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HAIR, WHOLE BLOOD, AND BLOOD-SOAKED CELLULOSE PAPER-BASED RISK ASSESSMENT OF MERCURY CONCENTRATIONS IN STRANDED CALIFORNIA PINNIPEDS

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ABSTRACT: Mercury (Hg) poses a health risk to wildlife populations and has been documented at relatively high concentrations in many marine mammals, including wild-caught pinnipeds along the central California, US coast. We measured total Hg concentrations ([THg]) in hair and blood of live-stranded harbor seals (HS; *Phoca vitulina*), California sea lions (CSL; *Zalophus californianus*), and northern elephant seals (NES; *Mirounga angustirostris*) in California to quantify species, temporal, and spatial variability in [THg] and assess the relationships between [THg] measured by different methods (blood vs. filter paper) and in different matrices (blood vs. hair). We compared [THg] with toxicologic thresholds of concern to aid in identification of at-risk individuals or groups and better understand how the use of different methods and matrices affects assumed toxicologic risk. There was a wide range of [THg] in blood (<0.01–1.13 μg/g) and hair (0.45–81.98 μg/g), and NES had higher [THg] compared with HS and CSL. All three species had individuals with [THg] that exceeded the lower threshold for one or both matrices, but only HS pups had [THg] exceeding upper thresholds. Spatial differences in [THg] were detected, with higher concentrations in HS pups from areas surrounding San Francisco Bay, but differences were dependent on sampling year and matrix. The relationship between [THg] in blood and filter paper (r^2=0.98) was strong, and differences had little influence on comparisons with toxicologic thresholds. Blood and hair [THg] were generally in agreement (r^2=0.72), but large mismatches for a few seals underscore the importance of combined sampling in adverse effects studies where accurate assessment of Hg exposure is crucial. The wide range of [THg] in stranded HS pups that exceeded published thresholds of concern makes them a promising candidate for adverse effects studies, particularly because different matrices represent Hg exposure across key developmental stages.

Key words: Elephant seal, harbor seal, sea lion, tissue correlations, total mercury, toxicology.

INTRODUCTION

Mercury (Hg) continues to be a contaminant of concern in terrestrial and aquatic ecosystems, in part because of increased atmospheric and point-source inputs associated with human activities in the last century (Wolfe et al. 1998; Wiener and Suchanek 2008; Driscoll et al. 2013; Eagles-Smith et al. 2018). Adverse effects from Hg exposure include neurologic, immunologic, and reproductive impairment (Clarkson and Magos 2006; Basu et al. 2007, 2010; Evers et al. 2008; Dietz et al. 2013); however, direct links between Hg exposure, tissue concentrations, and biologic effects can be challenging to establish for many wildlife populations (O'Hara and Hart 2018). These challenges arise in part because wild animals live in dynamic environments where they are exposed to multiple stressors that can affect physiology and development, and rigorous observations to detect clinical symptoms and adverse outcomes are rarely possible. In marine ecosystems, some marine mammals have very high tissue total Hg concentrations ([THg]) because of their ecology and life

history (Woshner et al. 2008; Knott et al. 2011; Rea et al. 2013; McHuron et al. 2014; Peterson et al. 2015; Reif et al. 2015), which has raised concern about the effect of this contaminant on individual health and population dynamics, especially given the myriad of stressors faced by wild animals that may reduce their resilience (Avila et al. 2018; Simmonds 2018). In vitro exposure to Hg at environmentally relevant concentrations has been associated with changes in immune function of multiple taxonomic groups of marine mammals (Desforges et al. 2016). High Hg concentrations and concern for potential adverse effects in wild populations have been reported for Steller sea lions (*Eumetopias jubatus*) in some areas of Alaska, US (Castellini et al. 2012; Rea et al. 2013; Kennedy et al. 2019) and bottlenose dolphins (*Tursiops truncatus*) from Florida, US (Schaefer et al. 2011; Damseaux et al. 2017). These concerns are based on comparisons with published thresholds for effects on wildlife (Scheuhammer et al. 2007; Evers et al. 2008), criteria related to risk of toxicosis in pinnipeds (Harley and O'Hara 2015; O'Hara and Hart 2018), and associations between health parameters and Hg concentrations.

