

Trace Element Concentrations in Liver of 16 Species of Cetaceans Stranded on Pacific Islands from 1997 through 2013

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Abstract The impacts of anthropogenic contaminants on marine ecosystems are a concern worldwide. Anthropogenic activities can enrich trace elements in marine biota to concentrations that may negatively impact organism health. Exposure to elevated concentrations of trace elements is considered a contributing factor in marine mammal population declines. Hawai'i is an increasingly important geographic location for global monitoring, yet trace element concentrations have not been quantified in Hawaiian cetaceans, and there is little trace element data for Pacific cetaceans. This study measured trace elements (Cr, Mn, Cu, Zn, As, Se, Sr, Cd, Sn, Hg, and Pb) in liver of 16 species of cetaceans that stranded on U.S. Pacific Islands from 1997 to 2013, using high resolution inductively coupled plasma mass spectrometry (HR-ICP-MS) ($n = 31$), and direct mercury analysis atomic absorption spectrometry (DMA-AAS) ($n = 43$). Concentration ranges ($\mu\text{g/g}$ wet mass fraction) for non-essential trace elements, such as Cd (0.0031–58.93) and Hg (0.0062–1571.75) were much greater than essential trace elements, such as Mn (0.590–17.31) and Zn (14.72–245.38). Differences were found among age classes in Cu, Zn, Hg, and Se

concentrations. The highest concentrations of Se, Cd, Sn, Hg, and Pb were found in one adult female false killer whale (*Pseudorca crassidens*) at concentrations that are known to affect health in marine mammals. The results of this study establish initial trace element concentration ranges for Pacific cetaceans in the Hawaiian Islands region, provide insights into contaminant exposure of these marine mammals, and contribute to a greater understanding of anthropogenic impacts in the Pacific Ocean.

Trace element contamination is a complex environmental and human safety concern. Trace elements (TEs) occur naturally in marine environments at very low concentrations while anthropogenic activities can enrich trace elements in marine ecosystems to high concentrations with deleterious results. TEs are not naturally geographically homogenous, and even small inputs into an ecosystem from human activities can cause large increases in the local biota due to bioaccumulation and biomagnification (Bard 1999; Das et al. 2003b). In the Pacific Ocean, trace elements are enter the marine environment in runoff from rural, industrial, and urbanized landmasses; through shipping activities; as a result of coal fire power plant fallout from Asia; and as residues produced from volcanic activity.

Many TEs have essential biological roles, and excess and/or deficiency can cause serious health effects. For example, manganese deficiency can lead to reproductive impairment, abnormal growth and development, abnormalities in bone and cartilage, and impaired glucose regulation (Hurley and Keen 1987). Copper is important in iron mobilization (Frieden 1980), is a catalyst in the peroxidation of membrane lipids (Stohs and Bagchi 1995), and is essential to cardiovascular health (Lynes et al. 2007). Zinc is essential for many cellular functions, and is the

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second most abundant trace element in mammals after iron (King 2006). Zinc is an important antioxidant (Stohs and Bagchi 1995) and plays a role in spermatogenesis, vitamin A metabolism, insulin regulation, energy metabolism, protein synthesis, cellular division, DNA transcription regulation, and the stabilization of macromolecules (Salgueiro et al. 2000). Selenium (Se) is an essential element that is a key component of many proteins and metalloenzymes. Selenium also is known to be closely involved with mercury detoxification (Kaneko and Ralston 2007).

Some TEs have no known essential biological role and can be toxic dependent on concentration and chemical species. For instance, cadmium interferes with calcium and vitamin D metabolism in the kidneys and in bone (Goyer 1997) and interferes with iron and Mn uptake and metabolism. Elevated Cd exposure also can cause immune deficiencies, neurological damage, hepatic and pulmonary systems impairment, testicular injury, and cancer (Klaassen et al. 1999; Shanker 2008). Mercury is the most widely studied nonessential trace element and in the methylated form (MeHg) it is a highly toxic neurotoxin. Mercury poses serious a public health concern when it is elevated in the marine environment and accumulates in commercially important marine organisms (Boening 2000). Lead is an omnipresent toxic metal with a long history of anthropogenic use and adversely affects cognitive and neurological development at even very low concentrations of exposure (Shanker 2008).

Some toxic trace elements are persistent in the marine environment and have the ability to accumulate in tissues and biomagnify in marine food webs (Anan et al. 2001; Dietz et al. 1998; Mackey et al. 1995; Zhao et al. 2013). As apex predators, many marine mammal species are exposed to high concentrations of anthropogenic contaminants, which increase their likelihood of experiencing negative health effects (Bossart 2011; Costa et al. 2012; Dietz et al. 1998). Trace element toxicity is known to cause a myriad of sublethal and lethal effects in marine mammals, such as suppression of the immune system, neurotoxicity, and general reduced fitness (De Guise et al. 1995; Kakuschke and Prange 2007; Lavery et al. 2009; Lynes et al. 2006, 2007; Pellisso et al. 2008; Siebert et al. 1999). The health status of marine mammals, such as cetaceans, is of global concern. Some marine mammal species are good sentinels for human health, because they consume many of the same species of fish caught by commercial fisheries for human consumption and share similar life history traits, such as long lifespan, low reproductive potential, late maturity, and high trophic level, which can make them particularly susceptible to the negative impacts of anthropogenic activities (Fair and Becker 2000).

In the U.S. Pacific Islands region, there have been very few studies examining anthropogenic contaminants in marine mammals. These studies are limited to persistent

organic pollutants in endangered Hawaiian monk seals (*Monachus schauinslandi*), endangered insular false killer whales (*Pseudorca crassidens*), and 16 species of stranded cetaceans (Bachman et al. 2014; Lopez et al. 2012; Willcox et al. 2004; Ylitalo et al. 2008, 2009). Trace element information for most marine mammal species across the Pacific is deficient and has limited sample sets. While trace elements have been examined extensively in stranded cetaceans in the Mediterranean Sea where Hg and other anthropogenic contaminants are elevated, there is far less data from other regions of the world. To date, trace element concentrations in U.S. Pacific Island associated marine mammal species have not been examined.

The purpose of this study was to examine in liver tissue a suite of trace elements of dietary and toxicological importance in a variety of cetacean species that have stranded on U.S. Pacific Islands. As a storage and detoxification organ, the liver is important in the sequestration of toxic nonessential trace elements and the homeostatic regulation of essential trace elements, making it the ideal tissue for trace element analysis in marine mammals. Trace element concentrations in liver were related to animal life history information. Differences were expected among age classes for essential trace element concentrations due to the dilution effect of growth and nonessential trace element concentrations as a result of bioaccumulation. Concentration differences also were expected among phylogenetically distinct groups due to differences in diet preference and foraging niche. Comparing trace element concentrations measured in this study to concentrations from studies in other regions, lower concentrations were expected in Pacific Island stranded cetaceans due to the remoteness of this region. Finally, results were used to assess the potential for negative health effects from nonessential trace elements in individual animals with concentrations above known effect thresholds.

Materials and Methods

Sample Collection and Processing

The Hawai'i Pacific University Marine Mammal Stranding Response Program provided liver samples from their sample archive for this study (NOAA permit#932-1905). All samples were from code 1 and 2 animals (live stranded or fresh dead, respectively) to minimize concerns associated with sample decomposition. Trace elements were measured in liver tissue collected from 1997 to 2013 in 16 cetacean species that stranded in the main Hawaiian Islands ($n = 41$), Guam ($n = 1$), and Saipan ($n = 1$) (Fig. 1; Table 1). Samples analyzed for all trace elements were collected from animals that stranded from 1997 to 2011 ($n = 31$) and for

