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Effects of Age, Colony, and Sex on Mercury Concentrations in California Sea Lions

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Abstract We measured total mercury (THg) concentrations in California sea lions (*Zalophus californianus*) and examined how concentrations varied with age class, colony, and sex. Because Hg exposure is primarily via diet, we used nitrogen (δ^{15}N) and carbon (δ^{13}C) stable isotopes to determine if intraspecific differences in THg concentrations could be explained by feeding ecology. Blood and hair were collected from 21 adult females and 57 juveniles from three colonies in central and southern California (San Nicolas, San Miguel, and Año Nuevo Islands). Total Hg concentrations ranged from 0.01 to 0.31 μg g^{-1} wet weight (ww) in blood and 0.74 to 21.00 μg g^{-1} dry weight (dw) in hair. Adult females had greater mean THg concentrations than juveniles in blood (0.15 vs. 0.03 μg g^{-1} ww) and hair (10.10 vs. 3.25 μg g^{-1} dw). Age class differences in THg concentrations did not appear to be driven by trophic level or habitat type because there were no differences in δ^{15}N or δ^{13}C values between adults and juveniles. Total Hg concentrations in adult females were 54 % (blood) and 24 % (hair) greater in females from San Miguel than females from San Nicolas Island, which may have been because sea lions from the two islands foraged in different areas. For juveniles, we detected some differences in THg concentrations with colony and sex, although these were likely due to sampling effects and not ecological differences. Overall, THg concentrations in California sea lions were within the range documented for other marine mammals and were generally below toxicity benchmarks for fish-eating wildlife.

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Mercury (Hg) contamination is of concern in terrestrial and marine environments because methylmercury (MeHg) can negatively affect the health of humans and wildlife (Scheuhammer et al. 2007; Mergler et al. 2007; Dietz et al. 2013). Direct links between Hg exposure and decreased wildlife health are difficult to establish for wild populations, but negative relationships between Hg concentrations and metrics of health and reproductive success have been documented for a variety of species (Basu et al. 2005; Evers et al. 2008; Goutte et al. 2014). Controlled dosing experiments on birds and mammals indicate that Hg exposure at environmentally relevant concentrations can alter behavior, impair reproduction, and decrease health (Basu et al. 2010; Frederick and Jayasena 2011; Carlson et al. 2014). Fish-eating wildlife and other upper trophic level consumers are at particular risk from MeHg because it is the primary form of Hg present in fish (Harris et al. 2003) and biomagnifies in food webs (Lavoie et al. 2013).

Pinnipeds (fur seals, sea lions, seals, and walrus) are a group of marine mammals that may be exposed to high concentrations of Hg as a result of their trophic position, habitat preferences, and relatively long life span. Mercury enters marine food webs primarily through atmospheric

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deposition and subsequent conversion to MeHg via abiotic and biotic mechanisms, although point source pollution may significantly contribute to Hg inputs in some areas (Fitzgerald et al. 2007). The predominant route of Hg exposure for pinnipeds is via dietary intake from prey, therefore Hg concentrations in tissues can be affected by foraging ecology and geographic location (Aubail et al. 2011; Rea et al. 2013; Peterson et al. 2015a). Once ingested, tissue concentrations may be influenced by factors that affect assimilation, excretion, and storage of Hg, such as the physiological status of the animal, age, and growth rate (Habran et al. 2011; Peterson et al. 2015b). There currently are no established thresholds for negative effects associated with Hg for marine mammals, although some pinnipeds have tissue concentrations that exceed effect guidelines for humans or thresholds for other mammals, including mustelids and ursids (Castellini et al. 2012; Dietz et al. 2013; Rea et al. 2013; McHuron et al. 2014). This has prompted concern regarding the impact of Hg on the health and population dynamics of harbor seals (*Phoca vitulina* [McHuron et al. 2014]), Steller sea lions (*Eumetopias jubatus* [Rea et al. 2013]), and northern elephant seals (*Mirounga angustirostris* [Peterson et al. 2015a]). Because many pinnipeds are top predators in marine ecosystems, an understanding of Hg exposure in these species may also provide an indication of Hg contamination in the environments they inhabit.