Hair and blood are two matrices commonly used to determine relative Hg exposure and assess the potential for adverse effects in pinnipeds. The majority of Hg in hair and blood is present as monomethylmercury ($MeHg^+$) from dietary exposure (Woshner et al. 2008; Dietz et al. 2011), which is one of the more toxic forms of Hg (Clarkson and Magos 2006). As a result, many researchers use the quick and inexpensive measurement of THg as a proxy for $MeHg^+$ for these tissue types. Pinnipeds undergo an annual pelage molt that lasts several weeks to months (timing varies among species and age classes; Ling 1970), which acts as an excretory route for Hg (Wang et al. 2014). Because hair is metabolically inert once grown, samples collected at any time within a molt year are representative of the same general time period, which is beneficial because it helps minimize the confounding effect of time on Hg concentrations. Many pinnipeds are born with a lanugo coat (hair grown in utero), and because $MeHg^+$ crosses the placenta and blood-brain barrier during this critical developmental phase, the fetus and neonate represent key cohorts of concern (O'Hara and Hart 2018). Because of the discrete nature of hair growth, there can be a temporal disconnect between Hg concentrations in blood and hair, necessitating an understanding of how tissue concentrations relate to each other and how potential differences affect risk assessment. These two tissues are often, but not always, in agreement, with the strength of the relationship between blood and hair [THg] affected by age class, weaning status, growth, and temporal distances in sampling of the two tissues (Peterson et al. 2016). Thus, there is a need for continued improvement in our understanding of how tissue concentrations relate to each other in specific species and age classes, particularly for neonates where gestational exposure to $MeHg^+$ via maternal diet occurs at a key phase for adverse effects (e.g., during neurogenesis).

We measured [THg] in whole blood (hereafter referred to as blood) and hair of three stranded pinniped species admitted to The Marine Mammal Center (TMMC; Sausalito, California, USA) to determine the relationships between blood and hair [THg] and evaluate the value of combined sampling (hair and blood) for risk assessment. Sampling of blood included traditional storage of frozen blood and collection of blood-soaked filter paper (FP) for comparison (Hansen et al. 2014). Additionally, we describe species, temporal, and geographic differences in blood and hair [THg] and how concentrations relate to published threshold values for toxicosis in wildlife, with a focus on harbor seal (HS) pups (*Phoca vitulina*). Previous studies on wild-caught pinnipeds from this region have documented relatively high [THg] with some spatial differences (McHuron et al. 2014, 2016; Peterson et al. 2016), which for harbor seals is likely due to historical contamination of estuaries (e.g., San Francisco Bay) resulting from mining activities (Conaway et al. 2003; Gehrke et al. 2011). Direct assessments of adverse outcomes in HS pups at TMMC

during a 1-yr pilot project suggested an association between neurologic impairment and blood but not hair [THg] (Van Hoomissen et al. 2015), necessitating an understanding of temporal and spatial variability in [THg] to aid in identification of at-risk individuals or groups. Our final objective was to compare [THg] in stranded HS pups with their wild counterparts to understand whether concentrations in stranded seals are representative of population-level exposure.

MATERIALS AND METHODS

Sample collection

Blood and hair samples were collected from live-stranded HS pups in 2008 and 2012–15, northern elephant seals (NES; *Mirounga angustirostris*) in 2013, and California sea lions (CSL; *Zalophus californianus*) in 2013 admitted to TMMC for rehabilitation. Samples were collected within 48 h of admission as described in Field et al. (2018), with the exception of 2008 samples, which were collected just before release. Information recorded at admittance included stranding location, age class, weight, and the presence of lanugo coat (HS only). One aliquot of mixed blood was applied to Advantec Nobuto™ FP (Toyo Roshi Kaisha, Tokyo, Japan) and air-dried (Curry et al. 2011; Hansen et al. 2014). A second aliquot was stored frozen at –20 C. Hair was stored frozen at –20 C in polyethylene bags until analysis. Hair samples were also collected from wild-caught HS pups in San Francisco Bay and Tomales Bay, California (May–June 2008), with methods described elsewhere (Greig et al. 2014). Samples were shipped to the Wildlife Toxicology Laboratory, University of Alaska Fairbanks for analysis of THg.