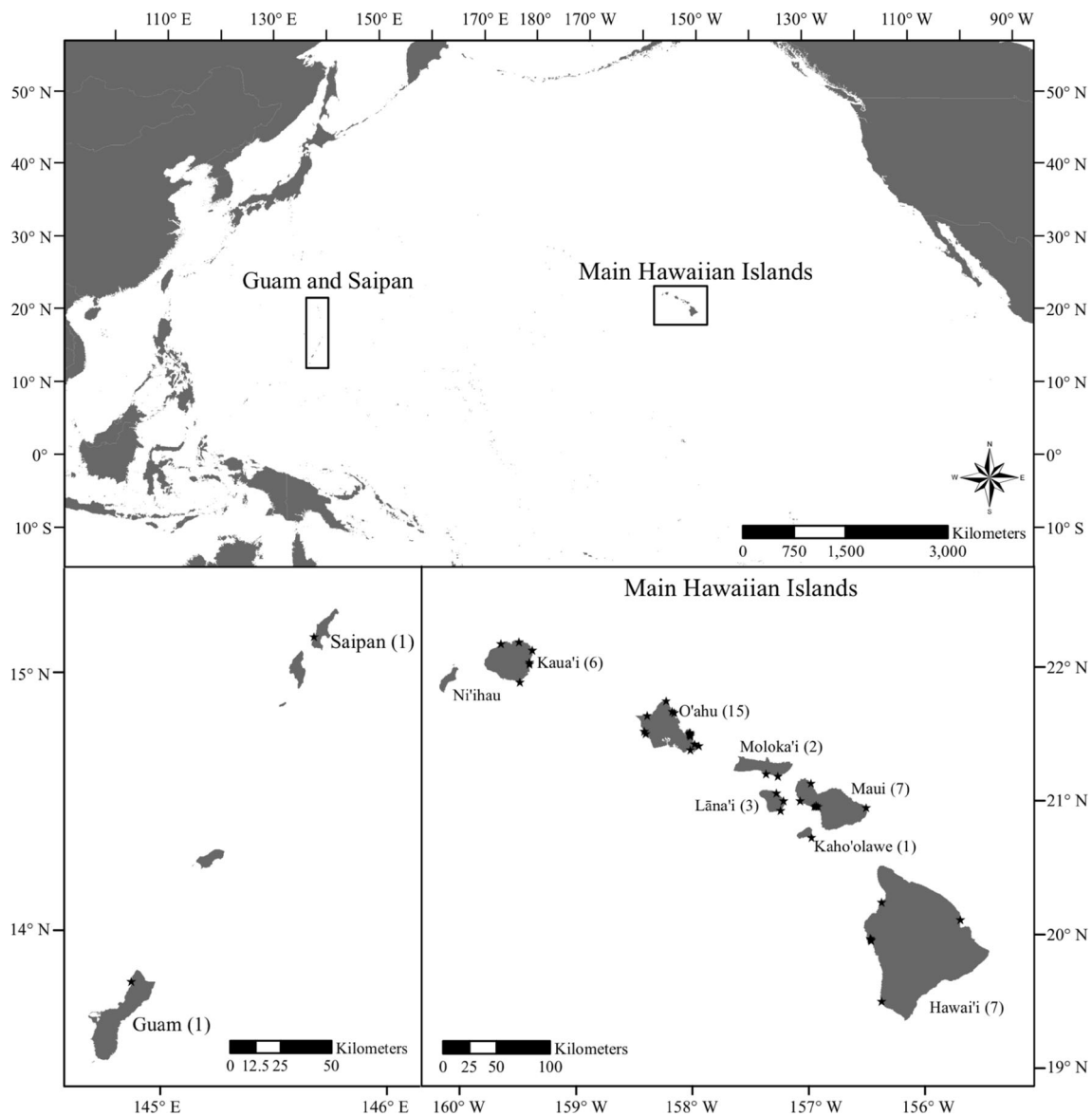


Fig. 1 Stranding locations of cetaceans in this study. Stars in the lower panels indicate the individual cetacean stranding locations and the number in parentheses indicates the number of strandings on each island

mercury (Hg) from 1997 to 2013 ($n = 43$). All 43 strandings were single animal events. Age class was estimated at the time of necropsy from visual observations of umbilicus, genital development, and total length: calf, unweaned animals that were nutritionally dependent on their mothers; juvenile, animals that were nutritionally independent but sexually immature; adult, sexually mature animals. Liver samples were removed and subsampled during necropsies with stainless-steel instruments and cut on polyethylene cutting boards. Tissue subsamples were individually wrapped in aluminum foil and stored at -20 or -80 °C until shipped for homogenization and analysis at the National Institute of Standards and Technology (NIST), Hollings Marine Laboratory, Charleston, SC.

Prior to homogenization, frozen liver samples were trimmed, rinsed with high purity deionized water (resistivity = $18 \text{ M}\Omega\text{-cm}$), placed in clean glass Petri dishes, and cut into smaller pieces that were suitable to fit inside the homogenization vials. Individual liver samples were cryogenically homogenized to produce a uniform sample composition of fresh frozen powder with a bench top homogenizer freezer/mill (SPEX SamplePrep, Metuchen, NJ). Samples were placed into a liquid nitrogen chilled vial with a stainless steel impactor, capped, placed in the mill, submerged in liquid nitrogen, and shaken at 10 Hz for 3 min. Homogenized powder was transferred into 15 mL acid cleaned polypropylene jars, and stored at -80 °C until analysis.

Table 1 Summary of sampled cetaceans, stranded on U.S. Pacific Islands 1997–2013

Species	Common name	Phylogenetic group	Sample ID	Sex	Age class	Stranding location	Year
<i>Feresa attenuata</i>	Pygmy killer whale	Dolphin	KW2009006	M	A	Maui	2009
<i>Indopacetus pacificus</i>	Longman's beaked whale	Beaked	KW2010005	M	J	Maui	2010
<i>Kogia breviceps</i>	Pygmy sperm whale	Sperm	15320-001	M	J	Kaua'i	2000
<i>Kogia sima</i>	Dwarf sperm whale	Sperm	15377-001	M	A	O'ahu	2000
<i>Megaptera novaeangliae</i>	Humpback whale	Baleen	KW2009012	M	A	Kaua'i	2009
			15063-001	F	C	Lāna'i	1998
			KW2013001	F	C	Lāna'i	2013
			KW2013002	M	C	O'ahu	2013
			KW2013007	M	C	O'ahu	2013
<i>Mesoplodon densirostris</i>	Blainville's beaked whale	Beaked	KW2013010	F	C	Lāna'i	2013
			KW2010012	M	J	Maui	2010
			KW2008010	M	A	Kaua'i	2008
<i>Orcinus orca</i>	Killer whale	Dolphin	KW2008010	M	A	Kaua'i	2008
<i>Peponocephala electra</i>	Melon-headed whale	Dolphin	KW2011002	M	C	O'ahu	2011
			KW2011011	M	A	Maui	2011
			KW2012003	M	A	O'ahu	2012
<i>Physeter macrocephalus</i>	Sperm whale	Sperm	KW2011008	F	C	O'ahu	2011
<i>Pseudorca crassidens</i>	False killer whale	Dolphin	KW2010019	F	A	Moloka'i	2010
<i>Stenella attenuata</i>	Pantropical-spotted dolphin	Dolphin	KW2009015	M	A	Hawai'i	2009
			KW2010011	M	C	Guam	2009
<i>Stenella coeruleoalba</i>	Striped dolphin	Dolphin	12470-001	M	A	O'ahu	1997
			KW2008006	F	C	Hawai'i	2008
			KW2009008	M	J	O'ahu	2009
			KW2009009	F	A	Maui	2009
			KW2009011	F	C	Maui	2009
			KW2010008	M	A	Hawai'i	2010
			KW2012002	F	A	O'ahu	2012
			KW2013006	M	J	O'ahu	2013
<i>Stenella longirostris</i>	Spinner dolphin	Dolphin	15028-001	M	C	O'ahu	1997
			KW2007004	M	J	O'ahu	2007
			KW2008004	M	A	Maui	2008
			KW2008009	F	A	Hawai'i	2008
			KW2008011	F	A	Hawai'i	2008
			KW2009004	F	C	O'ahu	2009
			KW2010006	M	J	O'ahu	2010
			KW2011013	M	C	Hawai'i	2011
			KW2011018	F	C	O'ahu	2011
			KW2013009	M	J	Hawai'i	2013
<i>Steno bredanensis</i>	Rough-toothed dolphin	Dolphin	KW2011021	M	A	Kaua'i	2011
<i>Tursiops truncatus</i>	Bottlenose dolphin	Dolphin	15121-001	M	C	O'ahu	1998
			KW2011001	F	J	Kaua'i	2011
			KW2011007	M	A	Kaua'i	2011
<i>Ziphius cavirostris</i>	Cuvier's beaked whale	Beaked	KW2008008	M	J	Moloka'i	2008
			KW2011016	M	A	Saipan	2011

M male, *F* female, *C* calf, *J* juvenile, *A* adult

Analytical Methods

Sample Preparation

Acid-assisted microwave digestion using PTFE pressurized vessels was utilized to digest liver samples, reference materials (RMs), and procedural blanks prior to performing trace element analysis (Bryan et al. 2012). The internal standard added to each sample contained Eu, Ru, Sc, and Y (NIST SRM 3100 series single-element standard solutions, Gaithersburg, MD). Sample digests were quantitatively transferred to 50-mL, acid-cleaned polypropylene centrifuge tubes and diluted to approximately 50 g using high-purity deionized water. Half of each sample solution was then transferred into another acid-cleaned, 50-mL polypropylene centrifuge tube, spiked with the multielement custom spike solutions, and each tube was diluted back to approximately 50 g with high-purity deionized water. For Hg measurements, tissue samples were aliquotted directly into nickel weigh boats and weighed. Some cetacean liver samples had Hg concentrations that were above the instrument detection window and required dilution. These samples were microwave digested and diluted following the same digestion procedures as above prior to Hg analysis and pipetted into quartz weigh boats for Hg analysis.

Calibration Methods and Sample Measurements

Trace element mass fraction measurements (except Hg) were collected using a Thermo high resolution inductively coupled plasma mass spectrometer (HR-ICP-MS) (Bremen, Germany) with a standard low-volume glass impact bead spray chamber (Peltier, cooled at +3 °C) and concentric glass nebulizer with the ICP-MS operating in low, medium, and high resolution. Single-point standard addition methods were used to measure multiple trace element mass fractions at the same time in cetacean liver samples (Christopher et al. 2005). This analytical quantification and validation scheme avoids matrix interferences by splitting a single sample and spiking one of the sample splits. The multielement custom spikes were prepared from NIST SRM 3100 series single-element standard solutions (NIST, Gaithersburg, MD).

The mass fraction of total Hg was measured by atomic absorption spectrometry (AAS) using a direct Hg analyzer (DMA 80, Milestone Scientific, Shelton, CT) with high purity oxygen as the carrier gas. External calibration methods were used to determine Hg mass fractions in samples (Bryan et al. 2012). QC03LH3 Pygmy Sperm Whale Liver Homogenate (NIST interlaboratory comparison exercise control material), SRM 1946 Lake Superior Fish Tissue, and SRM 3133 Mercury Standard Solution

(NIST, Gaithersburg, MD) were the RMs utilized to create the calibration curves. The slope and intercept from the calibration curves were used to calculate the Hg mass fraction in liver and RMs. Four cetacean liver samples were weighed and measured in triplicate to ensure sample homogeneity by mass fraction reproducibility (mean 4.3 % RSD; range 1.7–8.7 % RSD).

Quality Assurance

For multielement and Hg analysis methods, reference materials (RMs) were used to monitor measurement accuracy and precision by preparing and analyzing them alongside unknown liver samples. The liver and RM sample concentrations were corrected by subtracting the procedural blank concentrations. SRM 1577c Bovine Liver, SRM 1566b Oyster Tissue, and QC03LH3 Pygmy Sperm Whale Liver homogenate were processed and analyzed in triplicate concurrently with unknown samples for HR-ICP-MS multi-element measurement quality control. QC04LH4 White-sided Dolphin Liver Homogenate, QC03LH3 Pygmy Sperm Whale Liver Homogenate, and QC04ERM1 Egg Reference Material-1 were used as control materials and run concurrently each analytical batch for AAS measurement of Hg. Controls were chosen based on matrix and/or trace element concentrations that were similar to the cetacean liver samples in this study. Control measurements agreed well with the certified and reference values (Online Resource 1). Reported concentrations for all trace elements are presented on a wet mass fraction basis in $\mu\text{g/g}$.