California sea lions (*Zalophus californianus*) are one of the most abundant otariids (fur seals and sea lions) in the North Pacific, with a current population size of approximately 300,000 individuals (Carretta et al. 2014). They are found in neritic and offshore habitats from Islas Marias, Mexico, to British Columbia, Canada, and forage on a variety of fish and cephalopod species (Porras-Peters et al. 2008; Orr et al. 2011). California sea lions are year-round residents in the California Current Ecosystem; in the United States, adult females, juveniles, and pups typically remain on breeding rookeries in southern California for the majority of the year, whereas subadult and adult males migrate northward during the nonbreeding season (Peterson and Bartholomew 1967). The majority of studies on Hg exposure in this species have primarily focused on dead and/or stranded animals (Martin et al. 1976; Harper et al. 2007; Wintle et al. 2011), and although informative, these data may not be representative of exposure in free-ranging animals. We measured Hg concentrations in blood and hair of free-ranging adult female and juvenile male and female California sea lions at three colonies in southern and central California to (1) assess baseline concentrations of Hg, (2) examine how Hg concentrations varied with age class, colony, and sex, and (3) determine if any intraspecific differences in Hg concentrations could be explained by feeding ecology.
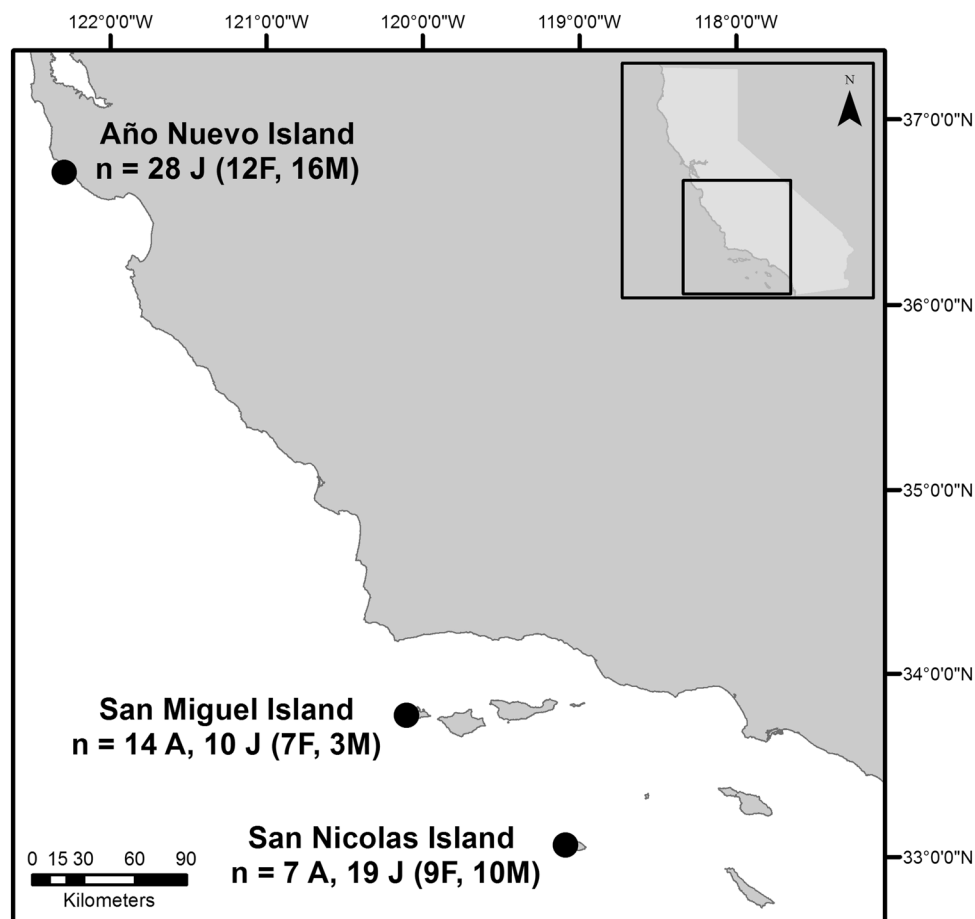
Methods

Sample Collection

Adult (female) and juvenile (male and female) California sea lions were sampled at San Nicolas Island, San Miguel Island, and Año Nuevo Island (Fig. 1). Age class determination of adults and juveniles was based on size, sex, time of year, and lactation/pup status. All adult females were lactating or observed nursing a pup. The estimated age of juvenile animals was between 1 and 3 years old with the exception of one approximately 5-year old male. Adult females were captured in May (San Miguel Island) or August (San Nicolas Island), whereas juveniles were captured in September (San Miguel and San Nicolas Islands) and October (Año Nuevo Island) of 2013.

All animals were captured using custom hoop nets and either chemically immobilized with an intramuscular injection of midazolam (0.15–0.20 mg kg^{-1} administered with 0.02 mg kg^{-1} of atropine) or anesthetized with isoflurane gas administered with oxygen via a field portable vaporizer. Blood samples were collected from the caudal gluteal vein into BD Vacutainers, Whirk-Paks, and Oster Pro are all registered trademarks® containing sodium heparin, and stored in a cooler with ice while in the field. Hair samples were collected from the dorsal pelvic region with an Oster Pro® battery-operated shaver and stored in individual Whirl-Paks®. The collection site was standardized because Hg concentrations in hair may vary among collection sites (McHuron et al. 2012). For all individuals, we noted whether the hair sample contained only old (unmolted) or new hair (molted) or a mixture of old and new hair (mixed molt). We used these classifications to assign molt status to each animal. In the laboratory, blood samples were gently remixed before measurement of hematocrit and removal of whole blood for Hg analysis. Samples were then centrifuged, and red blood cells (RBCs) were removed for stable isotope analysis. Blood samples were stored in polyethylene cryovials and frozen at −20 °C until analysis.

