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AGE, REGION, AND TEMPORAL PATTERNS OF TRACE ELEMENTS MEASURED IN STRANDED HARBOR SEALS (*PHOCA VITULINA RICHARDII*) FROM WASHINGTON INLAND WATERS

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ABSTRACT—We measured concentrations of aluminum (Al), arsenic (As), cadmium (Cd), copper (Cu), lead (Pb), nickel (Ni), total mercury (THg), methylmercury (MeHg), selenium (Se), silver (Ag), and zinc (Zn) in liver from Harbor Seals ($n = 31$) stranded dead in the inland waters of Washington State between 2004 and 2007. Results were compared by age class and region, and to results from past studies. Significant differences in concentration were detected among age classes, regions, and years. Adult seals (>3 y old) from this study had significantly higher concentrations of As, Cd, THg, MeHg, Ni, Se, and Ag than neonate pups. Seals from the eastern Strait of Juan de Fuca (SJF) had significantly higher concentrations of As, MeHg, Se, and Ag than seals from the southern Puget Sound (SPS). When pooled with past studies, significant age and regional differences were detected for Zn and Cu, respectively, where Zn decreased with age and Cu was higher in SJF than SPS seals. Lead was significantly lower in seals from the present study compared to past studies. Examining the concentrations of both essential and non-essential trace elements indicates that Harbor Seal tissues can be useful in detecting regional and temporal trends in contaminants.

Key words: contaminants, Harbor Seals, *Phoca vitulina*, Puget Sound, Strait of Juan de Fuca, trace elements, Washington State

Harbor Seals (*Phoca vitulina richardii*) are long-lived, non-migratory residents of Washington State (USA) and, as high trophic level foragers, can be used as indicators of contaminant bioavailability in the ecosystem (Huber and others 2012; Ross and others 2013). Past studies on metal contaminants and trace elements in stranded and live-captured Harbor Seals have identified regional differences in concentrations between areas within the Puget Sound and Strait of Juan de Fuca (Calambokidis and others 1984, 1991; Noël and others 2011). Similarly, persistent organic pollutants and other chemical contaminants have been shown to be at significantly higher levels in Washington Harbor Seals compared to those living in nearby waters of British Columbia, Canada (Ross and others 2004; Cullon and others 2005; Mos and others 2006; Ross and others 2013).

Although environmental management has curbed some contaminant inputs, anthropogenic pollutants are still dominant sources of trace elements and metal contaminants in the Puget Sound, Strait of Georgia, and eastern Strait of Juan de Fuca (Ecology and King County 2011). Investigating trends in contaminant loads of a long-lived resident species can provide valuable insights into regional and temporal trends of contaminants in the environment. Non-essential trace elements like mercury (Hg) may exist in the environment in highly toxic forms, such as methylmercury (MeHg), and are well known to have deleterious effects on seals and other wildlife (Law 1996). Imbalances of essential trace elements such as zinc (Zn) can also lead to compromised immune systems (Anan and others 2002), making trends and baselines of these elements worth monitoring.

In order to investigate trends in contaminant loads, we measured the concentrations of aluminum (Al), arsenic (As), cadmium (Cd), copper (Cu), lead (Pb), nickel (Ni), total mercury (THg), MeHg, selenium (Se), silver (Ag), and Zn in liver of Harbor Seals stranded dead in the southern Puget Sound (SPS) and eastern Strait of Juan de Fuca (SJF) between 2004 and 2007. We compared results for these 11 elements to historical data from Washington State (Calambokidis and others 1984, 1991), and compared concentrations by age class and region to confirm previously reported trends and assess age and region-specific contaminant exposure.

METHODS

Specimen Collection

We opportunistically collected Harbor Seal carcasses throughout Washington from 2004 through 2007 as part of routine marine mammal stranding response. The majority of samples used in this study were collected in SPS. Samples were also collected through targeted searches for dead Harbor Seal pups conducted during the pupping season from 2004 through 2006 at Gertrude Island (SPS) and at Smith and Minor Islands in the SJF. Necropsies were performed on carcasses in fresh to moderate postmortem condition. A portion of each liver was collected in a glass jar and frozen for trace metal analysis.

All seals collected in this study were classified as adults (greater than 3 y old, $n = 15$) or neonate pups (newborn, non-weaned, $n = 15$), except for 1 weaned pup (estimated to be 2 to 3 mo old). Adults averaged 154 cm standard length. Age determined by cementum annuli was available for some adults used in this study (Washington Department of Fish and Wildlife, unpubl. data); adults were aged 4 to 21 y, with a mean age of 13 y.