Statistical Analysis

JMP 11.0 (2013, SAS Institute Inc. Cary, NC) and Excel (Microsoft Inc, Redmond, WA) were used for all computational and statistical analyses. Data were transformed as needed to meet assumptions for parametric tests. When data sets could not be transformed to meet assumptions, nonparametric tests were used. A simple regression analysis was used to investigate possible linear covariance between trace elements in liver tissue, with an alpha level of 0.05. Only one significant untransformed data pairing (Hg/Se) met the parametric assumptions for Pearson's correlation test, using the Shapiro–Wilk test of residual distribution. When all the data were natural log i_j transformed, a majority of the results met the parametric assumptions for Pearson's, and correlations that did not meet those assumptions were reanalyzed using Kendall's Tau nonparametric correlation test.

Trace element concentration means were organized categorically to examine differences among multispecies age class and sex groupings. Phylogenetically related

species also were compiled resulting in baleen whale, sperm whale, beaked whale, and dolphin groups (Messenger and McGuire 1998). Data were transformed as needed and the Shapiro–Wilk Goodness-of-Fit W test was used to confirm normal distribution (Online Resource 2). Levene's homogeneity of variances test was used to ensure parametric assumptions were met for two-way factorial ANOVAs conducted to look for interactions and differences among sample groups. The post hoc Tukey–Kramer honestly significant difference (HSD) tests were used to identify where the differences lie among individual groups and the nonparametric Steel–Dwass all pairs multiple comparisons test was conducted for Sn, Hg, and Hg/Se molar ratios that could not be normalized by transformation, and as a follow-up on the entire data set (Online Resource 2). To correct for multiple tests, an alpha level of 0.05 was adjusted with the Holm–Bonferroni simple sequential rejective multiple test procedure for each family of tests (Holm 1979).

Results and Discussion

Trace Element Concentrations in Stranded Cetaceans

Concentrations of 11 trace elements (Cr, Mn, Cu, Zn, As, Se, Sr, Cd, Sn, Hg, and Pb) were measured in liver samples of 43 individual cetaceans representing 16 species that stranded on U.S. Pacific islands (Tables 2 and 3). While there are some possible biases associated with obtaining samples from stranded cetacean, such as difficulty in identifying the population of origin, decomposition altering biomarker measurements, and health status not being representation of the population or community of presumably healthy animals, stranded cetaceans provide a valuable snapshot of contaminant exposure in multiple species of these rare and highly protected animals (Aguilar and Borrell 1994). To date, this is the first known marine mammal trace element study in this region, and the first study to measure these trace elements in liver of a Longman's beaked whale (*Indopacetus pacificus*).

There was a large degree of variability in concentrations of each trace element across the sample set (Fig. 2). Concentrations of nonessential and ultra-trace elements, such as Hg and Cd, spanned 2–6 orders of magnitude, while essential trace element concentrations, such as Zn and Cu, spanned 1–2 orders of magnitude with the exception of Se, which spanned 3 orders of magnitude. The lesser degree of variability in known essential trace element concentrations within the sample set was expected, because these elements are tightly regulated biologically. Homeostatic processes cause essential trace elements to have much shorter

biological half-lives than nonessential trace elements that accumulate with much greater interspecies variability (Mackey et al. 1995).

Correlations Between Trace Elements

Certain trace elements covaried and correlations were observed between many trace element pairs (Table 4). Most of the significant correlations were identified with Pearson's correlation analyses ($df = 30$, $\alpha = 0.05$) with a critical coefficient value of $r = 0.349$. Correlations that did not meet parametric assumptions were confirmed with nonparametric Kendall's Tau test (Cu/Zn, Cd/Sn, Cd/Pb, and Sn/Pb). Linear correlations between trace elements can occur, because they share the same metabolic or regulatory pathways, they chemically interact with one another, or they bioaccumulate via the same processes (Mackey et al. 1995, 2003).

Many of the correlations found between trace elements have been observed in other cetacean studies. The strong positive correlations observed between Sn/Se and Sn/Hg were similar to the positive correlations between these elements reported by Mackey et al. (2003) in rough-toothed dolphins (*Steno bredanensis*). They postulated that the observed correlation between Sn and Se could indicate that Se has a regulatory or protective detoxification role against Sn, or that this correlation is simply a linear covariance with age, which also is likely the case with the Sn/Hg correlation (Mackey et al. 2003). Because Sn is not well represented in cetacean trace element literature, the strong positive correlations found in this study of Cd/Sn and Pb/Sn have not been reported elsewhere, and likely indicate similar patterns of age-related accumulation. The positive correlations between Cd/Hg and Cd/Se found are consistent with many other studies and are generally thought to be age-dependent. However, shared detoxification mechanisms also could contribute to these patterns (Agusa et al. 2008; Caurant et al. 1994; Monaci et al. 1998; Seixas et al. 2007). A strong positive Hg/Pb correlation was observed, similar to that identified in striped dolphins (*Stenella coeruleoalba*) by Agusa et al. (2008).

Four negative correlations were observed that corroborate findings in other studies: Cu/Hg, Cu/Cd, Zn/Se, and Zn/Hg (Agusa et al. 2008; Caurant et al. 1994; Roditi-Elasar et al. 2003). The negative correlation between Cu and Cd could be indicative of a competitive protein binding interaction as the same metallothionein isoform involved in Cu regulation and homeostasis is responsible for Cd sequestration (Caurant et al. 1994; Roditi-Elasar et al. 2003). Copper and Zn may covary, because they both have similar homeostatic regulatory mechanisms (Caurant et al.

Table 2 Trace element concentrations ($\mu\text{g/g}$, wet mass) in liver tissue of U.S. Pacific Island stranded cetaceans, stranded 1997–2013

Sample ID	Cr	Mn	Cu	Zn	As	Se	Sr	Cd	Sn	Hg	Pb	Hg:Se
12470-001	0.36	3.91	5.98	27.32	0.19	66.71	0.13	4.72	0.51	156.27 ^a	0.025	0.92
15028-001	0.077	2.57	29.59	65.59	2.39	0.88	0.58	0.0032	0.0077	0.85	0.0022	0.38
15063-001	0.68	1.40	156.13	112.52	0.028	0.92	0.12	0.0031	0.0088	0.0062	0.010	0.003
15121-001	3.86	2.32	10.32	39.34	0.23	7.28	0.12	0.0050	0.010	2.28	0.014	0.12
15320-001	2.61	2.40	5.12	27.78	3.49	3.12	0.51	6.00	0.044	1.61	0.018	0.20
15377-001	1.82	17.31	4.33	53.05	1.59	12.84	2.77	22.70	0.42	22.00 ^a	0.11	0.67
KW2007004	1.61	3.30	10.05	44.73	1.06	14.36	0.086	5.90	0.025	24.61 ^a	0.010	0.67
KW2008004	0.38	2.54	5.37	51.06	0.21	10.75	0.067	2.14	0.016	26.60 ^a	0.0049	0.97
KW2008006	0.79	4.19	19.64	69.03	0.39	2.16	0.062	0.0067	0.010	3.15	0.0040	0.57
KW2008008	1.50	2.01	7.35	45.89	3.71	3.02	0.50	37.51	0.041	5.85	0.020	0.76
KW2008009	0.61	4.02	4.81	47.57	0.72	10.94	0.083	7.35	0.0084	21.53 ^a	0.0058	0.77
KW2008010	0.34	1.82	5.16	41.39	0.043	105.81	0.18	0.72	0.047	263.76 ^a	0.0027	0.98
KW2008011	0.16	2.04	3.39	29.72	0.61	14.25	0.11	2.63	0.014	29.40 ^a	0.0069	0.81
KW2009004	1.18	4.66	2.77	68.74	1.60	1.96	0.18	0.18	0.010	0.58	0.0066	0.12
KW2009006	0.20	2.49	1.90	14.72	0.21	58.84	0.12	1.40	0.054	142.28 ^a	0.013	0.95
KW2009008	0.32	2.81	6.00	30.86	1.04	25.14	0.11	8.30	0.064	54.92 ^a	0.010	0.86
KW2009009	0.10	2.40	4.67	23.83	0.25	79.27	0.17	13.54	0.13	198.18 ^a	0.021	0.98
KW2009011	0.86	4.68	7.69	76.62	1.25	4.04	0.065	2.93	0.49	5.84	0.0060	0.57
KW2009012	0.28	1.26	3.26	24.19	0.66	9.09	0.42	27.35	0.087	9.25	0.026	0.40
KW2009015	0.058	3.78	6.75	66.11	1.38	37.09	0.17	2.42	0.010	95.21 ^a	0.0067	1.01
KW2010005	0.16	2.63	3.53	61.54	0.27	0.85	0.072	0.024	0.013	1.33	0.0039	0.61
KW2010006	0.11	3.48	7.37	98.52	1.20	2.72	0.087	0.79	0.0052	0.62	0.0053	0.09
KW2010008	0.054	3.51	7.83	51.68	2.22	35.84	0.11	25.64	0.058	77.96 ^a	0.0096	0.86
KW2010011	1.32	0.59	43.89	25.99	1.21	2.18	0.039	0.0034	0.0056	0.62	0.0078	0.11
KW2010012	0.20	4.34	6.84	70.14	0.16	4.05	0.080	19.06	0.030	9.81 ^a	0.024	0.95
KW2010019	0.28	3.11	12.23	32.65	1.81	633.06	0.063	58.93	1.16	1571.75 ^a	0.20	0.98
KW2011001	0.43	5.80	6.47	102.65	2.11	1.65	0.20	0.48	0.0052	1.53	0.0039	0.36
KW2011002	0.20	2.15	3.52	32.01	1.35	1.23	0.14	0.0046	0.0099	1.82	0.014	0.58
KW2011007	0.29	2.71	3.93	70.56	0.24	34.22	0.19	12.61	0.023	89.44 ^a	0.012	1.03
KW2011008	0.24	1.31	13.68	245.38	0.26	0.85	0.083	0.0064	0.0058	1.90	0.0075	0.88
KW2011011	0.12	1.42	4.12	22.84	1.18	86.74	0.071	12.18	0.12	204.49 ^a	0.034	0.93
KW2011013	–	–	–	–	–	–	–	–	–	1.13	–	–
KW2011016	–	–	–	–	–	–	–	–	–	10.77	–	–
KW2011018	–	–	–	–	–	–	–	–	–	0.52	–	–
KW2011021	–	–	–	–	–	–	–	–	–	224.84	–	–
KW2012002	–	–	–	–	–	–	–	–	–	22.58	–	–
KW2012003	–	–	–	–	–	–	–	–	–	277.98	–	–
KW2013001	–	–	–	–	–	–	–	–	–	0.012	–	–
KW2013002	–	–	–	–	–	–	–	–	–	0.023	–	–
KW2013006	–	–	–	–	–	–	–	–	–	141.40	–	–
KW2013007	–	–	–	–	–	–	–	–	–	0.0066	–	–
KW2013009	–	–	–	–	–	–	–	–	–	11.78	–	–
KW2013010	–	–	–	–	–	–	–	–	–	0.022	–	–

^a Samples analyzed for total Hg after dilution

1994; Lemos et al. 2013; Roditi-Elasar et al. 2003), and opposing age-related accumulation patterns could account for each of these negative trace element correlations.