Tissue Selection

We sampled blood and hair because these tissues can be collected nonlethally and are increasingly used to determine baseline Hg concentrations, assess toxicological risk, and examine intraspecific differences for pinnipeds. Blood Hg concentrations reflect recent exposure from diet and exchange with other tissues and organs (Lieske et al. 2011; Wang et al. 2014), although the dynamics of blood Hg in pinnipeds are not well understood. Mercury concentrations in hair are generally representative of blood

Fig. 1 Sampling locations (denoted by dots) of adult female (A) and juvenile (J [*M* male, *F* female]) California sea lions (*Z. californianus*) captured in central and southern California. Sample sizes separated by age class and sex are shown following each location

Hg concentrations during the period of hair growth (Wang et al. 2014), and pinnipeds renew their pelage during an annual molt that lasts weeks to several months depending on the species. For juvenile and adult female California sea lions, molt occurs from August to October (Peterson and Bartholomew 1967; Williams et al. 2007), although the timing of molt for each age class and the actual duration for individuals are not well-documented in this species. California sea lion pups are born with hair that is grown in utero, but undergo one postnatal molt at 3–4 months of age and potentially a second molt at 7–8 months (Melin et al. 2000; Orr et al. 2012). Hair Hg concentrations in this age class are therefore reflective of either Hg exposure in utero or from milk depending on the month of sample collection. Pups typically wean after 10–11 months (in April or May), but because molt does not begin until August or later, Hg concentrations in hair collected between these time periods are still reflective of lactational transfer. Once grown, Hg in hair is not bioavailable and is unaffected by ecological and physiological factors that occur outside of the molt period.

Sample Preparation and Analysis

Hair was washed in a 1 % Alconox solution (White Plains, New York, USA) using deionized water, sonicated to remove surface debris, and dried at 50 °C for 48 h. Whole blood (hereafter referred to as "blood") and hair samples were analyzed for total Hg (THg) using a DMA-80 Direct Mercury Analyzer (Milestone, Shelton, CT, USA) at the U.S. Geological Survey Dixon Field Station Mercury Laboratory following EPA method 7473 (USEPA 2000). We used THg an index of MeHg because it is strongly correlated with MeHg, and >80 % of Hg present in hair and blood is in the methylated form (Woshner et al. 2008; Dietz et al. 2011). Quality-assurance measures included certified reference materials (DORM-3, DOLT-3 or DOLT-4, or TORT-3; National Research Council of Canada, Ottawa, Canada), continuing calibration verifications, system and method blanks, and duplicate samples. Percentage recoveries (mean ± SE) were 100 ± 0.5 % for certified reference materials and 99 ± 0.8 % for calibration verifications. Duplicate samples of hair differed by an

absolute relative difference of 4 ± 1.5 %. Concentrations are presented in µg g^{-1} wet weight (ww) for blood and µg g^{-1} dry weight (dw) for hair.

Red blood cells were analyzed for stable isotopes of nitrogen (N) and carbon (C) to examine the relationship between Hg concentrations and feeding ecology. This compartment was chosen because the majority of Hg in whole blood is bound to RBCs (Correa et al. 2013), and because RBCs integrate dietary information over a longer time period than serum or plasma (several months vs. days to weeks [Hilderbrand et al. 1992; Zhao et al. 2006]). Red blood cells were freeze-dried for 48 h, homogenized, and weighed (0.5 ± 0.05 mg) into tin capsules for stable isotope analysis. Samples were analyzed using a Carlo-ErbaNE2500 CHNS-O Analyzer coupled to a Thermo Finnigan DELTAplus XP Isotope Ratio Mass Spectrometer via a Thermo Finnigan ConFlo III at the University of California Santa Cruz Stable Isotope Laboratory. Results are expressed as a ratio using delta (δ) notation in units of parts per thousand (‰) and calculated from the equation $\delta X = [R_{sample}/R_{standard}) - 1] \times 1000$ where $X = {}^{15}N$ or ^{13}C and $R = {}^{15}N/{}^{14}N$ or $^{13}C/^{12}C$ in the sample and standard. The standard for C was Vienna-Pee Belemnite Limestone and atmospheric N$_2$(air) for N. Replicates of an internal laboratory standard were used to assess precision and were 0.11 ‰ for $\delta^{15}N$ and 0.07 ‰ for $\delta^{13}C$. Duplicate RBC samples were also run and were within 0.09 ‰ for $\delta^{15}N$ and 0.15 ‰ for $\delta^{13}C$.