Trace Element Analysis

Trace elements were analyzed by Columbia Analytical Services (CAS) located in Kelso, Washington. Prior to analysis, each sample was homogenized, sub-sampled, and freeze-dried. The freeze-dried material was further ground to a homogenous meal before aliquoting for acid digestion. The digestion for all elements other than THg was performed in concentrated nitric acid using a closed vessel system under elevated temperature and pressure. For THg, the homogenized sample was digested in nitric acid, followed with strong oxidizers to convert all Hg to the mercuric ion state prior to final reduction to the elemental state with purging into an atomic absorption cell for measurement.

All metals, other than those exceptions listed below, were analyzed by inductively coupled plasma-mass spectroscopy (Method 6020, US EPA 1990). Method detection limits (MDLs) in mg/kg dry weight (dw) for these elements were: 0.4 for Al; 0.05 for As; 0.01 for Cd; 0.03 for Cu; 0.005 for Pb; 0.03 for Ni; 0.006 for Ag; and 0.06 for Zn. THg was analyzed by cold-vapor atomic absorption (Method 7471A, US EPA 1994a) with a MDL of 0.015 mg/kg dw. The standard operating procedure used by CAS for MeHg was a modification of EPA Method 1630 (US EPA 2001) with a MDL of 0.005 mg/kg dw. The modification included an extraction of the tissue with alcoholic KOH prior to ethylation of the monomethylmercuric ion and subsequent chromatographic separation. As the various Hg compounds eluted from the gas chromatograh, they were thermally converted to elemental Hg, concentrated via amalgamation on a gold trap, thermally desorbed, and detected by atomic fluorescence spectroscopy. Se was analyzed by atomic absorption using 2 methods: samples submitted to the lab in 2007 used graphite furnace atomic absorption (Method 7740, US EPA 1986) with a MDL of 0.5 mg/kg dw; samples submitted to the lab in 2008 used gaseous hydride atomic absorption (Method 7742, US EPA 1994b) with a MDL of 0.02 mg/kg dw.

All trace element analyses followed quality control measures as specified by the EPA methods used. Quality control measures for MeHg followed the requirements in EPA Method 1630, but were adapted to tissue as Method 1630 is written for aqueous samples. For the Standard Reference Materials, the laboratory analyzed National Research Council of Canada DORM-2 and DOLT-3 for all analytes

TABLE 1. Results of Two-Way ANOVA and backwards, stepwise General Linear Model, using age class and region as categorical variables and year as a continuous variable. NS indicates not significant where $P \geq 0.05$. Blank cells show variables that were not included in the final GLM model because they were not significant ($P \geq 0.05$). MeHg was not analyzed in the past studies and is not included in the GLM.

	Two-Way ANOVA				General Linear Model[a]				
Metals	$n =$	Age class	Region	Age class *region	$n =$	R^2	Age class	Region	Year
As	30	0.00	0.00	0.02	43	0.45	0.00	0.02	
Cu	30	NS	NS	NS	57	0.10		0.02	
Pb	30	NS	NS	NS	57	0.64	0.00		0.00
THg	30	0.00	NS	NS	57	0.59	0.00		
MeHg	23	0.00	0.00	0.02					
Se	30	0.00	0.02	0.04	43	0.43	0.00		
Ag	30	0.00	0.00	0.01	57	0.28	0.00	0.01	
Zn	30	NS	NS	NS	57	0.39	0.00		

[a] Includes samples from Calambokidis and others (1984, 1991).

other than MeHg. Only DOLT-3 was analyzed for MeHg. Method blanks, matrix spikes, and standard reference materials were analyzed with each analytical batch and all fell within normal control limits with the exception of one duplicate showing Al and one showing THg with above normal relative percent differences (RPDs). All concentrations in text and tables are reported in dry weight (dw) units. For comparison to publications reported in wet weight (ww) units, we converted our results to ww using the percent solids of each sample provided by CAS. Mean percent solids of the samples was 28%.

