not reported (Saeki et al., 2001). A second

study investigated the metal loads of vanadium in juvenile and adult Steller sea lion liver tissue and found a correlation between liver vanadium levels and age (Saeki et al., 1999). A third study reported cadmium and zinc levels in livers and kidneys of juvenile and adult Steller sea lions, and also found a correlation with age (Hamanaka et al., 1982). Another study measured zinc and iron in adult Steller sea lion canine teeth to assess fluctuations in metal accumulation over the course of individual lifetimes (Ando-Mizobata, 2006). These data indicate that adults are exposed and metals accumulate with age.

By contrast, only two studies have considered Steller sea lion pups. Beckmen et al. (2002) found relatively low mercury concentrations in hair samples collected from pups and juveniles of both populations. The other study found detectable levels of aluminum, arsenic, cadmium, chromium, copper, iron, lead, mercury, selenium and silver in the livers of eight pups (Sydeman and Jarman, 1998). These data present a clear indication that exposure has occurred, but the picture is incomplete as only four tissues have been considered, hair, liver, kidney and teeth while other important organs such as the lung, skin, testes and ovary have not been considered. This absence of data limits interpretation of the potential extent of exposure to metals in Steller sea lions, as typical in mammals there is a differential accumulation of metals in different organs. Sampling of a single organ may not give an accurate indication of the extent of exposure or the potential impact to the animal's physiologic processes. For example, a study in bowhead whales (*Baelaena mysticetus*) revealed that cadmium concentrations in the liver are relatively low at 11 ug/g, but cadmium concentrations in the kidney cortex can reach up to 200–300 ug/g (Bratton et al., 1997). Consequently, our understanding of the extent of metal exposure and bioaccumulation in Steller sea lions remains incomplete. Accordingly, to further assess the extent of exposure, this study investigated seven different metals in nine different organs. Because mammals are particularly sensitive to many metals during development, we specifically focused on pups as a measure of developmental exposure and an indirect measure of adult exposure and considered sex and regional differences to establish a baseline of exposure to these metals.

2. Materials and methods

2.1. Collection of Steller sea lion tissue samples

Tissues were obtained opportunistically from free-ranging animals found dead by field researchers investigating other aspects of Steller sea lion life history and physiology. Between 5 and 15 g of each tissue was collected with a Teflon knife and placed in plastic vials and stored at −70 °C until analysis. Location and sex were recorded for each animal (Fig. 1). Since the Steller sea lion population is geographically separated at 145° W longitude, samples obtained west of 145° W longitude were classified as the Western population and any samples collected east of 145° W longitude were classified as the Eastern population. Samples came from intact recently dead pups. No developmental abnormalities were observed in the pups and the causes of death included cervical dislocation (1), euthanized due to emaciation (1), suffocation (1), drowning in cesspool (5), aspiration of milk (2), infection (1), and unknown (14).

2.2. Determination of metal concentrations in tissues from Steller sea lions

Wet samples were homogenized, weighed to the nearest 0.0001 g, transferred to polypropylene tubes and digested with a mixture of 3 ml trace metal grade nitric acid, 1 ml hydrochloric acid and 2 ml ultra-pure hydrogen peroxide at atmospheric pressure in a CEM MARS-5 microwave oven. No liquid was added to the tissues during homogenization. Skin was rinsed to remove external particulate contaminants. Following digestion, samples were diluted to volume with Milli-Q 18 M Ohm water and prepared as necessary for analysis. Each group of tissue samples included the following quality control samples: method blanks, spiked blanks, certified reference materials, duplicate samples and spiked samples. Quality assurance and quality control information is listed in Table 1.

Mercury concentrations were determined by cold vapor atomic absorption, using a Cetac 7500 QuickTrace Hg analyzer. Silver, arsenic, cadmium, and vanadium concentrations were determined by inductively coupled plasma-mass spectroscopy (ICP-MS) using a Perkin Elmer/Sciex DRC 2 instrument equipped with a dynamic reaction cell to remove molecular ion interferences. Prior to analysis, digestates were diluted 10× with Milli-Q water in order to reduce the nitric acid level to approximately 2%. Because of molecular ion interferences, arsenic and vanadium were determined in "dynamic reaction cell (DRC) mode" using ammonia as the reaction gas.