Hg analysis

Hair was washed and freeze-dried (FreeZone Plus 6, Labconco, Kansas City, Missouri, USA) using 1% Triton™ X-100 followed by five rinses with ultrapure water (Castellini et al. 2012; Van Hoomissen et al. 2015). Air-dried, blood-soaked FPs were prepared for analysis as described in Hansen et al. (2014). In brief, the narrow blood-absorbing end of the FP was cut at the junction of the narrow and wide end and freeze-dried for 48 h to remove water that may have absorbed during storage and transport because of atmospheric humidity. Matched blood aliquots were stored frozen until analysis. We determined [THg] with a direct mercury analyzer (DMA-80, Milestone Inc., Shelton, Connecticut; USEPA 1998) for hair and blood (Castellini et al. 2012; Rea et al. 2013) and for FP (Hansen et al. 2014). Sample mass was 0.010–0.020 g dry weight (dw) of hair, 0.075–0.100 g wet weight (ww) of blood, and 0.065–0.075 mg of FP (dried strip and blood). Each FP absorbed approximately 100 μL of blood. Mass of dried blood on the FP was determined by subtracting the mean (±SD) mass of an unsoaked, dried FP (0.047±0.002 g) from the total mass of the soaked FP. For comparison with blood, the mass of dried blood on the blood-soaked FP was converted to wet weight assuming a blood water content of 75% for HS, 73% for NES, and 78% for CSL (Castellini and Castellini 1989; Hansen et al. 2014). Quality control included blanks, liquid standards, and standard reference materials in each analytic batch. Mean recoveries from 2012 ranged between 94.6% and 102.2% (Hansen et al. 2014). For 2013–15 samples, the recoveries for liquid standards were 98.1±3.1% (0.1 μg/g) and 103.1±3.6% (1.0 μg/g); the recoveries for standard reference materials were 96.1±4.7% (0.0353 μg/g; Seronorm™, Westbury, New York, USA) for blood, and 96.6±7.2% (0.573 μg/g; IAEA-086, National Research Council, Ottawa, Ontario, Canada) and 101.8±6.9% (23.2 μg/g; IAEA-085) for hair. Detection limit was 0.025 μg/g dw for hair and 0.005 μg/g ww for blood and FP.

Statistical analyses

We used linear regressions to examine the relationships between [THg] in blood and FP and between [THg] in blood and hair, and values from O'Hara et al. (2008) to assess the predictive strength of these relationships ($r^2 \leq 0.35$=none, 0.36–0.55=weak, 0.56–0.75=moderate, and >0.75=strongly predictive). We used a Student's *t*-test to determine whether the slope of the relationship was different from 1.0 (O'Hara et al. 2018) and paired Student's *t*-tests to assess differences in [THg] between blood and FP. An analysis of covariance was used to determine whether [THg] in paired blood and FP samples differed among species, with blood THg included as a covariate to account for potential species differences in [THg] that could influence the magnitude of the offset between the two methods (i.e., we were interested in species differences unrelated to absolute [THg]). We used an analysis of variance to test for the effect of year on differences in [THg] determined from blood and FP in HS pups, the only species with data across multiple years. Post hoc comparisons were conducted by Tukey's honest significant difference test.

We used analyses of variance to examine species differences in [THg] and the influence of year and stranding location on [THg] of HS pups. The stranding location of each seal was assigned to one of two geographic regions based

TABLE 1. Age class, mean admittance mass, and the number of blood and hair samples collected from stranded pinnipeds admitted to The Marine Mammal Center (Sausalito, California, USA), including California sea lions (*Zalophus californianus*) in 2013, harbor seals (*Phoca vitulina*) in 2008 and 2012–15, and northern elephant seals (*Mirounga angustirostris*) in 2013. Sample sizes are also shown for wild-caught harbor seals.