The strongest trace element correlation observed in this study was a positive correlation between Hg and Se in liver (Fig. 3). This correlation in marine mammal liver tissue

Table 3 Median (range) and mean \pm SE (or single measurement) of trace elements ($\mu\text{g/g}$, wet mass) in liver tissue of 16 species of stranded cetaceans

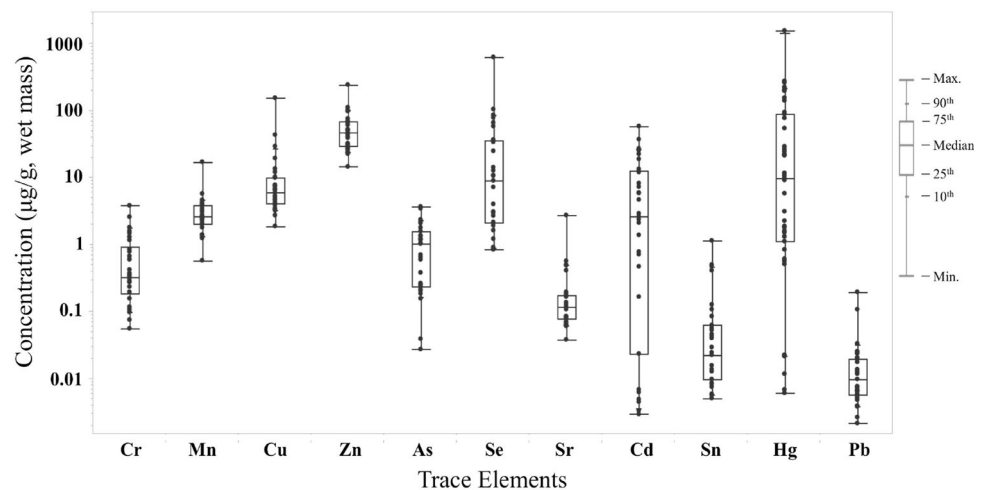
Common name	<i>n</i>	Cr	Mn	Cu	Zn	As	Se
Blainville's beaked whale	1	0.2	4.34	6.84	70.14	0.16	4.05
Bottlenose dolphin	3	0.43 (0.29–3.86)	2.71 (2.32–5.80)	6.47 (3.93–10.32)	70.56 (39.34–102.65)	0.24 (0.22–2.11)	7.28 (1.65–34.22)
Cuvier's beaked whale	TE = 1 Hg = 2	1.53 \pm 1.17 1.50	3.61 \pm 1.10 2.01	6.91 \pm 1.86 7.35	70.85 \pm 18.28 45.89	0.86 \pm 0.63 3.71	14.39 \pm 10.05 3.02
Dwarf sperm whale	2	1.05 (0.28–1.82)	9.28 (1.26–17.31)	3.80(3.26–4.34)	38.62 (24.19–53.05)	1.12 (0.66–1.59)	10.96 (9.09–12.83)
		1.05 \pm 0.77	9.28 \pm 8.02	3.80 \pm 0.54	38.62 \pm 14.43	1.12 \pm 0.47	10.96 \pm 1.87
False killer whale	1	0.28	3.11	12.23	32.65	1.81	633.06
Humpback whale	TE = 1 Hg = 5	0.68	1.40	156.13	112.52	0.03	0.92
Killer whale	1	0.34	1.82	5.16	41.39	0.04	105.81
Longman's beaked whale	1	0.16	2.63	3.53	61.54	0.27	0.85
Melon-headed whale	TE = 2 Hg = 3	0.16 (0.12–0.20)	1.78 (1.42–2.15)	3.82 (3.52–4.12)	27.42 (22.84–32.01)	1.26 (1.18–1.35)	43.99 (1.23–86.74)
		0.16 \pm 0.04	1.78 \pm 0.37	3.82 \pm 0.30	27.42 \pm 4.59	1.26 \pm 0.09	43.99 \pm 42.75
Pantropical-spotted dolphin	2	0.69 (0.06–1.32)	2.18 (0.59–3.78)	25.32 (6.75–43.89)	46.05 (25.99–66.11)	1.29 (1.21–1.38)	19.64 (2.18–37.09)
		0.69 \pm 0.63	2.18 \pm 1.59	25.32 \pm 18.57	46.05 \pm 20.06	1.29 \pm 0.09	19.64 \pm 17.46
Pygmy killer whale	1	0.20	2.49	1.90	14.72	0.21	58.84
Pygmy sperm whale	1	2.61	2.40	5.12	27.78	3.49	3.12
Rough-toothed dolphin	1	–	–	–	–	–	–
Sperm whale	1	0.24	1.31	13.68	245.38	0.26	0.85
Spinner dolphin	TE = 7 Hg = 10	0.38 (0.08–1.61)	3.30 (2.04–4.66)	5.37 (2.77–29.60)	51.06 (29.72–98.52)	1.06 (0.21–2.39)	10.75 (0.88–14.36)
		0.59 \pm 0.23	3.23 \pm 0.35	9.05 \pm 3.55	57.99 \pm 8.38	1.11 \pm 0.27	7.98 \pm 2.24
Striped dolphin	TE = 6 Hg = 8	0.34 (0.05–0.86)	3.71 (2.40–4.68)	6.85 (4.67–19.64)	41.27 (23.83–76.62)	0.71 (0.19–2.22)	30.49 (2.16–76.27)
		0.41 \pm 0.14	3.58 \pm 0.35	8.63 \pm 2.25	46.56 \pm 9.25	0.89 \pm 0.32	35.53 \pm 13.04
All samples	TE = 31 Hg = 43	0.32 (0.05–3.86)	2.63 (0.59–17.30)	6.00 (1.90–156.13)	47.57 (14.72–245.38)	1.04 (0.03–3.71)	9.09 (0.85–633.06)
		0.68 \pm 0.15	3.32 \pm 0.51	13.34 \pm 5.00	57.23 \pm 7.69	1.07 \pm 0.17	41.03 \pm 20.40
Common name	<i>n</i>	Sr	Cd	Sn	Hg	Pb	Hg:Se
Blainville's beaked whale	1	0.08	19.06	0.03	9.81	0.02	0.95
Bottlenose dolphin	3	0.19 (0.12–0.20)	0.48 (0.005–12.61)	0.01 (0.005–0.02)	2.28 (1.53–89.44)	0.01 (0.004–0.01)	0.36 (0.12–1.03)
		0.17 \pm 0.03	4.37 \pm 4.12	0.01 \pm 0.005	31.08 \pm 25.27	0.01 \pm 0.003	0.51 \pm 0.27
Cuvier's beaked whale	TE = 1 Hg = 2	0.50	37.51	0.04	8.31 (5.85–10.77)	0.02	0.76
					8.31 \pm 2.46		
Dwarf sperm whale	2	1.59 (0.42–2.77)	25.02 (22.70–27.35)	0.25 (0.09–0.42)	15.63 (9.25–22.00)	0.07 (0.03–0.10)	0.54 (0.40–0.67)
		1.59 \pm 1.18	25.02 \pm 2.33	0.25 \pm 0.17	15.63 \pm 6.4	0.07 \pm 0.04	0.54 \pm 0.14
False killer whale	1	0.06	58.93	1.16	1571.75	0.20	0.98
Humpback whale	TE = 1 Hg = 5	0.12	0.003	0.009	0.012 (0.006–0.023)	0.01	0.003
					0.01 \pm 0.004		