The remaining elements, aluminum and lead, were determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES), using a Spectro CirOS instrument equipped with an axial torch.
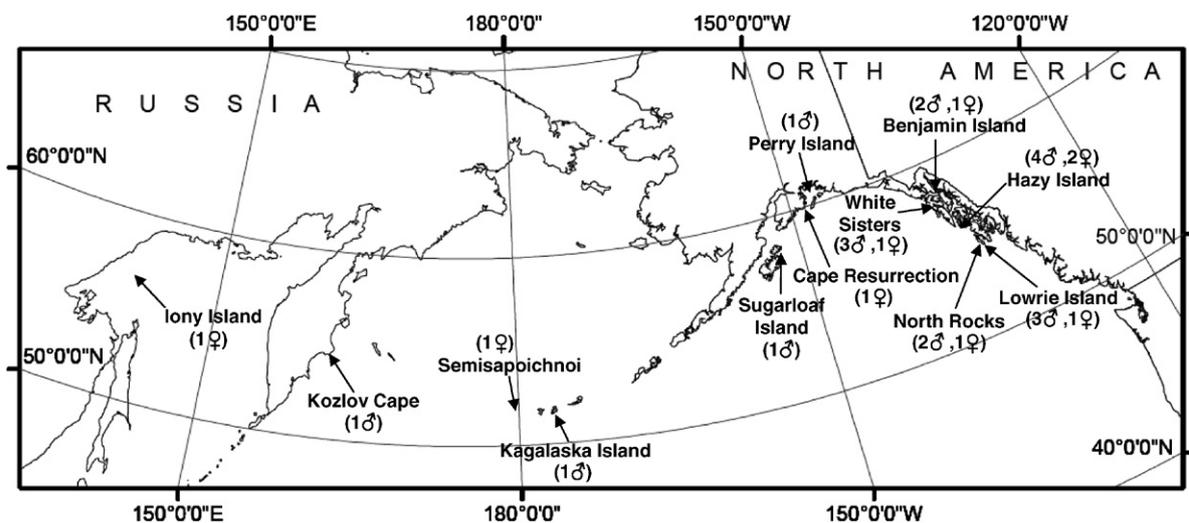

Fig. 1. Locations of collected samples.

Table 1
Quality assurance and quality control (QA/QC) data for element analyses

Element	MDL [a] (ppm)	Blank (ppm)	Duplicate (RPD)[b]	LCS recovery (%)[c]	Spike recovery (%)[d]	Standard reference material recovery (%)			
						2976 [e]	1577b [f]	DOLT-2 [g]	DORM-2 [h]
Ag	0.004	0.001	6.5 (6)[i]	100 (16)	101 (16)		112 (3)	99 (11)	86 (2)
Al	0.544	0.114		107 (17)	103 (18)	70 (2)		83 (12)	
As	0.148	0.020	11.0 (1)	97 (16)	104 (17)	109 (2)		85 (11)	93 (3)
Cd	0.005	0.0005	2.3 (4)	102 (16)	104 (17)	102 (2)	94 (6)	98 (11)	
Hg	0.003	0.0002	9.6 (18)	97 (14)	94 (13)	105 (2)		95 (11)	93 (2)
Pb	0.007	0.002		101 (16)	102 (10)	105 (2)	88 (3)	124 (11)	
V	0.135	0.009		104 (17)	103 (18)	99 (2)	86 (3)	104 (11)	93 (2)

[a] Method detection limit.
[b] Duplicate values are valid when average concentration is not less than $3 \times$ MDL.
[c] Laboratory control sample (LCS) values are valid when the observed concentration is not less than $3 \times$ MDL.
[d] Spike values are valid when spike level values are valid when the observed concentration is not less than $3 \times$ MDL.
[e] 2976, NIST Mussel tissue SRM.
[f] 1577b, NIST Bovine liver SRM.
[g] DOLT-2, NRC Dogfish liver SRM.
[h] DORM-2, NRC Dogfish muscle SRM.
[i] (n), Number of valid observation.