Statistical Analysis

We used Analysis of Variance (ANOVA) or Analysis of Covariance to determine if THg concentrations in blood and hair differed with age class, colony, and sex. We first compared THg concentrations from adult females and juveniles at San Miguel and San Nicolas Islands (the two colonies where both age classes were sampled) to assess whether there were any differences in THg concentrations between age classes. We subsequently used separate analyses for each age class to determine how THg concentrations varied with colony, and for juveniles we also examined how THg concentrations varied with sex. Juveniles varied in their molt status, but due to small sample sizes of juveniles in each molt category at any given island (Table 1), we did not include molt as a factor in juvenile Hg comparisons. We quantified hematocrit for all animals but did not include it as a covariate in any of the blood THg comparisons because there was no relationship with THg concentrations for either age class; additionally, hematocrit concentrations overlapped for adults (range 30.0–53.5, $\bar{x} = 45.0$) and juveniles (range 29.5–51.0, $\bar{x} = 45.6$).

Nitrogen ($\delta^{15}N$) was included as a covariate in the blood analyses to account for the potential relationship between Hg

and trophic level, except when comparing adults with juveniles because of a significant interaction between age class and $\delta^{15}N$. For this reason, we used an ANOVA to test for any differences in $\delta^{15}N$ values between adults and juveniles at San Miguel and San Nicolas Islands. Carbon ($\delta^{13}C$) was excluded as a covariate because of minimal overlap in the distribution of $\delta^{13}C$ values among colonies, especially for juveniles. Instead, we used ANOVAs to determine if there were differences in $\delta^{13}C$ values (indicative of habitat use) with age class (adults vs. juveniles), colony (within each age class), and sex (male vs. female juveniles).

Residual plots were used to examine the assumptions of normality and homoscedasticity. Total Hg concentrations were natural-log-transformed to meet these assumptions, and we report back-transformed mean concentrations for statistical comparisons in text. Results were considered significant at $\alpha = 0.05$. All statistical analyses were performed in R v 3.01 (R Development Core Team).

Results

Total Hg concentrations in adult female sea lions ranged from 0.05 to 0.31 µg g^{-1} ww in blood ($n = 19$) and 5.10 to 21.00 µg g^{-1} in hair ($n = 21$), whereas THg concentrations in juveniles ranged from 0.01 to 0.06 µg g^{-1} ww in blood ($n = 57$) and 0.74 to 9.57 µg g^{-1} dw in hair ($n = 56$; Table 1). THg concentrations differed between age classes for both blood ($F_{1,45} = 103.20$, $p < 0.01$) and hair ($F_{1,47} = 51.38$, $p < 0.01$). Mean THg concentrations in adult females were 0.15 µg g^{-1} ww in blood and 10.10 µg g^{-1} dw in hair compared with 0.03 µg g^{-1} ww and 3.25 µg g^{-1} dw in juveniles (Fig. 2).

The effect of colony on THg concentrations was not the same for both age classes. For adult females, THg concentrations were affected by colony (blood: $F_{1,16} = 4.82$, $p = 0.04$; hair: $F_{1,19} = 8.26$, $p < 0.01$). Females at San Miguel had greater THg concentrations in blood ($\bar{x} = 0.18$ µg g^{-1} ww) and hair ($\bar{x} = 11.69$ µg g^{-1} dw) than females from San Nicolas Island ($\bar{x} = 0.11$ µg g^{-1} ww and 7.55 µg g^{-1} dw). For juveniles, there was a significant interaction between colony and sex for both blood and hair (blood: $F_{2,49} = 5.97$, $p < 0.01$; hair: $F_{2,50} = 3.41$, $p = 0.04$). Because of this interaction, we used Tukey's Honest Significant Difference test to determine if THg concentrations for each sex differed among colonies and if concentrations differed between male and female juveniles at the same colony. There were no differences in blood THg concentrations with sex or colony ($p > 0.05$ for each comparison) except for juvenile males at San Miguel Island. Blood THg concentrations in juvenile males from San Miguel Island ($n = 3$; $\bar{x} = 0.01$ µg g^{-1} ww) were less than those in juvenile females from this island