Table 3 continued

Common name	<i>n</i>	Sr	Cd	Sn	Hg	Pb	Hg:Se
Killer whale	1	0.18	0.72	0.05	263.76	0.003	0.98
Longman's beaked whale	1	0.07	0.02	0.01	1.33	0.004	0.61
Melon-headed whale	TE = 2	0.11 (0.01–0.14)	6.09 (0.005–12.18)	0.062 (0.01–0.12)	204.49 (1.82–277.98)	0.024 (0.01–0.034)	0.75 (0.58–0.93)
	Hg = 3	0.11 ± 0.04	6.09 ± 6.09	0.06 ± 0.05	161.43 ± 82.58	0.024 ± 0.010	0.75 ± 0.17
Pantropical-spotted dolphin	2	0.10 (0.04–0.17)	1.21 (0.003–2.42)	0.008 (0.006–0.01)	47.91 (0.61–95.21)	0.007 (0.007–0.008)	0.56 (0.11–1.01)
		0.10 ± 0.06	1.21 ± 1.21	0.008 ± 0.002	47.91 ± 47.30	0.007 ± 0.0005	0.56 ± 0.45
Pygmy killer whale	1	0.12	1.40	0.05	142.3	0.01	0.95
Pygmy sperm whale	1	0.51	6.00	0.04	1.61	0.02	0.20
Rough-toothed dolphin	1	–	–	–	224.84	–	–
Sperm whale	1	0.08	0.01	0.01	1.89	0.01	0.88
Spinner dolphin	TE = 7	0.09 (0.08–0.58)	2.14 (0.003–7.35)	0.01 (0.005–0.03)	6.45 (0.52–39.27)	0.006 (0.002–0.01)	0.67 (0.09–0.97)
	Hg = 10	0.17 ± 0.07	2.71 ± 1.08	0.012 ± 0.003	11.76 ± 3.94	0.006 ± 0.001	0.55 ± 0.13
Striped dolphin	TE = 6	0.11 (0.06–0.17)	6.51 (0.007–25.64)	0.10 (0.01–0.49)	77.96 (3.20–198.18)	0.01 (0.004–0.025)	0.86 (0.57–0.98)
	Hg = 8	0.11 ± 0.02	9.19 ± 3.80	0.21 ± 0.09	82.54 ± 26.33	0.012 ± 0.003	0.79 ± 0.07
All samples	TE = 31	0.12 (0.04–2.77)	2.63 (0.003–58.93)	0.02 (0.005–1.15)	9.81 (0.006–1571.75)	0.10 (0.002–0.20)	0.76 (0.003–1.03)
	Hg = 43	0.25 ± 0.09	8.89 ± 2.42	0.11 ± 0.04	86.43 ± 38.27	0.02 ± 0.007	0.65 ± 0.06

TE all trace elements measured, and Hg:Se ratio, Hg mercury only measured

Fig. 2 Summary of trace element liver concentrations in U.S. Pacific Island stranded cetaceans



was first reported by Koeman et al. (1973) and has since been observed in virtually every marine mammal liver trace element study. The Hg/Se correlation has been previously observed by Roditi-Elasar et al. (2003) in Mediterranean bottlenose dolphins, Lemos et al. (2013) in seven cetacean species on the coast of Brazil, Agusa et al. (2008) in striped dolphins on the coasts of Japan, and Mackey et al. (1995) in pilot whales (*Globicephala melas*),

harbor porpoises (*Phocoena phocoena*), and white-sided dolphins (*Lagenorhynchus acutus*) sampled from Atlantic coasts of the United States. Mercury and Se interact on a molecular level with a strong affinity to produce toxicologically inert mercury-selenide crystals (HgSe) that are stored primarily in liver tissue (Bard 1999; Das et al. 2003b; Lailson-Brito et al. 2012; Nigro and Leonzio 1996; Yang et al. 2007).

Table 4 Results of correlation analyses between trace element concentrations in liver of stranded cetaceans ($n = 31$)

	Cr	Mn	Cu	Zn	As	Se	Sr	Cd	Sn	Hg
Mn	0.0941 $p = 0.615$									
Cu	0.1666 $p = 0.370$	-0.3116 $p = 0.088$								
Zn	-0.0234 $p = 0.900$	0.2249 $p = 0.224$	0.0252^a $p = 0.025^*$							
As	0.0513 $p = 0.784$	0.2391 $p = 0.195$	-0.1744 $p = 0.348$	-0.1034 $p = 0.580$						
Se	-0.2400 $p = 0.193$	0.0880 $p = 0.638$	-0.3511 $p = 0.053$	-0.5572 $p = 0.001^*$	-0.0883 $p = 0.637$					
Sr	0.1864 $p = 0.315$	0.3810 $p = 0.035^*$	-0.1896 $p = 0.307$	-0.0456 $p = 0.808$	0.2881 $p = 0.116$	-0.0690 $p = 0.712$				
Cd	-0.1135 $p = 0.543$	0.3433 $p = 0.059$	-0.5309 $p = 0.002^*$	-0.3472 $p = 0.056$	0.2498 $p = 0.175$	0.6685 $p < 0.001^*$	0.2165 $p = 0.242$			
Sn	0.0320 $p = 0.864$	0.2839 $p = 0.122$	-0.2604 $p = 0.157$	-0.4417 $p = 0.013^*$	0.0826 $p = 0.659$	0.6662 $p < 0.001^*$	0.1961 $p = 0.290$	0.5079^a $p < 0.001^*$		
Hg	-0.3193 $p = 0.080$	0.1724 $p = 0.354$	-0.5053 $p = 0.004^*$	-0.4926 $p = 0.005^*$	-0.0153 $p = 0.935$	0.9242 $p < 0.001^*$	-0.0640 $p = 0.732$	0.7103 $p < 0.001^*$	0.6286 $p < 0.001^*$	
Pb	0.1618 $p = 0.385$	0.1527 $p = 0.412$	-0.1430 $p = 0.443$	-0.3917 $p = 0.029^*$	0.1627 $p = 0.382$	0.4952 $p = 0.005^*$	0.2551 $p = 0.166$	0.3899^a $p = 0.002^*$	0.4691^a $p = 0.0002^*$	0.4096 $p = 0.022^*$

The bold values indicate a significant correlation between trace elements

* Significant correlation between trace elements ($p < 0.05$)

^a Correlations calculated with non-parametric Kendall's Tau

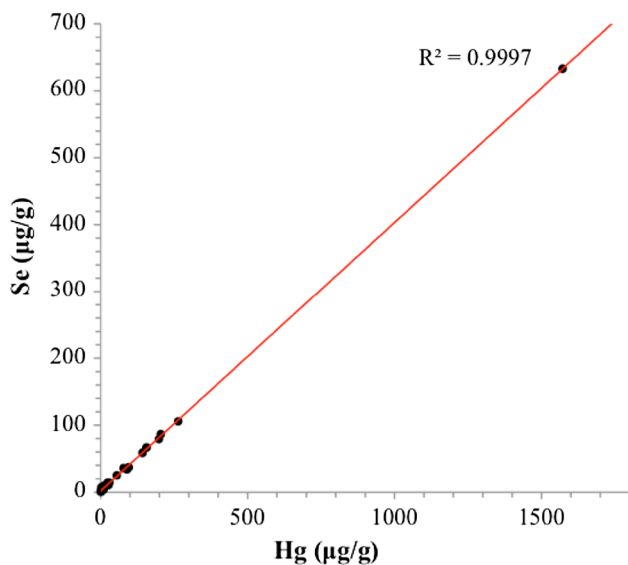


Fig. 3 Linear relationship between Hg and Se concentrations in stranded cetacean livers ($R^2 = 0.9997$). Pearson's correlation coefficient $r = 0.9998$, $p < 0.0001$, $df = 30$. $R^2 = 0.995$ when the highest point excluded

Trace Element Trends Relative to Age Class and Sex

Significant differences in concentrations between age classes for several trace elements were found, while differences between genders were not observed in this data set. The greatest concentrations of most essential trace elements, such as Cu and Zn, were observed in calves, and the lowest concentrations were found in adults, with the exception of Mn and Se. This is likely due to ontogenetic metabolism differences and dilution effects related to growth (Baer and Thomas 1991; Caurant et al. 1994; Wagemann et al. 1998). The opposite trend was observed in nonessential trace element concentrations, such as Hg and Cd, with the lowest concentrations occurring in calves, and the greatest concentrations in adults, because of bioaccumulation and biomagnification over time with age and trophic position (Aguilar et al. 1999).

To investigate age-related trends more closely two-way factorial ANOVA and Steel–Dwass multiple comparisons tests were conducted for the life history factors of age class, sex, and phylogenetic grouping of species (Table 1).

Multiple comparisons resulted in the use of varying alpha levels with each trace element for each family of tests. No interactions were found among age and sex groups. The only factor found to be driving the model differences was age. Notable differences were found among age classes for Cu, Zn, Se, Cd, Sn, and Hg liver concentrations, and Hg/Se molar ratios. Both Cu and Zn liver concentrations had an inverse relationship with age; however, the results were not statistically significant (Fig. 4a, b). Copper concentrations decreased with age and although calves had a greater mean concentration than older age classes the difference was not significant. Zinc concentrations showed the same trend as Cu with calves having a greater mean concentration than older age classes. The ANOVA results indicated a difference in the means for age class, and an interaction with sex, but after making Holm–Bonferonni sequential adjustments for multiple comparisons to the alpha the post hoc results were not significant (Online Resource 2). Copper and Zn are both essential trace elements important in growth and

development. Both absorption and retention rates for these elements may be significantly greater in calves prior to weaning because greater concentrations of Cu and Zn are required for rapid cell differentiation, postnatal growth, and repair processes (Caurant et al. 1994; Mason et al. 1981; Quaipe et al. 1986; Sabolic et al. 2010). Differences in ontogenetic metabolism and the dilution effect of increasing body size as animals mature likely accounts for the inverse Cu and Zn concentration relationship with age (Baer and Thomas 1991; Caurant et al. 1994; Kunito et al. 2004; Sabolic et al. 2010; Wagemann et al. 1998).