2.3. Statistics

The unbalanced two-way ANOVA was used to determine whether there was a difference in the average metal concentrations in each organ for each of the following situations: east vs. west regardless of gender, east male vs. west male and east female vs. west female. The interaction between region and gender was also tested. All non-detected values have been considered in the analysis. A method described by Aboueissa and Stoline (2004) was used for adjusting the non-detected values. Since most of our data sets had multiple detection limits in each data set, all non-detected observations were replaced by the average of their detection limits.

3. Results and discussion

3.1. Sea lion population characterization

One hundred and sixty-two samples from 27 different pups were analyzed for their metal content. There were a total of 25 blubber samples, 22 brain samples, 16 heart samples, 26 kidney samples, 26 liver samples, 24 lung samples, 6 muscle samples, 2 ovary samples and 15 testes samples. Forty-nine samples from 9 female sea lions and 113 samples from 18 male sea lions were analyzed. Nineteen animals and 123 samples were from the Eastern population and 8 animals and 39 samples were from the Western population.

3.2. Element loads in tissues from all animals

We sought to investigate tissue burdens of numerous elements in multiple organ systems in Steller sea lion pups. Vanadium was the only metal that was not detectable in any tissues other than one blubber sample. These data contrast with a previous study that showed that vanadium was present in organs of Steller sea lions (Saeki et al., 1999). However, this discrepancy may be due to differences in age classes sampled. All of our samples were collected from pups, whereas the majority of animals collected in Saeki et al. (1999) were juveniles or adults, and they showed vanadium concentrations correlated with age.

Of the seven metals we tested, total mercury appears to be of the highest concern. Methylmercury is the most toxic form of mercury (Law, 2006); however due to difficulties in measuring specific forms of mercury in tissues we measured only total mercury levels. Mercury was present in 159 of the 162 samples with

the highest concentrations of mercury in the liver, ranging from 0.17 to 9.38 ug/g wet weight. These values fall within the range of those previously reported for Steller sea lion (4.8–73 ug/g dry weight which is roughly equivalent (assuming 70% moisture) to 1.44–21.9 ug/g wet weight) and Northern fur seals (7.65–10.8 ug/g wet weight) (Sydeman and Jarman, 1998; Zeisler et al., 1993).

Mercury is thought to be hepatotoxic in pinnipeds (Ross and Troisi, 2001). Mercury also targets pinniped testes by altering biosynthesis of steroids in gray seals (Freeman and Sangalang, 1977). An *in vivo* study of harp seals showed that one of the main causes of death for dietary mercury exposure was renal failure after exposure to 25 mg of mercury/kg/day for 20 days. Mercury levels in the livers of exposed animals were 143 and 142 ug/g (Ronald et al., 1977). Harp seals exposed to 0.25 mg of mercury/kg/day for 60 days had a decreased appetite and reduced activity but exhibited no "neurological dysfunction" (Ronald et al., 1977). Tissue mercury level in the liver in animals exposed to 0.25 mg/kg/day were 64 and 82.5 ug/g mercury (Ronald et al., 1977). A study in rats exposed to 1.5 mg of mercury/kg for two days had mercury levels in the kidney of 11.94 ug/g (dos Santos et al., 2007). These animals showed changes in their motor activity (dos Santos et al., 2007). In addition, mercury is more toxic to developing nervous systems (Goyer and Clarkson, 2001); therefore, mercury may be a metal of particular concern for Steller sea lion pups.

It should be noted that the upper limit of these values reaches 9.38 ug/g in pups which is more than 9-times the current action level (1 ppm or 1 ug/g) for mercury in fish for human consumption. The World Health Organization states that women with maternal hair concentrations between 10 and 20 ug/g have a 5% risk of their infants developing neurological disorder due to methylmercury exposure (van Oostdam et al., 1999), which raises concern for the sea lion pups. This level also raises concern for the health of subsistence hunters because their diet includes the liver from juvenile Steller sea lions which could have even higher concentrations of mercury. The maximum kidney, lung and hair mercury concentrations also reached the action level. Of the metals considered, mercury is the only metal with an action level for food.