Table 1 Mean total mercury concentrations (±SD) in blood (µg g^{-1} wet weight; top value) and hair (µg g^{-1} dry weight; bottom value) of adult and juvenile California sea lions (*Z. californianus*) from three islands in central and southern California
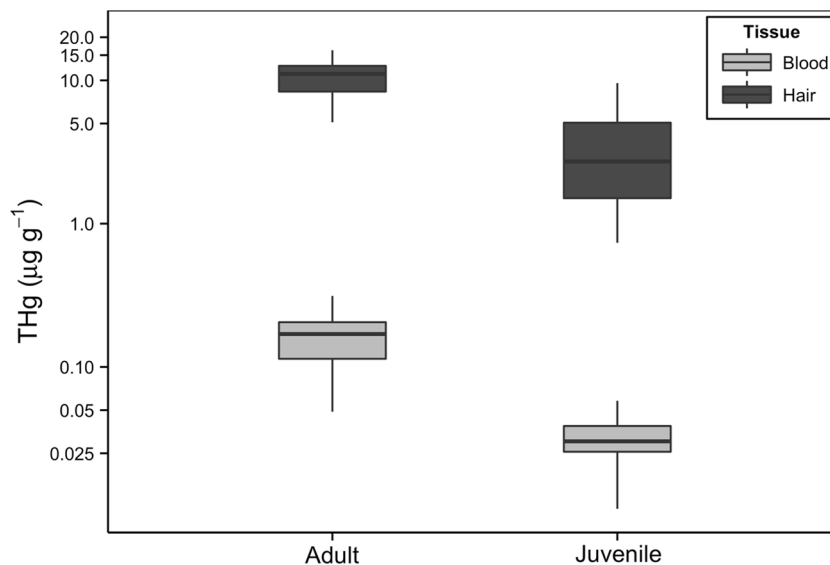
	Año Nuevo Island			San Miguel Island			San Nicolas Island		
	Old	New	Mixed	Old	New	Mixed	Old	New	Mixed
Juvenile females	0.03 ± 0.002	0.03 ± 0.01	NA	NA	0.03 ± 0.003	0.04 ± 0.008	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.008
	3.0 ± 1.8	4.1 ± 1.3			5.1 ± 0.9	5.6 ± 1.6	2.7 ± 2.3	4.9 ± 2.2	3.7 ± 2.8
	(2Y, 1J)	(1Y, 8J)			(3J)	(4J)	(2J)	(2J)	(5J)
Juvenile males	0.03 ± 0.007	0.03 ± 0.01	NA	NA	0.01	0.01 ± 0.003	0.04 ± 0.04	0.03 ± 0.01	0.04 ± 0.02
	1.5 ± 0.4	5.0 ± 4.4			1.2	4.1 ± 2.0	1.4 ± 0.3	3.4 ± 1.2	4.1 ± 2.0
	(10Y, 3J)	(3J)			(1Y)	(2Y)	(1Y, 1J)	(3J)	(1Y, 4J[a])
Adult females	NA	NA	NA	0.2 ± 0.07	NA	NA	0.1 ± 0.06	NA	NA
				12.1 ± 3.7			8.1 ± 3.4		
				(14[b])			(7)		

Concentrations are separated by island and within island by age class, sex, and molt status (old = unmolted, new = newly molted, and mixed = mix of molted and unmolted hair). Sample sizes are shown in parentheses and for juveniles are further separated into yearlings (Y, 1–2 years) and juveniles (J, 2–3 years) based on estimated ages. Footnotes have been provided when samples sizes differed between the two tissue types

[a] There were only three hair samples from juveniles in this category

[b] There were only 12 blood samples from adult females in this category

Fig. 2 Total mercury (THg) concentrations (µg g^{-1}) in whole blood (ww) and hair (dw) of adult and juvenile California sea lions (*Z. californianus*) from central and southern California. Concentrations in both tissues differed between adults and juveniles ($p < 0.01$). Sample sizes are the same for both tissues unless otherwise indicated and are as follows: San Miguel Island (12A$_{blood}$, 14A$_{hair}$, 10 J), and San Nicolas Island (7A, 19J$_{blood}$, 18J$_{hair}$). *A* adult; *J* juvenile

($\bar{x} = 0.03$ µg g^{-1} ww; $p < 0.01$) and males from the other two islands ($\bar{x} = 0.03$ µg g^{-1} ww at each island; $p < 0.01$). Hair THg concentrations in juveniles were not affected by colony ($p > 0.05$ for each sex), but differed between males and females at two of the islands ($p < 0.05$ for each island). Juvenile females from San Miguel ($\bar{x} = 5.27$ µg g^{-}) and Año Nuevo Islands ($\bar{x} = 3.57$ µg g^{-1} dw) had greater hair THg concentrations than juvenile males from those islands ($\bar{x} = 1.38$ and 1.64 µg g^{-1} dw, respectively; Fig. 3).

The relationship between blood THg concentrations and δ^{15}N values in RBCs was also not the same for adults and

juveniles. Blood THg concentrations in adult females were affected by δ^{15}N values ($F_{1,16} = 4.73$, $p = 0.04$), but linear regressions indicated that the strength of this relationship was weak for adult females at both colonies ($r^2 = 0.14$ or 0.16). For juveniles, there was no effect of δ^{15}N values on blood THg concentrations ($F_{1,49} = 0.27$, $p = 0.27$).

In general, there were few differences in δ^{15}N or δ^{13}C values in RBCs with age class, colony, or sex (Fig. 4). Adult females from San Nicolas and San Miguel Islands had δ^{15}N values that ranged from 15.3 to 16.1 ‰ ($\bar{x} = 16.1$) and δ^{13}C values from −18.6 to −17.3 ‰