Statistical Analysis

We used a Two-Way ANOVA in Systat Statistical and Graphing Software (Systat Software, Inc., San Jose, CA) to examine differences in element concentrations by age class, region, and their interaction effects (age class*region). Only adult and neonate pups had suitable sample sizes for these comparisons. Residual plots, Shapiro-Wilk, and Bartlett's tests were used to evaluate data for normal distribution, heterogeneity of variances, and outliers; all data were transformed prior to analysis with a transformation of log10(x+1). Due to a high number of samples that fell below detection limits, Al, Cd, and Ni concentrations were examined using Fisher's exact tests in the program R (R Development Core Team 2008) to compare the proportion of detected and non-detected samples by age class and region. To control for interaction effects between variables in the Fisher's exact tests, age class was compared using adult and neonate seals from SPS only and region was compared using neonates only.

Data from the current study was compared to 2 past studies (Calambokidis and others 1984, 1991) using a backwards, stepwise, General Linear Model (GLM) in Systat. Age class and region were modeled in the GLM as categorical variables and year was used as a continuous variable. Samples from the past studies included adults (Calambokidis and others 1984, $n = 2$), subadults aged 1 to 3 y (Calambokidis and others 1984, $n = 5$), weaned pups (Calambokidis and others 1984, $n = 5$), and neonate pups (Calambokidis and others 1984, $n = 2$; Calambokidis and others 1991, $n = 12$). All age classes were modeled as separate categories. All data were transformed as described above. Aluminum, Cd, and Ni were not analyzed in the GLM due to the high number of non-detected samples from the current sample set. The earliest publication, Calambokidis and others 1984, did not include As, Ni, or Se.

RESULTS

Age Class

Concentrations of As, THg, MeHg, Se, and Ag were significantly higher in adults than in neonate pups (Two-Way ANOVA, Table 1). When we included samples from past analyses, Pb and Zn also differed significantly by age class (GLM, Table 1), where Pb increased and Zn decreased with age. We found that among seals from SPS, adults were significantly more likely than pups to have detectable levels of Cd

TABLE 2 Trace element concentrations (mg/kg dw) in liver of stranded Harbor Seals collected 2004 to 2007. ND = not detected; NA = sample not analyzed; A = adult, PW = weaned pup, PN = neonate pup.

Sample	Age	Region	Al	As	Cd	Cu	Pb	Hg	MeHg	Ni	Se	Ag	Zn
CRC-583	PN	SJF	1.2	0.54	0.01	58	0.34	1.2	0.45	0.37	4	0.14	318
CRC-586	PN	SJF	2.9	1.2	ND	167	0.25	7.4	2.4	0.17	3.6	1.5	656
CRC-596	PN	SJF	0.5	0.65	ND	52	0.02	0.57	0.48	0.04	3.8	0.23	339
CRC-622	PN	SJF	10	0.35	ND	25	0.015	0.89	NA	ND	4.5	0.08	211
CRC-629	PN	SJF	26	0.92	ND	169	0.014	3.2	NA	0.035	5.3	0.63	745
CRC-636	PN	SJF	1	0.82	ND	62	0.017	1.5	0.59	0.03	3.5	0.52	206
CRC-640	PN	SJF	1.6	1.4	ND	20	0.052	19	NA	0.03	2.9	1	359
CRC-648	PN	SJF	74	0.75	0.03	20	0.021	3.2	NA	0.03	3.1	0.17	404
CRC-649	A	SJF	ND	5	230	101	0.068	476	6.1	0.12	201	3.5	229
CRC-817	A	SPS	0.7	1.5	5	44	0.067	127	2.1	0.13	35	0.82	286
CRC-825	A	SPS	0.7	0.25	0.55	4.1	0.02	0.85	0.36	0.04	0.52	0.012	21
EI07-01	A	SPS	ND	0.74	1	20	0.024	45	0.67	ND	7.6	0.2	52
GI04-09	PN	SPS	0.5	0.52	0.01	6.4	0.036	1.08	NA	ND	2.4	0.047	77
GI04-28	PN	SPS	ND	0.34	ND	18	0.034	0.78	NA	0.05	3.7	0.074	199
GI06-12	PN	SPS	20	0.49	ND	28	0.02	2	0.56	0.03	3.1	0.21	259
GI06-16	A	SPS	ND	4	3.9	40	0.073	516	0.84	0.04	163	1.5	164
GI06-17	PN	SPS	0.8	0.36	ND	37	0.018	2.5	0.69	ND	2.7	0.21	271
GI06-20	PN	SPS	19	0.44	ND	28	0.012	0.69	0.5	0.07	2.4	0.09	136
GI06-25	PN	SPS	1.5	0.34	ND	132	0.014	2.4	0.46	ND	2.5	0.42	465
GI06-55	PN	SPS	0.5	0.3	ND	161	0.037	2.2	NA	ND	3	0.2	598
GI06-58	PW	SPS	ND	0.36	ND	74	0.022	1.2	NA	ND	2	0.2	273
GI07-01	A	SPS	2	0.6	2.4	53	0.03	68	1.2	0.06	13	0.29	270
GI07-23	A	SPS	4	0.92	1.1	13	0.039	32	0.82	0.05	6.4	0.16	45
GI07-29	A	SPS	0.4	2.2	3.1	37	0.04	314	2.8	0.08	93	1.4	165
GI07-39	A	SPS	ND	0.86	1.7	34	0.048	27	1.6	0.1	3.5	0.14	168
GI07-43	A	SPS	ND	0.79	3.3	29	0.062	26	1.7	0.18	6.4	0.17	180
GI07-44	A	SPS	0.5	1.6	1.3	34	0.061	105	2.3	0.06	23	0.72	222
WDFW0207-03	A	SPS	ND	0.91	0.59	17	0.31	20	0.7	0.14	2.5	0.082	43
WDFW0607-31	A	SPS	ND	1.2	0.46	46	0.029	56	1.6	0.07	11	0.22	124
WDFW1107-08	A	SPS	1	1.2	1.8	41	0.04	104	1.3	0.08	32	0.65	108
WDFW1207-01	A	SPS	ND	1.4	4.8	36	0.04	198	2.1	0.06	51	0.49	125