It has been reported that marine mammals often have elevated levels of selenium to detoxify mercury allowing them to tolerate higher mercury exposures (O'Hara and O'Shea, 2001). The exact mechanism of detoxification of methylmercury by selenium is unclear but it appears to form a complex with mercury, demethylate methylated mercury, transport mercury away from sensitive organs and prevent oxidative damage (O'Hara and O'Shea, 2001). We found that the molar selenium concentrations in the pups were

either greater than or equal to the molar concentrations of mercury. Therefore, if this mechanism is active in pups the elevated mercury levels may not be inducing toxic effects.

Aluminum was detected in approximately one-fourth of all the samples with the majority of detected samples and highest concentrations in the blubber (0.37–46 ug/g wet weight). Aluminum concentrations are rarely considered in marine mammal tissues. Only one report indicated concentrations of 16–35 ug/g dry weight in Steller sea lion liver (Sydeman and Jarman, 1998) which is roughly comparable to 4.9–10.5 ug/g wet weight. These concentrations are slightly higher than our levels of 1.03–4.98 ug/g wet weight aluminum in the liver.

Aluminum is a neurotoxicant but the degree of toxicity varies widely depending on species (Savory et al., 2006). Mice are relatively insensitive to aluminum neurotoxicity while rabbits are highly sensitive and experience severe neurological changes (Savory et al., 2006). One study in rats found that administration of 4.29, 8.59 and 17.18 mg/100g aluminum induced brain aluminum levels of 1.728, 2.043 and 2.371 ug/g wet weight, respectively (Abd El-Rahman, 2003). These exposed animals experienced significant brain alterations, including neuronal degeneration of the cerebral cortex, neurofibrillary degeneration and demyelination and increased levels of the excitatory neurotransmitters, glutamate and glutamine, and decreased level of the inhibitory neurotransmitter, GABA (Abd El-Rahman, 2003). In our study, we reported brain aluminum levels ranging from 0.52 to 6.27 ug/g wet weight. The sensitivity of Steller sea lions to aluminum remains unknown; however, aluminum could be of particular concern for developing pups.

Cadmium concentrations were relatively low in Steller sea lion pups and detected in only 13 samples with the highest concentrations in the kidney (0.006–4.25 ug/g wet weight). Cadmium was only detected in two liver samples with concentrations of only 0.005 and 0.40 ug/g wet weight. However, Sydeman and Jarman (1998) reported relatively high concentrations of cadmium in the livers of Steller sea lion pups. The explanation for this is uncertain, though in that study only one animal was reported, and thus, it is unclear if this is an anomalous animal or reflective of a population.

Arsenic was detected in approximately half of the samples with the brain having the highest concentrations (0.28–1.58 ug/g wet weight). Limited data exist on the abundance and toxicity of arsenic in marine mammals, however, the concentrations found in brain and other organs may be harmful. It has been shown that arsenic exposure alters *in vitro* biosynthesis of steroids in the adrenals and testes of gray seals (Freeman and Sangalang, 1977). We found arsenic concentrations in the liver ranged between 0.039 and 0.27 ug/g wet weight. Previous reports on marine animals revealed high arsenic concentrations in the liver of black-footed albatross (0.8–13 ug/g wet mass) and hawksbill turtle (0.66–7.5 ug/g wet mass); however there was no discussion of the harmful effects that arsenic may have on either species (Fujihara et al., 2003). Ingestion of arsenic-contaminated water is known to cause skin, liver, lung, kidney and bladder cancer in humans (Smith et al., 1992) although effects in marine mammals are poorly understood.

Lead was detected in approximately two-thirds of samples at relatively low concentrations with concentrations in the liver ranging between 0.003 and 0.1 ug/g wet weight. These concentrations are consistent with data reported in the Steller sea lion liver by Sydeman and Jarman (1998) and are consistent with those in livers from other marine mammals (Zeisler et al., 1993).