Fig. 3 Total mercury (THg) concentrations in hair (μg g^{-1} dw) of male and female juvenile California sea lions (*Z. californianus*) from three islands in central and southern California. Different letters (*a, b*) correspond to significant differences at $p \leq 0.05$. Sample sizes are as follows: Año Nuevo Island (16 M, 12F), San Miguel Island (3 M, 7F), and San Nicolas Island (9 M, 9F). *M* male; *F* female
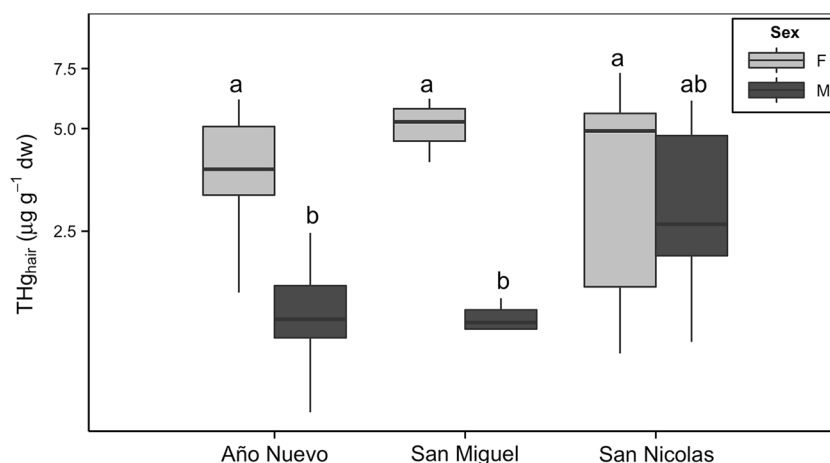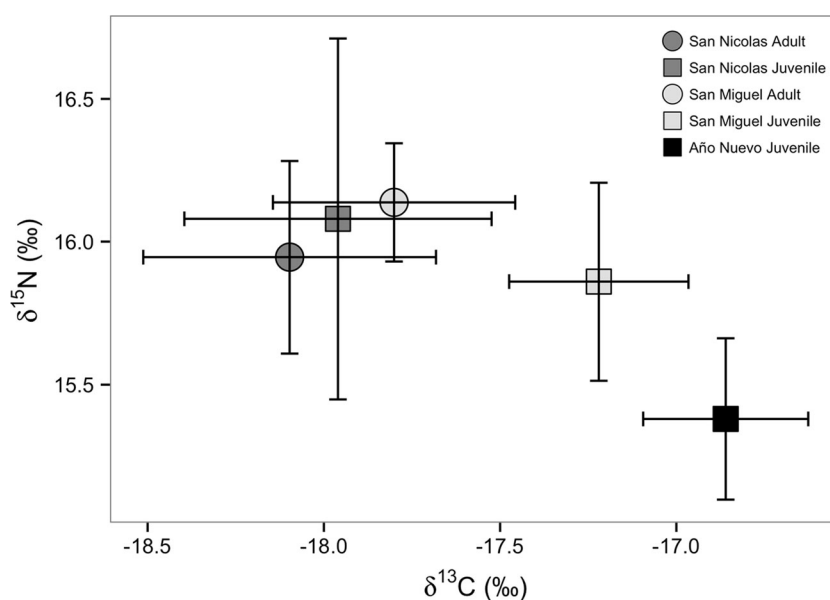
Fig. 4 Mean δ^{15}N and δ^{13}C values ($\pm$SD) in red blood cells of adult (female) and juvenile (male and female) California sea lions (*Z. californianus*) from three islands in central and southern California. Sample sizes are as follows: Año Nuevo Island (28 J), San Miguel Island (12A, 10 J), and San Nicolas Island (7A, 19 J). *A* adult; *J* juvenile

($\bar{x} = -17.9$). Juveniles from all three islands had δ^{15}N values that ranged from 14.9 to 18.0 ‰ ($\bar{x} = 15.7$) and δ^{13}C values from -18.5 to -16.4 ‰ ($\bar{x} = -17.3$). There was no difference in δ^{15}N or δ^{13}C values between adults and juveniles at San Miguel and San Nicolas Islands (δ^{15}N: $F_{1,46} = 0.21$, $p = 0.65$; δ^{13}C: $F_{1,46} = 2.11$, $p = 0.15$). There also was no significant difference in δ^{13}C values between adult females at the two islands ($F_{1,17} = 2.83$, $p = 0.11$). In contrast to adult females, δ^{13}C values for juveniles differed among the three islands ($F_{2,50} = 66.06$, $p < 0.01$). Juveniles from Año Nuevo Island were the most enriched in ^{13}C ($\bar{x} = -16.9$ ‰) followed by San Miguel ($\bar{x} = -17.2$ ‰) and San Nicolas Island ($\bar{x} = -18.0$ ‰). There was no difference in δ^{13}C values between male and female juveniles ($F_{1,50} = 0.18$, $p = 0.68$).

Discussion

Baseline THg Concentrations

California sea lions from southern and central California had THg concentrations in blood and hair within the range documented for other marine mammals, including other pinnipeds from the eastern North Pacific (Beckmen et al. 2002; Das et al. 2008; Woshner et al. 2008; Cardona-Marek et al. 2009; Aubail et al. 2011; Habran et al. 2011; Castellini et al. 2012; McHuron et al. 2014). In general, blood and hair THg concentrations in California sea lions were less than those in harbor seals (0.06–1.19 μg g^{-1} ww and 2.69–144.31 μg g^{-1} dw, respectively) and northern elephant seals (0.18–1.27 μg g^{-1} ww and 2.83–75.23 μg g^{-1} dw, respectively), which are the two other species for which THg

concentrations have been reported in free-ranging pinnipeds sampled in California (McHuron et al. 2014; Peterson et al. 2015a, b). These interspecific trends are consistent with a study by Wintle et al. (2011) that found stranded California sea lions had the lowest mean muscle THg concentrations compared with stranded harbor seals, northern elephant seals, and Steller sea lions.