and Ni (Fisher's exact test, $P = 0.00$ and $P = 0.03$, respectively). A Cd concentration of 230 mg/kg was measured in a single adult female Harbor Seal in this study. This value is a high outlier as compared to other adult seals collected that had a range of 0.46 to 5 mg/kg of Cd (Table 2).

Region

Concentrations of As, MeHg, Se, and Ag were significantly higher in SJF than in SPS seals (Two-Way ANOVA, Table 2). When pooled with past samples, concentrations of Cu were also significantly higher in SJF than SPS seals, whereas Se was no longer significantly different between regions (GLM, Table 2).

Year

When pooled with past samples, concentrations of Pb differed significantly by year (GLM, Table 2), where Pb concentrations have significantly decreased over time. Controlling for age, the mean concentration from 2 neonate pups collected in the earliest publication (Calambokidis and others 1984) was 2-times greater than mean concentrations of neonates from 1990 (Calambokidis and others 1991, $n = 12$) and 4-times greater than the mean of neonates from the present study ($n = 15$).

Mercury, Selenium, and Methylmercury Ratios

We calculated the molar ratio of THg to Se (THg:Se) and the ratio of THg to MeHg (THg:MeHg) for Harbor Seal adults and neonates from the present study. The majority of adult Harbor Seals in this study (13 of 15) had THg:Se ratios >1 (Fig. 1a), although seals with the highest THg concentrations (476 and 516 mg/kg) had THg:Se ratios closest to 1 (0.93 and 1.25, respectively). Pups had higher concentrations of MeHg than adult seals (Table 3) and correspondingly higher ratios of MeHg to THg (Fig. 1b).

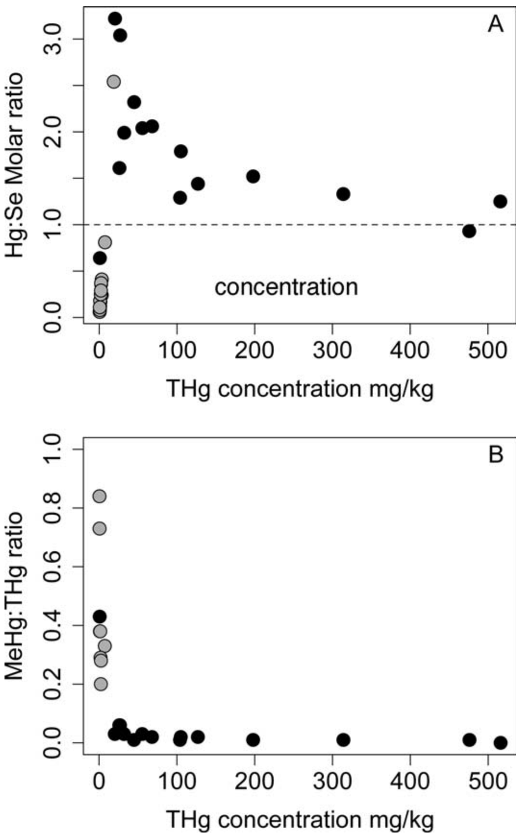

FIGURE 1. A: molar ratio of mercury to selenium (Hg:Se); and B: ratio of methylmercury to total mercury (MeHg:THg), relative to concentration of total mercury (THg) in Harbor Seal liver. ● = Adult; ○ = Pup.