Silver was detected in one-third of the samples with the liver having a concentration range of 0.027–0.19 ug/g wet weight. However, the silver concentrations we report are much lower than those previously observed. Specifically, Saeki et al. (2001) reported concentrations of 0.1–1.04 ug/g wet weight, which is substantially higher than the concentrations we found (Saeki et al., 2001). This difference is again largely due to age since we sampled pups, while that study considered concentrations in adults, demonstrating that silver exposure continues with age and is consistent with their find-

Table 2
Tissue burdens in Western vs. Eastern Steller sea lions pups

Organ	Region	N[a]	Metal (ug/g)						
			Ag[b]	Al	As	Cd	Hg	Pb	V
Blubber	West	7	ND[c]	11.03±17.2[e] (3)	0.77±0.54 (7)	0.0074±0.0022 (1)	0.12±0.24 (7)	0.088±0.16 (2)	1.77 (1)
	East	18	0.0047±0.0003 (2)	1.30±0.51 (11)	0.50±0.36 (18)	0.0066±0.0002 (1)	0.036±0.027 (18)	0.028±0.019 (16)	ND
Brain	West	6	0.0080±0.0023 (3)	ND	ND	ND	0.053±0.047 (5)	0.030±0.033 (4)	ND
	East	17	0.011±0.0067 (14)	1.37±1.27 (2)	ND	ND	0.088±0.059 (17)	0.013±0.020 (7)	ND
Heart	West	4	ND	ND	0.19±0.13 (3)	0.012±0.0078 (1)	0.066±0.065 (4)	ND	ND
	East	12	ND	ND	0.20±0.080 (7)	ND	0.10±0.066 (12)	0.014±0.0093 (5)	ND
Kidney	West	7	0.0055±0.0021 (4)	ND	0.17±0.062 (4)	0.62±1.60 (2)	0.66±0.28[e] (7)	0.019±0.018 (4)	ND
	East	19	0.0072±0.010 (7)	1.53±2.01 (3)	0.17±0.037 (9)	0.0069±0.0033 (2)	0.32±0.18 (19)	0.012±0.012 (11)	ND
Liver	West	7	0.91±0.042 (7)	1.02±0.004 (1)	0.18±0.074 (4)	0.062±0.15 (2)	2.41±3.19[e] (7)	0.037±0.039[e] (7)	ND
	East	19	0.078±0.052 (17)	1.23±0.91 (1)	0.16±0.041 (7)	ND	0.55±0.26 (19)	0.0097±0.0034 (9)	ND
Lung	West	5	ND	0.97±0.34 (3)	ND	ND	0.073±0.063 (4)	0.15±0.28 (4)	ND
	East	19	0.0079±0.014 (3)	1.68±2.45 (2)	0.15±0.032 (3)	0.0064±0.00060 (2)	0.17±0.31 (19)	0.021±0.038 (14)	ND
Muscle	West	0	NA[d]	NA	NA	NA	NA	NA	NA
	East	6	0.012±0.0032 (1)	0.84±0.35 (4)	0.22±0.023 (4)	0.0061±0.0035 (1)	0.22±0.13 (6)	0.029±0.021 (6)	ND
Ovary	West	1	ND	ND	ND	ND	0.0285 (1)	0.014 (1)	ND
	East	1	ND	3.73 (1)	ND	ND	0.057 (1)	0.016 (1)	ND
Testes	West	3	0.011±0.00020 (1)	4.70±5.98 (2)	ND	ND	0.096±0.070 (3)	0.013±0.00084 (1)	ND
	East	12	0.011±0.00020 (1)	1.40±0.19 (4)	0.40±0.10 (1)	0.014±0.0017 (1)	0.082±0.061 (12)	0.012±0.0046 (6)	ND

[a] Number of samples analyzed.

[b] Data reflect an average of detectable samples±standard error. (*n*), Equals the number of samples with detectable levels.

[c] ND, not detectable.

[d] NA, not applicable.

[e] Statistically significant compared to east ($p < 0.05$).

ings that silver concentrations in marine mammals correlate with age.

3.3. Influence of region on tissue concentrations

We further analyzed the data based on region. The Western and Eastern populations had similar tissue metal levels (Table 2), with only three statistically significant differences. The Western

population had statistically higher concentrations of mercury in the kidney ($p = 0.0013$) and liver ($p = 0.00131$) and lead in the liver ($p = 0.0075$).