Cadmium accumulated significantly with age (Fig. 4d). Calves had a lower mean concentration than juveniles and adults, which is consistent with findings from past cetacean studies of this nonessential trace element (statistical values in Online Resource 2). Law et al. (1992) found that Cd concentrations in striped dolphin and Dall's porpoise fetuses and calves in the North Atlantic had negligible or very low concentrations of Cd compared with their adult

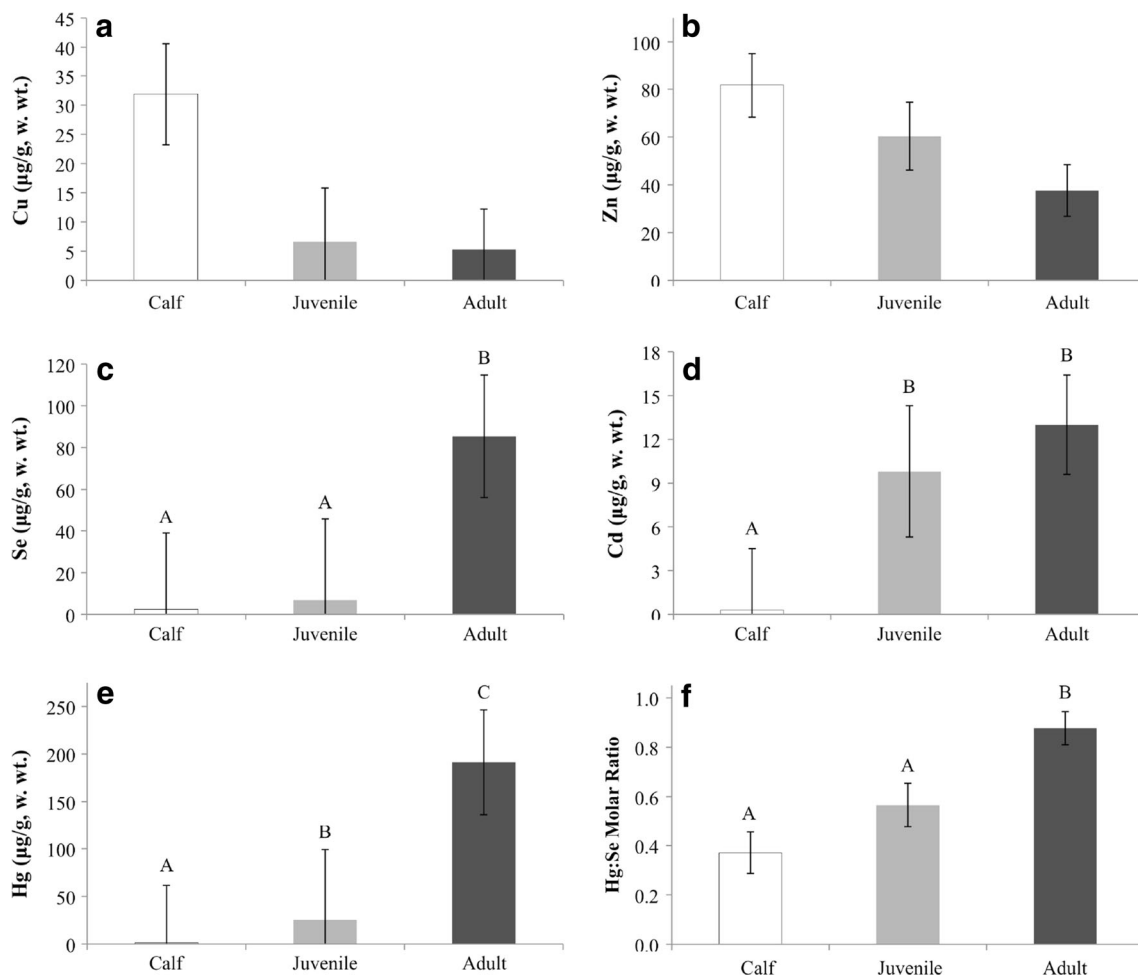


Fig. 4 Trace element age class differences in U.S. Pacific Island stranded cetaceans. Mean (\pm SE), letters above error bars denote significantly different means. $\alpha = 0.05$ with Holm–Bonferonni

sequential adjustments for multiple comparisons. **a** copper; **b** zinc; **c** cadmium; **d** selenium; **e** mercury; **f** Hg:Se molar ratios

mothers. Lahaye et al. (2006) observed a rapid increase in Cd concentrations of Mediterranean striped dolphin calves after birth that reached a plateau after 2 years. Both of these studies suggest that there is lactational transfer of Cd as well as age-related accumulation of Cd.

Selenium and Hg concentrations both increased significantly with increasing age class, similar to the trends observed with Cd. The significant increase of Se concentration with age resulted in calves and juveniles having significantly lower Se concentrations than adults (Fig. 4c). Mercury concentrations followed the same significant trend, calves had a lower mean concentration than juveniles and adults, and juveniles had significantly lower concentrations than adults (Fig. 4e) (statistical values in Online Resource 2). The chemical relationship between Hg and Se is one of the most well known examples of heavy metal interaction (Civin-Aralar and Furness 1991). Elevated concentrations of Hg and Se have been reported in marine mammals with no observed overt signs of Hg or Se poisoning. This has led to the conclusion that the molar ratio of Hg to Se in liver may be more important in assessing the potential for health effects rather than the individual concentrations of these elements (Civin-Aralar and Furness 1991; Das et al. 2003b). An animal with a liver molar excess of Se ($\text{Hg:Se} < 1$) can be considered at lower risk of Hg toxicity, whereas an animal with a molar excess of Hg ($\text{Hg:Se} > 1$) is at a greater risk of Hg toxicity. Molar ratios of Hg:Se in this study set spanned from almost 0 in the humpback whale calf to 1.03 in an adult bottlenose dolphin (*Tursiops truncatus*) (Table 2). This sample set had an average Hg:Se ratio of 0.65 and a median value of 0.76. A clear increasing trend of Hg:Se ratios with increasing age class was observed, with the Hg:Se molar ratios of calves and juveniles being significantly lower than the molar ratios of adults (Fig. 4f) (statistical values in Online Resource 2). As animals mature, they demethylate MeHg from their diet more efficiently, which then binds with protein-bound Se to form insoluble and toxicologically inert HgSe crystals that accumulate in liver tissue, and in the case of high Hg exposure a close to 1:1 molar ratio of Hg:Se is maintained in adult animals (Caurant et al. 1996; Civin-Aralar and Furness 1991; Itano et al. 1984; Koeman and van de Ven 1975; Martoja and Berry 1981; Nigro and Leonzio 1996; Yang et al. 2007). This remarkable capacity to demethylate and sequester Hg with Se in the liver may give cetaceans a greater tolerance to dietary Hg exposure than terrestrial animals (Betti and Nigro 1996; Das et al. 2003b; Himeno et al. 1989).

Trace Element Concentrations Relative to Phylogenetic Group

To highlight differences in trace element concentrations due to differential diet preferences, feeding strategies, and

trophic levels, phylogenetic groupings of species were explored. Differences in trace element concentrations among species groups (baleen whales $n = 5$, sperm whales $n = 4$, beaked whales $n = 4$, and dolphins $n = 30$, Table 1) were difficult to statistically assess because of small species sample sizes resulting in incomplete representation, and the strong age-related differences shown above. Differences in Hg, Se, and Cd concentrations were expected among species groups, and whereas not statistically significant, when phylogenetic groups were separated into age classes some interesting trends were observed. Sperm and baleen whale calves had the lowest Se and nonessential trace element concentrations relative to calves in other species groups, whereas dolphin and sperm whale juveniles and adults had the greatest concentrations of nonessential trace elements relative to the juveniles and adults of other species groups. Adult and juvenile beaked whales fell in the middle for most element concentrations compared with other species groups of the same age classes, except for Cd, which was on the high end of the concentration range for all species.

Trace element concentration patterns that arise by phylogenetic grouping are likely to reflect the diet, trophic level, and feeding strategies of the species of which each group is comprised. Baleen whales that filter feed to collect and consume very small fish and crustaceans feed much lower on the foodweb than large delphinids that feed on larger predatory fish species; therefore, baleen whales were expected to have much lower concentrations of nonessential trace elements. Because calves are dependent on their mother's milk for sustenance and young weaned cetaceans forage alongside adult members of their population, the younger age classes likely reflect the trophic level of the adult portion of their population. For example, the lowest concentrations of Hg in this sample set were found in baleen whale calf samples, whereas sperm and dolphin calves had much greater concentrations of Hg reflecting the lower trophic level of baleen whales and the higher trophic level of sperm and dolphin species. The trace element concentrations of juvenile phylogenetic groups had very similar patterns as their adult counterparts. Dolphin juveniles and adults had the greatest concentrations of As and Hg, sperm whale juveniles and adults had the greatest concentration of Cr, Se, and Sr, and the highest concentrations of Cd were found in sperm whale adults and the beaked whale adult and juveniles. Other studies have found links between diet composition and trace element concentrations on a population level. Delphinid populations with diets comprised primarily of pelagic fish accumulated greater concentrations of Hg than populations in the same region that consumed cephalopods, which are at a lower trophic level (Lahaye et al. 2006; Svensson et al. 1992; Watanabe et al. 2002). Cephalopods also are a source of Cd

for cetaceans, and populations that mainly consume cephalopods tend to accumulate greater concentrations of Cd than piscivorous populations (Honda and Tatsukawa 1983; Lahaye et al. 2006). Deep diving cetaceans, such as sperm and beaked whales that feed primarily on cephalopods, are exposed to greater concentrations of Cd, As, and Cr, because these elements are naturally enriched in cephalopods (Bustamante et al. 1998, 2002; Dorneles et al. 2007; Kubota et al. 2001; Lahaye et al. 2006).

Geographic Comparison of Trace Element Concentrations

Intraspecies comparisons of trace elements across studies were difficult to make due to small sample sizes in this study and in the literature. In general, trace element concentrations for cetacean species in this study were similar to those observed in other areas of the Pacific, and within the ranges measured in other regions of the world for most trace elements (Table 5). Bottlenose and spinner dolphins had lower Sn and Pb concentrations than other regions in the world. The most elevated Hg concentrations observed in the literature are observed in high trophic level adult cetaceans or in cetaceans living in regions with elevated Hg concentrations. The greatest concentrations of Hg in this study were measured in an adult male killer whale KW2008010 (264 µg/g wet mass fraction) and a false killer whale KW2010019 (1572 µg/g wet mass fraction). These Hg concentrations are most comparable with concentrations measured in the same dolphin species stranded on the Pacific coast of British Columbia, Canada (Table 5). Baird et al. (1989) reported an adult male false killer whale with a Hg concentration in liver (728 µg/g wet mass fraction), and Langelier et al. (1990) reported Hg liver concentrations of 1272 µg/g wet mass fraction in a killer whale and 1614 µg/g wet mass fraction in an adult female false killer whale. The Hg concentrations observed in this study are generally much lower in comparison to those observed in the Mediterranean where there are naturally occurring Hg deposits (cinnabar or native Hg ore, HgS) that cause resident wildlife to accumulate extreme Hg concentrations, such as the liver Hg concentration of 3945 µg/g wet mass fraction observed in a bottlenose dolphin by Leonzio et al. (1992) (Table 5).