Species	Age class[a]	Mass ± SD (kg)	No.	
			Blood	Hair
California sea lion	Pup–adult	16.6±5.8–100.2±78.5[b]	19	5
Harbor seal	Pup	9.5±7.1	79	199, 26 (wild)
Northern elephant seal	Pup–yearling	43.0±16.7	30	

[a] Pup refers to seals stranded or captured during the pupping season or shortly thereafter.
[b] Pup and adult mass.

on the findings of McHuron et al. (2014): San Francisco County or counties bordering San Francisco Bay (Sonoma, Marin, Contra Costa, San Mateo) and counties outside of this area. Stranding location is likely a good proxy for birth location because most HS pups strand within the first few weeks of life, in part because of malnutrition (Colegrove et al. 2005), and are unlikely to have the energy stores or experience to undertake significant dispersal from natal sites. We used a Student's *t*-test to determine whether there were differences between [THg] in hair from stranded vs. captive pups sampled in the same year (2008) and from the same geographic area.

Lower and upper toxicologic thresholds of concern were selected from the literature to assess how the use of different methods (blood vs. FP) and matrices (blood vs. hair) affected the overall conclusion with respect to an individual's toxicologic risk and to understand how these risks varied among species. We used threshold values of 0.2 μg/g and 0.5 μg/g ww for blood and 20 μg/g and 30 μg/g dw for hair (O'Hara and Hart 2018). Point estimates and 95% confidence intervals (CIs) for prevalence (percentage above or below a threshold value; Rickard et al. 1999) were calculated for each species by the *binconf* function in the R package Hmisc (Harrell 2018).

All statistical analyses were conducted by R version 3.5.0 (R Development Core Team 2018). Statistical assumptions were assessed by residual and quantile-quantile plots and were met for all analyses. Statistical significance was concluded at $P \leq 0.05$. Total Hg concentrations are presented as microgram per gram ww (blood, FP) and microgram per gram dw (hair).

RESULTS

A total of 228 (blood) and 230 (hair) samples were analyzed for THg (Table 1). The majority of samples were collected from dependent or newly weaned pups with mass at admittance ranging from 5.5 to 227 kg (Table 1).

There was a strong positive relationship between [THg] in blood and FP (r^2=0.98, $P<0.001$; Fig. 1), with a slope that was statistically different from 1.0 (P=0.050). Differences between blood and FP [THg] ranged from –0.08 to 0.10 μg/g, with a mean ± SD of 0.003±0.19 μg/g across all species and years (n=229). The mean difference in [THg] between blood and FP was significantly different from zero (t=2.16, P=0.032), but this difference was largely driven by greater discrepancies in 2012 and 2013; year-specific analyses indicated no difference from zero in 2014 (t=–0.36, P=0.721) and 2015 (t=–1.06, P=0.291). Agreement between the two methods was 97.4% and 100% for the 0.2 and 0.5 μg/g thresholds, respectively. Differences among species in the relationship between [THg] in blood and FP were not significant (intercept: $F_{2,63}$=0.41, P=0.669; slope: $F_{2,61}$=0.14, P=0.873; 2013 only), but the effect of year was significant ($F_{3,175}$=4.72, P=0.003; HS only). This result was primarily driven by differences between 2015 and 2012 (P=0.010) and, to a lesser extent, differences between 2012 or 2013 and the remaining years (2012, P=0.061; 2013, P=0.068), with greater similarity between [THg] in more recent years.