Considering the data further and comparing gender and region revealed additional differences. The Western male and Eastern male populations had seven statistically significant differences between them (Table 3). The Western male population had statistically higher concentrations of aluminum in the blubber ($p = 0.0016$) and

Table 3
Tissue burdens in Western vs. Eastern male Steller sea lions pups

Organ	Region	N^a	Metal (ug/g)						
			Ag[b]	Al	As	Cd	Hg	Pb	V
Blubber	West	4	ND[c]	17.92±21.0[e] (2)	0.67±0.61 (4)	0.0065±0.00034 (1)	0.19±0.32[e] (4)	0.024±0.0075 (1)	1.77(1)
	East	12	0.0047±0.0004 (2)	1.33±0.50 (8)	0.44±0.15 (12)	0.0081±0.0029[f] (1)	0.043±0.031 (11)	0.031±0.022 (11)	ND
Brain	West	2	0.0062±0.0016 (1)	ND	ND	ND	0.028±0.0032 (2)	0.037±0.048 (1)	ND
	East	12	0.012±0.0072(10)	1.53±1.49 (1)	ND	ND	0.099±0.065 (12)	0.010±0.017 (5)	ND
Heart	West	2	ND	ND	0.12±0.055 (1)	ND	0.028±0.0057 (2)	ND	ND
	East	9	ND	ND	0.20±0.093 (5)	ND	0.11±0.075 (9)	0.13±0.011(5)	ND
Kidney	West	4	0.0065±0.0020 (2)	ND	0.14±0.047 (2)	0.015±0.017 (1)	0.70±0.15[e] (4)	0.017±0.015 (2)	ND
	East	14	0.0048±0.0011 (4)	1.05±0.25 (2)	0.17±0.042 (7)	0.0062±0.00013 (1)	0.32±0.19 (14)	0.011±0.010 (9)	ND
Liver	West	4	0.10±0.38 (4)	1.02±0.005 (1)	0.15±0.075 (2)	0.0060±0.00096 (1)	1.44±1.07 (4)	0.036±0.032[e] (4)	ND
	East	13	0.080±0.56 (12)	ND	0.16±0.036 (4)	ND	0.59±0.28 (13)	0.010±0.0031 (6)	ND
Lung	West	4	ND	0.97±0.39 (2)	ND	ND	0.084±0.067 (3)	0.18±0.31[e] (3)	ND
	East	14	0.0090±0.016(2)	ND	0.15±0.028 (2)	ND	0.21±0.35 (14)	0.019±0.034 (10)	ND
Muscle	West	0	NA[d]	NA	NA	NA	NA	NA	NA
	East	4	0.011±0.0039 (1)	0.81±0.43 (2)	0.22±0.029 (4)	0.0068±0.0043 (1)	0.26±0.15 (4)	0.035±0.024 (4)	ND
Testes	West	3	0.011±0.0002 (1)	4.70±5.98[e] (2)	ND	ND	0.096±0.070 (3)	0.013±0.00084 (1)	ND
	East	12	0.011±0.0002 (1)	1.40±0.19 (4)	0.40±0.10 (1)	0.014±0.0017 (1)	0.082±0.061 (12)	0.012±0.0046 (6)	ND

[a] Number of samples analyzed.
[b] Data reflect an average of detectable samples±standard error. (n), Equals the number of samples with detectable levels.
[c] ND, not detectable.
[d] NA, not applicable.
[e] Statistically significant compared to east ($p < 0.05$).
[f] Statistically significant compared to west ($p < 0.05$).