DISCUSSION

Our findings were consistent with past literature reporting that elements such as As, Cd, Hg, MeHg, Se, and Ag increased with age and prey preference (Law 1996; Kubota and others 2001; Saeki and others 2001). Zinc, which is required for tissue development and proper immune function, is commonly reported to be higher in young animals (Robbins 1993), as was illustrated by pooling the Harbor Seals from this and past studies.

The regional differences we observed in As concentrations in Harbor Seals may be related to changes in local availability. The largest current source of As in the Puget Sound basin is from point-source air emissions, although other inputs include natural sources such as weathering of rocks and soil, and historic sources such as remnants from the Asarco smelter (Ecology and King County 2011). Given that regional differences in As concentrations were not observed in seals in the past (Calambokidis and others 1991), the large-scale and ongoing cleanup of the Asarco Smelter Plant in SPS (Ecology 2012) could be contributing to the significantly lower concentrations of As in SPS seals compared to SJF seals.

Similarly, the regional differences we noted in Cu and Ag could reflect on-going regional emissions trends. Copper contaminants in the Puget Sound are primarily attributed to surface runoff (Ecology and King County 2011); however, runoff from boat yards can also lead to increased concentrations of Cu in marine waters and sediments due to weathering of antifouling paints used on boats (Johnson and others 2006; Johnson 2007). Consistent with our findings in Harbor Seals, Cu concentrations in bottom sediments collected from a boat yard fallout site in SJF (Port Townsend Marina, Port Townsend, WA) were more than 6-times greater than those from SPS (Swanton Boatworks, Olympia, WA) (Johnson and others 2006).

For Ag, regional differences in concentrations were previously reported in neonate Harbor Seals (Calambokidis and others 1991), but few other regional comparisons are available. Concentrations of Ag in Harbor Seals from this study appear within range of current Ag concentrations measured in sediment from north, central, and south Puget Sound (Long and others 1999, 2000, 2002). In marine mammals, concentrations of Ag have been correlated with concentrations of Se, which, similar to its association with Hg, appears to reduce the toxicity of Ag (Saeki and others 2001; Ikemoto and others 2004). It is possible that the higher concentrations of Ag observed in SJF seals are associated with the higher concentrations of Se observed in SJF seals (Table 2).

The observed decrease in Pb concentrations over time mirrors the decline of Pb in sediments following bans on products such as leaded gasoline and lead paint during the 1970s to 1990s (Lefkovitz and others 1997). In contrast to past studies that have documented higher Pb concentrations in juvenile than adult Harbor Seals (Agusa and others 2011), we found that when pooled with past samples Pb concentrations were

TABLE 3. Mean concentrations (mg/kg dw) ± standard deviations and range of trace elements in stranded Harbor Seals (2004–2007), separated by age and region. Means for Al, Cd, and Ni are not presented due to the number of samples that fell below the detected limit. Means do not include weaned pup, GI06-58. AD = adult; PN = neonate pup.

Test	As	Cu	Pb	Hg	MeHg	Se	Ag	Zn
Age class								
AD (n = 15)	1.5±1.3 (0.25–5)	37±22 (4.1–101)	0.064±0.071 (0.02–0.31)	141±165 (0.85–516)	1.7±1.4 (0.36–6.1)	43±62 (0.52–201)	0.69±0.90 (0.012–3.5)	147±84 (21–286)
PN (n = 15)	0.63±0.34 (0.3–1.4)	66±60 (6.4–169)	0.059±0.096 (0.012–0.34)	3.2±4.6 (0.57–19)	0.77±0.68 (n=8) (0.45–2.4)	3.4±0.82 (2.4–5.3)	0.37±0.40 (0.47–1.5)	349±194 (77–745)
Region								
SPS (n = 21)	1.0±0.86 (0.25–4)	41±38 (4.1–161)	0.05±0.062 (0.012–0.31)	79±127 (0.69–516)	1.2±0.73 (n=18) (0.36–2.8)	22±39 (0.52–163)	0.39±0.42 (0.012–1.5)	189±140 (21–598)
SJF (n = 9)	1.3±1.4 (0.35–5)	75±59 (20–169)	0.088±0.12 (0.014–0.34)	57±157 (0.57–479)	2.0±2.4 (n=5) (0.45–6.1)	26±66 (2.9–201)	0.86±1.1 (0.08–3.5)	385±193 (206–745)
Neonates								
SPS (n = 7)	0.39±0.085 (0.3–0.52)	59±61 (6.4–161)	0.024±0.11 (0.012–0.037)	1.6±0.77 (0.69–2.5)	0.55±0.10 (n=4) (0.46–0.69)	2.8±0.48 (2.4–3.7)	0.18±0.13 (0.047–0.42)	286±185 (77–598)
SJF (n = 8)	0.82±0.33 (0.35–1.4)	72±62 (20–169)	0.090±0.13 (0.014–0.34)	4.6±6.1 (0.57–19)	0.99±0.97 (n=4) (0.45–2.4)	3.8±0.77 (2.9–5.3)	0.53±0.50 (0.08–1.5)	405±196 (206–745)