Age Class

The greatest differences we found in THg concentrations were between age classes, with adult female California sea lions having concentrations in blood and hair almost five times greater than juveniles. Mercury bioaccumulates within individuals primarily because of slow excretion rates (Hg intake > Hg excretion) coupled with storage of Hg in internal tissues and organs. As a result, it is possible that adults had greater THg concentrations simply because they were older, and therefore had a greater time period over which to accumulate Hg. Several studies on marine mammals have found positive relationships between THg concentrations in blood or hair and age (Ikemoto et al. 2004; Stavros et al. 2008; Woshner et al. 2008; Peterson et al. 2015a); however, other studies on marine mammals found no such relationship (Ikemoto et al. 2004; Stavros et al. 2008; Agusa et al. 2011; St Louis et al. 2011). For example, Ikemoto et al. (2004) found a positive correlation between hair THg concentrations and age in Baikal seals (*Pusa sibirica*) but no correlation for northern fur seals (*Callorhinus ursinus*). Similar conflicting results have been documented for other mammals and birds (Ben-David et al. 2001; Evers et al. 2005). This may be because at relatively low exposure levels animals can efficiently demethylate and/or depurate Hg at a rate similar to ingestion (Evers et al. 1998; Fevold et al. 2003), or because ecological factors that affect Hg concentrations obscure the signal of age (Stavros et al. 2008; Cardona-Marek et al. 2009). Peterson et al. (2015a) found that although age affected blood THg concentrations of adult female northern elephant seals, it was relatively unimportant compared with ecological factors and only bioaccumulated at a rate of approximately 0.01 ± 0.01 μg g^{-1} ww/y.

Foraging behavior influences the bioaccumulation of Hg (Cardona-Marek et al. 2009; Rea et al. 2013; Peterson et al. 2015a); however, juvenile California sea lions forage on the same prey species, size classes, and in similar habitats as adult females (Orr et al. 2011, 2012). We did not detect any difference in $\delta^{15}N$ or $\delta^{13}C$ values between adults and juveniles, indicating that animals in our study foraged at similar trophic levels and in similar habitats regardless of age class. While similarity in $\delta^{15}N$ or $\delta^{13}C$ values does not necessarily indicate that juveniles and adults foraged in the same location, broad overlap has been observed in the at-

sea distribution of juveniles and adults (Melin et al. 2008; Orr et al. 2012; Kuhn and Costa 2014).

Despite similar foraging behavior, these two age classes differ in their energetic demands, which could influence Hg exposure. Adult females typically give birth to a single pup annually in June and nurse that pup for 10–11 months (Melin et al. 2000). During lactation, females are central-place foragers, alternating foraging trips to sea with periods of onshore nursing at the rookery. Lactation is energetically expensive, and female otariids may increase their foraging effort during this time period to acquire more prey (Costa and Gales 2003; Hoskins and Arnould 2013). A captive study by Williams et al. (2007) found that the greatest rate of food intake for nursing California sea lions occurred during peak lactation. Because the majority of Hg exposure occurs from dietary intake, these potential differences in consumption rates could result in greater Hg exposure and contribute to the observed higher THg concentrations in adults compared with juveniles.

Growth rate, specifically the faster growth rates in juvenile animals compared with adults, also may have contributed to the differences in THg concentrations between these two age classes. Rapid growth can reduce circulating Hg concentrations through mass dilution and incorporation of Hg into newly developed tissues (Ackerman et al. 2011; Habran et al. 2011). In northern elephant seal pups, THg concentrations in blood decreased by approximately 50 % from early to late lactation, presumably due to a threefold to fourfold increase in body mass (Habran et al. 2011; Peterson et al. 2015b). Otariids in general have much longer lactation durations and slower growth rates than phocids (Burns et al. 2004; Schulz and Bowen 2004), indicating that the dynamics of mass dilution may differ between these two families. Growth rates in otariids are more rapid in young pups compared with older pups and juveniles; however, because length and mass asymptote with age, juvenile animals overall have faster growth rates than adults (Winship et al. 2001; Burns et al. 2004; Childerhouse et al. 2010).