Table 4
Tissue burdens in Western vs. Eastern female Steller sea lions pups

Organ	Region	N^a	Metal (ug/g)						
			Ag[b]	Al	As	Cd	Hg	Pb	V
Blubber	West	3	ND[c]	1.85±0.20(1)	0.91±0.53 (3)	ND	0.028±0.013 (3)	0.17±0.25 (1)	ND
	East	6	ND	1.24±0.59 (3)	0.63±0.60 (6)	ND	0.020±0.0028 (5)	0.023±0.014 (5)	ND
Brain	West	3	0.0092±0.0021 (2)	ND	ND	ND	0.070±0.057(3)	0.025±0.031 (3)	ND
	East	5	0.0084±0.0048 (4)	0.99±0.26 (1)	ND	ND	0.064±0.031 (5)	0.019±0.028 (2)	ND
Heart	West	2	ND	ND	0.26±0.17 (2)	0.015±0.011[e](1)	0.10±0.081 (2)	ND	ND
	East	3	ND	ND	0.18±0.025 (2)	ND	0.093±0.034 (3)	ND	ND
Kidney	West	3	0.0042±0.0017 (2)	ND	0.20±0.069 (2)	1.42±2.45[e] (1)	0.62±0.44 (3)	0.022±0.025 (2)	ND
	East	5	0.014±0.019 (3)	1.53±2.01 (1)	0.17±0.018 (2)	0.0091±0.0065 (1)	0.30±0.13 (5)	0.017±0.018 (2)	ND
Liver	West	3	0.077±0.052 (3)	ND	0.23±0.054 (2)	0.14±0.22[e] (1)	3.70±4.95[e] (3)	0.037±0.056 (3)	ND
	East	6	0.072±0.046 (5)	1.68±1.62 (1)	0.17±0.052 (3)	ND	0.46±0.19(6)	0.0092±0.0042 (3)	ND
Lung	West	1	ND	0.97 (1)	ND	ND	0.030 (1)	0.13 (1)	ND
	East	5	0.0047±0.000012 (1)	3.15±4.84 (2)	0.17±0.39 (1)	0.0058±0.0010 (2)	0.048±0.020 (5)	0.029±0.052 (4)	ND
Muscle	West	0	NA[d]	NA	NA	NA	NA	NA	NA
	East	2	ND	0.89±0.20 (2)	ND	ND	0.15±0.061 (2)	0.018±0.0040 (2)	ND
Ovary	West	1	ND	ND	ND	ND	0.0285 (1)	0.014 (1)	ND
	East	1	ND	3.73 (1)	ND	ND	0.057 (1)	0.016 (1)	ND

[a] Number of samples analyzed.
[b] Data reflect an average of detectable samples±standard error. (n), Equals the number of samples with detectable levels.
[c] ND, not detectable.
[d] NA, not applicable.
[e] Statistically significant compared to east ($p < 0.05$).

testes ($p=0.0489$), mercury in the blubber ($p=0.0484$) and kidney ($p=0.0053$) and lead in the liver ($p=0.0369$) and lung ($p=0.0309$). The Eastern male population only had higher concentrations of cadmium in the blubber ($p=0.0265$) (Table 3). The Western female population had statistically higher concentrations of cadmium in the heart ($p=0.0199$), kidney ($p=0.0158$) and liver ($p=0.0128$) and mercury in the liver ($p=0.0077$) (Table 4). Only aluminum in the blubber ($p=0.0393$) and cadmium in the liver ($p=0.0461$) had a statistically significant interaction between sex and region.

These data are the first comparisons of elemental concentrations in the Western and Eastern stocks of Alaskan Steller sea lions. The data suggest that with a few exceptions there are not substantial differences in tissue levels between the two populations, at least with respect to pups. It does not appear that these animals are experiencing different exposures as pups, however, as noted for several contaminants (e.g. silver, vanadium and cadmium) exposures as juveniles or adults can be significantly greater. Thus, it would be premature to conclude that the exposure of other age classes is also similar between the populations. Future work should consider elemental concentrations in juveniles and adults.

It would be premature to conclude that these exposure levels are 'safe' or non-toxic simply, because they do not differ between the two populations. It is possible that the level of some of these contaminants may pose a threat to the health of both populations and may be contributing to the decline in the pup population. It should be noted that while the Western population has fallen dramatically and is now endangered, the Eastern population has been experiencing a 3% growth over the last 30 years; however, this growth rate is less than expected for maximum growth in pinnipeds. Interpreting the potential toxicity of these concentrations is difficult as there are very few data investigating the toxic effects of contaminants in Steller sea lions or marine mammals in general. Comparisons to rodent and human studies are also difficult as these studies usually focus on an administered dose and not tissue burdens, and it is currently not possible to predict the level of exposure based on measured tissue concentrations. Thus, the data are presented here as a baseline study of metal concentrations in Alaskan Steller sea lion pups in several different organs with future toxicology studies needed to help put these data in a fuller context.

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