significantly higher in adults than in pups. Among the seals from the current study, the means and ranges of adults and pups appear similar (Table 3); however, with outliers removed (0.25 and 0.34 mg/kg in neonates, and 0.31 mg/kg in 1 adult), the amount of Pb present in adults was nearly double that of pups ($\bar{x}$ = 0.046, s = 0.018 adults; $\bar{x}$ = 0.024, s = 0.012 neonates).

Total mercury concentrations in Harbor Seals from this study were within ranges of concentrations reported in stranded and harvested Harbor Seals from other areas of the US, including Alaska (Miles and others 1992; Marino and others 2011), California (Brookens and others 2007, 2008), and the Northeast (Lake and others 1995; Shaw 2002). One adult Harbor Seal stranded in California in 1971 had a higher liver Hg concentration (700 mg/kg ww) than reported in the previously listed publications; however, the possibility of sample contamination was cited (Anas 1974). High accumulation of Hg as seen in adult seals is consistent with the pervasiveness of this contaminant in Puget Sound fish (West and others 2001) and sediments, which exceed state sediment standards at multiple locations throughout Puget Sound (Long and others 1999, 2000, 2002; Ecology and King County 2011).

Seals and other marine mammals have several detoxification mechanisms to help alleviate the toxic effects of non-essential trace elements (for examples see, Saeki and others 2001; Das and others 2001; Brookens and others 2008), including demethylation of organic MeHg (Ikemoto and others 2004). Therefore, rather than using contaminant concentrations to indicate health hazards, the focus often falls on the balance and interactions between elements. Marine mammals with high liver Hg concentrations have often been reported to have a 1:1 Hg:Se molar ratio in the liver, as Se acts to detoxify demethylated inorganic mercury (IHg) by forming mercury selenide (HgSe) complexes (Ikemoto and others 2004). It has been suggested that detoxification by Se is triggered by some threshold amount of Hg (Woshner and others 2001). It is likely that THg in the majority of adult seals in this study had not yet reached threshold levels and therefore only the animals with the highest THg concentrations had molar ratios close to 1:1. In pups this detoxification mechanism is not fully developed (Wagemann and others 1988) and, as observed in previous studies (Brookens and others 2007), neonates in this study had higher ratios of toxic MeHg to THg than observed in adults.

The higher concentrations of Se in SJF seals could allow SJF pups to better cope with high maternal MeHg transfer. Mercury concentrations in neonates reflect maternal transfer of MeHg (the form of Hg that transfers through the food chain) via the placenta and milk (Wagemann and others 1988; Law 1996). One adult female collected from SJF had the highest MeHg concentration measured in this study (6.1 mg/kg). This female was lactating and recently post-partum, suggesting that her pup was exposed to high concentrations of MeHg. Future investigations of MeHg concentrations in adult females could be useful for indicating the risk of MeHg toxicity to neonate pups and for understanding regional differences in pup mortality rates (Steiger and others 1989).

Conclusions

The observed regional and temporal differences in trace element concentrations detected in Harbor Seals in this study speak to their usefulness as indicators of concentrations in local ecosystems. Tissue samples are more easily collected from dead stranded than from live seals and can include internal organs, such as liver, that reflect long-term accumulation. Continued monitoring of essential and non-essential trace elements in stranded seals can be useful for detecting changes and long-term trends in contamination of marine wildlife.

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