Trace Element Case Studies in Individual Animals

A number of individual animals in this study had elevated nonessential trace element liver concentrations that could cause detrimental health effects. These elevated concentrations give insight into the challenges populations of some cetacean species may be experiencing in the Pacific Islands region and across the Pacific Ocean, and highlight differences in cetacean ecology between species and

among populations. Several individuals in this study had Cd and Hg liver concentrations that exceeded thresholds for toxicity (Tables 2 and 3). Eleven animals had liver concentrations of Cd that could cause kidney damage according to observations made by Lavery et al. (2009) in Southern Australian bottlenose dolphins (*Tursiops aduncus*) (5–37 µg/g wet mass fraction), and five of those animals had Cd concentrations within the effect range of 20–200 µg/g wet mass fraction in liver extrapolated for marine mammals from human studies (Table 2) (Fant et al. 2001; Fujise et al. 1988; Law 1996). The greatest concentrations of Cd were measured in adult false killer whale KW2010019; juvenile Cuvier's beaked whale KW2008008; two adult dwarf sperm whales 15377 and KW2009012; and adult striped dolphin KW2010008 (Table 2). Other studies have found elevated concentrations of Cd in marine mammal liver tissues without obvious indications of Cd toxicity, suggesting that marine mammals have a highly efficient detoxification mechanism for Cd and a greater capacity to internally mitigate Cd toxicity than terrestrial mammals. Solid granules composed of Cd, calcium, and phosphorous have been observed in kidney tissues of Atlantic white-sided dolphins, suggesting a sequestration mechanism (Gallien et al. 2001). Greenland ringed seals (*Phoca hispida*) were found to exceed kidney Cd concentration limits of 100–200 µg/g wet mass fraction without evidence of renal dysfunction (Dietz et al. 1998).

Twelve animals had Hg concentrations greater than the 60 µg/g wet mass fraction effect threshold for liver and lymph cellular breakdown observed by Rawson et al. (1993) in Atlantic bottlenose dolphins and pilot whales, nine of these animals were within the maximum detoxification range of 100–400 µg/g for mammals (Piotrowski and Coleman 1980), and a false killer whale surpassed this range. It is important to note that Se was measured in nine of these animals, and the resulting Hg/Se molar ratios were close to 1:1 or less, ranging from 0.86 to 1.03 (Table 2), indicating a low likelihood of systemic Hg toxicity (Betti and Nigro 1996; Das et al. 2003b; Ikemoto et al. 2004; Palmisano et al. 1995; Rawson et al. 1993; Wagemann et al. 1984). The false killer whale with the greatest concentration of Hg in this study (1572 µg/g wet mass) was a 24-year-old adult female, KW2010019, from the endangered insular population (Table 3). False killer whales are a high trophic level species and Hawaiian populations have been observed feeding on the same predatory pelagic fish targeted by the Hawaiian commercial long-line fishery, such as mahi-mahi (*Coryphaena hippurus*) and tuna species (*Thunnus spp.*), as well as various species of cephalopod, seabird, and other cetaceans (Baird 2002, 2009; Baird et al. 2008). This concentration far exceeds the effect threshold for marine mammals of 60 µg/g wet mass fraction (Rawson et al. 1993). However, this animal also had

Table 5 Cetacean trace element liver concentrations ($\mu\text{g/g}$, wet mass) by region

Species	Region or coast	Cr	Mn	Cu	Zn	As	Se
Blainville's beaked whale	This study	0.20	4.34	6.84	70.14	0.16	4.05
	UK ^{1, 2}	0.63	–	5.6	41	98	2.5
Bottlenose dolphin	This study	0.29–3.86	2.32–5.80	3.93–10.32	39.34–102.65	0.22–2.11	1.65–34.22
	Mediterranean ^{3–13}	0.02–0.36	1.3–6.5	0.7–80.7	8.5–154.8	–	–
	Atlantic ^{14–21}	<DL–0.1	2.0–3.7	0.1–79.0	11.5–271.1	<DL–1.6	0.2–207.6
	Japan and China ^{22–23}	<DL–53.3	–	2.5–23.4	16.2–89.0	0.5–6.9	0.1–12.2
	Australia ^{24–26}	1.0–1.2	–	4.9–85	26.2–144	0.2–0.8	<DL–253.4
Cuvier's beaked whale	India ²⁷	–	–	–	–	–	–
	This study	1.50	2.01	7.35	45.89	3.71	3.02
Dwarf sperm whale	Mediterranean ^{3, 28, 29}	0.08–0.12	0.98	0.8–1.8	11.7–16.3	–	110.6
	Argentina ³⁰	–	–	–	–	–	–
False killer whale	This study	0.28	3.11	12.23	32.65	1.81	633.06
	Brazil ¹⁵	–	–	–	–	–	–
Humpback whale	Pacific, Canada ^{31,32}	–	6.6	15	154	<DL	153
	Australia ²⁴	–	–	–	–	–	–
	This study	0.68	1.40	156.13	112.52	0.028	0.92
Killer whale	Australia ³³	–	–	9–141	35–102	–	1.68–2.25
	This study	0.34	1.82	5.16	41.39	0.04	105.81
Longman's beaked whale	Atlantic ^{1,34}	0.8	5.5	8.3–28.8	48.0–986.3	0.6	26.6–31.0
	Japan ^{22,35–37}	–	1.4–3.6	6.0–16.3	59.8–93.5	0.3–0.4	1.2–32.9
	Pacific, Canada ³²	–	–	–	–	–	–
	Australia ²⁴	–	–	–	–	–	–
Melon-headed whale	This study	0.12–0.20	1.42–2.15	3.52–4.12	22.84–32.01	1.18–1.35	1.23–86.74
Pantropical spotted dolphin	Japan ^{35,38}	–	–	–	37.1	1.1–5.8	–
	Australia ²⁶	0.2–0.4	–	2.1–4.9	22–47	0.3–0.8	6.2–58
Pygmy killer whale	This study	0.06–1.32	0.59–3.78	6.75–43.89	25.99–66.11	1.21–1.38	2.18–37.09
	Brazil ¹⁵	–	–	–	–	–	–
	Pacific ^{39,40}	–	–	–	–	–	0.8–4.2
Pygmy sperm whale	Australia ²⁴	–	–	–	–	–	–
	This study	0.20	2.49	1.90	14.72	0.21	58.84
Rough-toothed dolphin	Brazil ³⁴	–	3.3	6.0	75.1	–	72.3
	This study	2.61	2.40	5.12	27.78	3.49	3.12
	Atlantic ^{2,41–44}	0.6–4.1	0.8–4.1	2.1–144.4	10.1–163.2	0.2	2.0–21.6
Sperm whale	S. Pacific ^{24,45}	<DL	1.1–1.2	1.9–4.1	16.9–18	–	4.5–5.7
	This study	–	–	–	–	–	–
	Atlantic ^{15,19,46}	–	2.8–5.1	3.6–14.6	32–116	0.3–0.6	2.9–133.3
Sperm whale	Japan ²²	–	–	–	–	–	–
	This study	0.24	1.31	13.68	245.38	0.26	0.85
	Mediterranean ^{3,4,47}	0.1–0.3	0.5–0.8	0.4–6.0	9.6–48.6	–	6.0–31.7
	North Sea ^{48–52}	<DL–0.8	–	1.6–30.6	2.1–37.5	0.7	1.7–12.9
	Mexico ⁵³	–	2.9	48.6	107	–	–
Australia ²⁴	–	–	–	–	–	–	

Table 5 continued

Species	Region or coast	Cr	Mn	Cu	Zn	As	Se
Spinner dolphin	This study	0.08–1.61	2.04–4.66	2.77–29.60	29.72–98.52	0.21–2.39	0.88–14.36
	Brazil ¹⁵	–	–	–	–	–	–
	Mexico ^{54,55}	–	–	–	–	–	1.0–2.4
	W. Pacific ^{22,40}	–	3.3	5.8	33.6	–	–
Striped dolphin	This study	0.05–0.86	2.40–4.68	4.67–19.64	23.83–76.62	0.19–2.22	2.16–76.27
	Mediterranean ^{4–7,12,56–66}	0.02–0.8	0.03–5.9	1.1–39.2	8.1–133.2	–	1.9–331.5
	Atlantic ^{2,15,17,18,62,67–71}	<DL–0.7	3.7	2.1–81.6	9.9–116	0.4–0.7	56.0–57.0
	Japan ^{72–74}	0.2	2.6	10.1	38.7	–	42.0
	Baltic Sea ⁷⁵	<DL	2.2	3.4–3.6	29.1–37.5	<DL	1.3–1.4
Species	Region or coast	Sr	Cd	Sn	Hg	Pb	Hg:Se
Blainville's beaked whale	This study	0.08	19.06	0.03	9.81	0.02	0.95
	UK ^{1, 2}	–	6.2	–	248	0.05	1.0
Bottlenose dolphin	This study	0.12–0.20	0.005–12.61	0.005–0.023	1.53–89.44	0.004–0.01	0.12–1.03
	Mediterranean ^{3–13}	–	<DL–1.2	–	0.5–3940	<DL–3.6	–
	Atlantic ^{14–21}	–	<DL–20	<DL–2.9	<DL–421.2	<DL–3.1	0.3–1.4
	Japan and China ^{22–23}	–	0.3–16.4	2.1–12.2	<DL–90	<DL–1.6	2.9
	Australia ^{24–26}	–	<DL–20	–	0.1–771.9	0.04–1.0	0.2–1.2
India ²⁷	–	–	0.1–0.5	–	–	–	
Cuvier's beaked whale	This study	0.50	37.51	0.04	5.85–10.77	0.02	0.76
	Mediterranean ^{3, 28, 29}	–	2.7–18.5	–	259–1419	0.28–0.84	0.9
	Argentina ³⁰	–	<DL	–	0.1	–	–
Dwarf sperm whale	This study	0.42–2.77	22.70–27.35	0.09–0.42	9.25–22.00	0.03–0.10	0.40–0.67
	Japan ²²	–	–	0.9	–	–	–
False killer whale	This study	0.06	58.93	1.16	1571.75	0.20	0.98
	Brazil ¹⁵	–	–	0.3	–	–	–
	Pacific, Canada ^{31,32}	–	1.1	–	728–1614	<DL	1.9
	Australia ²⁴	–	0.05–75.8	–	41–479	0.05–0.5	–
Humpback whale	This study	0.12	0.003	0.009	0.006–0.023	0.01	0.003
	Australia ³³	–	–	–	–	–	–
Killer whale	This study	0.18	0.72	0.05	263.76	0.003	0.98
	Atlantic ^{1,34}	–	1.8–3.7	–	88.0–120.7	<DL	1.1
	Japan ^{22,35–37}	–	<DL–11.5	1.6–1.9	0.3–97.8	–	0.1–1.2
	Pacific, Canada ³²	–	–	–	1272	–	–
Australia ²⁴	–	<DL	–	1.5	–	–	
Longman's beaked whale	This study	0.07	0.02	0.01	1.33	0.004	0.61
Melon-headed whale	This study	0.01–0.14	0.005–12.18	0.01–0.12	1.82–277.98	0.014–0.034	0.58–0.93
	Japan ^{35,38}	–	12.6	–	147.9	–	–
	Australia ²⁶	–	8.0–21	–	13–141	<DL–0.1	0.7–1.0
Pantropical spotted dolphin	This study	0.04–0.17	0.003–2.42	0.006–0.01	0.61–95.21	0.007–0.008	0.11–1.01
	Brazil ¹⁵	–	–	<DL–0.2	–	–	–
	Pacific ^{39,40}	–	–	–	0.1–12.0	–	–
	Australia ²⁴	–	–	–	5.8–30.6	<DL	–
Pygmy killer whale	This study	0.12	1.40	0.05	142.28	0.01	0.95
	Brazil ³⁴	–	0.9	–	297.8	–	–
Pygmy sperm whale	This study	0.51	6.00	0.04	1.61	0.02	0.20
	Atlantic ^{2,41–44}	–	0.2–7.6	–	0.4–56.9	0.1	0.01–1.0
	S. Pacific ^{24,45}	–	6.3–14.3	–	1.7–17.2	0.1	–