Due to the opportunistic nature of our study, samples from adults and juveniles were not collected at the same time periods, and sample collection for juveniles overlapped with the molting period. As a result, juveniles were in varying in stages of molt (35 % unmolted, 23 % partially molted, and 42 % fully molted), whereas all adults were unmolted at the time of sampling. Temporal differences in sample collection can influence blood Hg concentrations because the half-life of Hg in whole blood is approximately 7 weeks (Lieske et al. 2011); therefore, blood collected at different times may not represent the same time period. Molt can also affect blood THg concentrations because animals are able to depurate Hg into their hair as it grows (Wang et al. 2014). Despite this, we

do not believe that these sampling effects were the cause of differences in THg concentrations between adult females and juveniles because (1) females at San Nicolas Island were sampled within one month of juveniles and still had much greater THg concentrations than juveniles; and (2) we detected the same trend in hair as in blood (adults > juveniles), and hair should be representative of circulating THg concentrations during a similar time period in both age classes (i.e., juveniles and adults molt around the same time).

Colony

We detected geographic differences in blood and hair THg concentrations for adult females, but those differences were largely absent for juveniles. The exception to this was males at San Miguel Island, which had lower blood THg concentrations than juvenile males from the other two islands, but this may reflect our inability to accurately represent the range of Hg exposure with only three juvenile males sampled at this island. Geographic differences in tissue Hg concentrations are often the result of differences in Hg contamination and/or MeHg production among foraging areas (Ackerman et al. 2007; St Louis et al. 2011; McHuron et al. 2014; Peterson et al. 2015a). This may have been the cause of colony differences for adult females because even though they appeared to forage in similar habitats, as indicated by $\delta^{13}C$ values, telemetry studies indicate that they do not forage in the same geographic locations. Females from San Nicolas Island primarily forage in the southern California Bight, whereas those from San Miguel tend to forage north of the Bight (Melin et al. 2008; Kuhn and Costa 2014). Females at the two islands were sampled approximately 3.5 months apart, which could have affected blood concentrations as mentioned previously; however, we detected the same trend in hair, which should be grown at approximately the same time at the two colonies. Because sea lions alter their foraging behavior based on prey availability and abundance (Weise and Harvey 2008; Kuhn and Costa 2014), the trend toward higher Hg concentrations in females from San Miguel may not be consistent among years.

In contrast to adult females, there were differences in $\delta^{13}C$ values among juveniles at the three colonies, yet we did not detect similar trends in THg concentrations. This may have been because juveniles of different ages were combined into one age class, coupled with the fact that we could not test for the effect of molt status. Yearling and older juvenile animals may differ physiologically (e.g., metabolic rates, oxygen stores), which could affect tissue THg concentrations and obscure any effect of colony.

Sex

Juvenile male and female sea lions differed in THg concentrations at some colonies, but these may have been a result of sampling and not ecological effects. At San Miguel Island, females had greater blood THg concentrations than males, which was likely due to small sample size for males at this island as mentioned previously. Juvenile females at San Miguel and Año Nuevo Islands had greater hair THg concentrations than males, although given that blood THg concentrations were largely similar between sexes, these differences may have been driven by unequal distributions of younger and older juveniles coupled with molt status. For example, 10 of the 16 males sampled at Año Nuevo Island were unmolted yearlings, whereas 9 of the 12 females were estimated to be 2–3 years old. Because the majority of yearling males had not molted, their hair was grown as a pup when their primary source of Hg exposure was from milk (as opposed to prey). Mercury concentrations in the hair of pups grown during lactation is often relatively low (Castellini et al. 2012), which may be due to limited transfer of Hg from mother to pup during lactation (Wagemann et al. 1988; Habran et al. 2011). The age and molt distribution was similar among juveniles at San Nicolas Island, which was the only island where we did not detect any sex differences in hair THg concentrations.

THg Concentrations and $\delta^{15}N$

There was evidence of slight Hg biomagnification within the food web/s used by adult females, as females with higher $\delta^{15}N$ values tended to have greater circulating THg concentrations irrespective of their capture location. The strength of this relationship was relatively weak, and we did not detect any relationship for juveniles. Although Hg biomagnifies in food webs (Lavoie et al. 2013), our observations were not particularly surprising because intraspecific relationships between blood THg concentrations and $\delta^{15}N$ values for marine mammals are often weak or undetected (Woshner et al. 2008; Cardona-Marek et al. 2009; McHuron et al. 2014; Peterson et al. 2015a). This may be because within a species, $\delta^{15}N$ values often do not span more than one trophic level, and the signal of biomagnification is therefore masked by other factors that influence either Hg concentrations or isotope values.

Conclusions

This study provides baseline measurements on Hg concentrations in California sea lions and shows that adults have much higher THg concentrations than juveniles. Total

Hg concentrations in both age classes were generally less than commonly cited toxicity benchmarks for humans and wildlife (Basu et al. 2009; Dietz et al. 2013; Rea et al. 2013), although it is largely unknown how applicable these Hg benchmarks are to marine mammals. Given their trophic position and use of both neritic and offshore habitats, California sea lions may be a useful species to monitor Hg contamination in the California Current Ecosystem. California sea lions also may be a good model species to better understand the ecological drivers responsible for Hg exposure in marine systems because they exhibit considerable variability in foraging behavior and, as indicated by our study, are exposed to a range of Hg concentrations.

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