Table 5 continued

Species	Region or coast	Sr	Cd	Sn	Hg	Pb	Hg:Se
Rough-toothed dolphin	This study	–	–	–	224.84	–	–
	Atlantic ^{15,19,46}	–	0.01–1.0	<DL–7.3	3.4–496.2	–	–
	Japan ²²	–	–	0.1–0.8	–	–	–
Sperm whale	This study	0.08	0.01	0.01	1.89	0.01	0.88
	Mediterranean ^{3,4,47}	–	0.1–1.5	–	0.004–119	<DL	0.9–1.4
	North Sea ^{48–52}	–	15.6–52.5	–	3.2–39.6	<DL–1.0	1.2
	Mexico ⁵³	–	7.7	–	–	4.2	–
	Australia ²⁴	–	1.2–11.2	–	1.3–34.0	<DL–0.6	–
Spinner dolphin	This study	0.08–0.58	0.003–7.35	0.005–0.03	0.52–39.27	0.002–0.01	0.09–0.97
	Brazil ¹⁵	–	–	<DL–0.3	–	–	–
	Mexico ^{54,55}	–	–	0.05–0.4	0.8–1.8	–	–
	W. Pacific ^{22,40}	–	7.3	–	20.1–105	1.0	–
Striped dolphin	This study	0.06–0.17	0.007–25.64	0.01–0.49	3.20–198.18	0.004–0.025	0.57–0.98
	Mediterranean ^{4–7,12,56–66}	–	<DL–9.0	0.3–4.2	0.8–1320	<DL–5.4	0.47–1.27
	Atlantic ^{2,15,17,18,62,67–71}	0.1	0.1–29.7	0.4	0.9–317	<DL–1.6	0.01–1.0
	Japan ^{72–74}	0.6	5.1	–	1.7–485	0.02	1.2
	Baltic Sea ⁷⁵	0.03–0.05	0.8–1.2	–	4.1–5.6	<DL	–

Single values indicate single animals sampled, ranges indicate multiple

<DL = Below the detection limit

^a Measurements converted from dry mass to wet mass assuming 70 % moisture (Yang and Miyazaki 2003), indicated in the list of references

¹ Law et al. (1997a), ²Law et al. (2001), ³Augier et al. (1998)^a, ⁴Bellante et al. (2009)^a, ⁵Bellante et al. (2012)^a, ⁶Frodello et al. (2000)^a, ⁷Frodello and Marchand (2001)^a, ⁸Frodello et al. (2002a)^a, ⁹Leonzio et al. (1992), ¹⁰Roditi-Elasar et al. (2003), ¹¹Shoham-Frider et al. (2009), ¹²Storelli and Marcotrigiano (2002), ¹³Viale (1994), ¹⁴Beck et al. (1997), ¹⁵Dorneles et al. (2008), ¹⁶Geraci (1989), ¹⁷Holsbeek et al. (1998)^a, ¹⁸Law et al. (1992), ¹⁹Lemos et al. (2013), ²⁰Meador et al. (1999)^a, ²¹Wood and Van Vleet (1996)^a, ²²Hai Le et al. (1999)^a, ²³Parsons and Chan (2001)^a, ²⁴Kemper et al. (1994), ²⁵Lavery et al. (2008)^a, ²⁶Law et al. (2003), ²⁷Lee and Mok (1995), ²⁸Frodello et al. (2002b)^a, ²⁹Storelli et al. (1999), ³⁰Marcovecchio et al. (1992), ³¹Baird et al. (1989), ³²Langelier et al. (1990), ³³Holyoake et al. (2012)^a, ³⁴Lemos et al. (2013), ³⁵Kubota et al. (2001), ³⁶Endo et al. (2006), ³⁷Endo et al. (2007), ³⁸Hirata et al. (2010), ³⁹Andre et al. (1991b), ⁴⁰Chen et al. (2002), ⁴¹Bryan et al. (2012), ⁴²Marcovecchio et al. (1990), ⁴³Odell and Asper (1976), ⁴⁴Henry and Best (1999), ⁴⁵Bustamante et al. (2003)^a, ⁴⁶Mackey et al. (2003), ⁴⁷Mazzariol et al. (2011), ⁴⁸Bouqueneau et al. (1997)^a, ⁴⁹Holsbeek et al. (1999)^a, ⁵⁰Joiris et al. (1997), ⁵¹Law (1996), ⁵²Law et al. (1997b), ⁵³Ruelas-Inzunza and Páez-Osuna (2002)^a, ⁵⁴Ruelas et al. (2000)^a, ⁵⁵Ruelas and Páez-Osuna (2002)^a, ⁵⁶Augier et al. (1993), ⁵⁷Augier et al. (2001)^a, ⁵⁸Capelli et al. (2000)^a, ⁵⁹Cardellicchio et al. (2000)^a, ⁶⁰Cardellicchio et al. (2002a)^a, ⁶¹Cardellicchio et al. (2002b)^a, ⁶²Lahaye et al. (2006)^a, ⁶³Monaci et al. (1998)^a, ⁶⁴Roditi-Elasar et al. (2003), ⁶⁵Storelli and Marcotrigiano (2002), ⁶⁶Viale (1994), ⁶⁷Andre et al. (1991a), ⁶⁸Das et al. (2000)^a, ⁶⁹Das et al. (2003a)^a, ⁷⁰Kunito et al. (2004)^a, ⁷¹Law et al. (1991), ⁷²Agusa et al. (2008)^a, ⁷³Honda et al. (1983), ⁷⁴Itano et al. (1984), ⁷⁵Ciesielski et al. (2006)

Hg/Se molar ratio of 0.98, which may indicate an ability to tolerate elevated Hg concentrations without toxic effects. It is important to note that the greatest concentrations of three other potentially toxic trace elements, Cd, Sn, and Pb, also were observed in this animal. These concentrations warrant further histological and biomolecular study of tissues, such as liver, kidney, and bone for indications of toxicity, and the analysis of tissues from other Hawaiian false killer whales as the opportunities arise. This species may be an important indicator of accumulating anthropogenic contaminants in fish targeted commercially by the Hawaiian long-line fishery, posing a health risk to human consumers. Mercury and Se concentrations have been studied in the fish species caught in this fishery (Kaneko and Ralston 2007), but the findings of this study may warrant a closer look for the sake of public safety. The Hawaiian insular

population of false killer whales, of which this sample is a known member, was found to have elevated concentrations of persistent organic pollutants and ongoing commercial fishery interactions (Bachman et al. 2014; Baird and Gorgone 2005; Forney and Kobayashi 2007; Ylitalo et al. 2009). The levels of potentially toxic trace elements observed in this sample brings further attention to the complex series of pressures this population is facing as it continues to decline.

Conclusions

This study established initial trace element concentration ranges for 11 trace elements in the liver tissue of 16 species of Pacific cetaceans stranded in the main Hawaiian Islands,

Saipan, and Guam. In this opportunistic sample set, trace element correlations agreed well with the literature; significant age-related trends were found in Se, Cd, and Hg concentrations, and Hg:Se molar ratios; and whereas no significant sex or phylogenetic group differences were found, some interesting trends were observed. In general, trace element concentrations in this study were most similar to concentrations observed elsewhere in the Pacific and fell within ranges reported in other oceans, with the exception of the Mediterranean. Concentrations of Cd and Hg in a number of Hawaiian cetacean individuals indicate a possible toxicity risk to these Hawaiian cetacean populations, warranting additional study to further our understanding of the effects of elevated trace element concentrations in cetaceans in this region of the Pacific Ocean.

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Disclaimer Certain commercial products and instruments are identified in this paper to adequately specify the experimental procedures. Such identification does not imply recommendation or endorsement by National Institute of Standards and Technology, nor does it imply that the items mentioned are the best for the intended purpose.

Compliance with Ethical Standards

Conflict of Interest None.

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