Total Hg concentration in hair was a moderately good predictor of blood [THg] in HS pups (r^2=0.72, $P<0.001$; Fig. 2); the inclusion of coat status (lanugo vs. adult) did

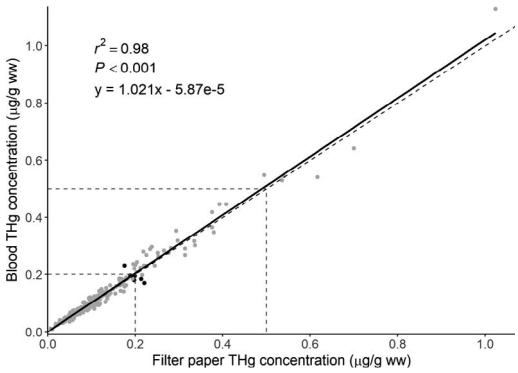

FIGURE 1. The relationship between total mercury (THg) concentrations in whole blood and filter paper for stranded harbor seals (*Phoca vitulina*), northern elephant seals (*Mirounga angustirostris*), and California sea lions (*Zalophus californianus*). The dashed black line indicates a 1:1 relationship, and the black dots correspond to the values that differed in their classification with respect to selected toxicologic thresholds of concern (0.2 and 0.5 μg/g wet weight, dashed gray lines).

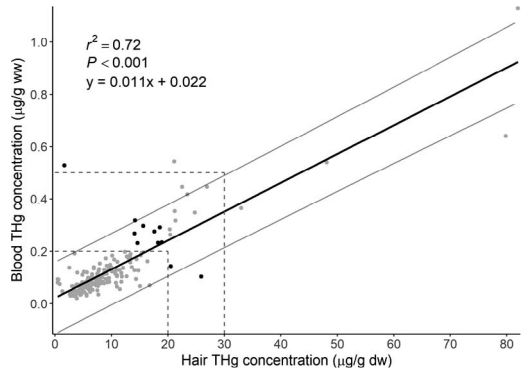

FIGURE 2. The relationship between total mercury (THg) concentrations in whole blood and hair of stranded harbor seal pups (*Phoca vitulina*). The solid gray lines represent the 95% prediction intervals. Points are color-coded on the basis of classification agreement with respect to selected lower (0.2 and 20 μg/g) and upper (0.5 and 30 μg/g) toxicologic thresholds of concern in the blood and hair (dashed gray lines), with black corresponding to points of disagreement between matrices.

not improve this relationship. Agreement between the two tissues was generally good with respect to selected toxicologic thresholds; only 6.3% of seals had [THg] in the two tissues that were classified differently with respect to the lower thresholds (0.2 and 20 μg/g), which decreased to 1.7% for the upper thresholds (0.5 and 30 μg/g). When differences existed, blood [THg] typically exceeded the selected threshold, whereas hair [THg] did not. In most cases, [THg] values were close to the threshold concentration, but three seals had a relatively large mismatch between [THg] in the two tissues (Fig. 2).

There was a wide range in [THg] in blood and hair of stranded seals, with concentrations ranging from <0.01 to 1.13 μg/g in blood and 0.45 to 81.98 μg/g in hair (Table 2). Northern elephant seals had higher blood [THg] compared with HS and CSL ($F_{2,64}$=39.63, P<0.001), although HS had the highest maximum [THg] across all species (Table 2). All three species had individuals with blood [THg] that exceeded the lower toxicologic threshold, with nonoverlapping CIs between NES (40% of seals) and the other two species (HS: 12.3%, CSL: 5.3%), but only HS had concentrations that exceeded the upper threshold (Table 2). Harbor seals had higher mean [THg] in hair than CSL (8.89 vs. 1.69 μg/g, 2013 only) with nonoverlapping CIs (CSL: 1.30–2.02, HS: 4.66–25.92, 2013 only). None of the CSL had hair concentrations exceeding selected toxicologic thresholds, but 10.0% and 4.0% of HS had values exceeding the lower and upper thresholds, respectively. No statistical comparisons were made between species because of the small sample size for sea lions (n=5).

Trends in spatial and temporal differences in hair [THg] of stranded HS pups were inconsistent across stranding locations and years (interaction term, $F_{4,189}$=4.29, P=0.002). Harbor seals that stranded in 2008 and 2013 in San Francisco and surrounding counties had higher concentrations than seals that stranded in counties outside of this area (P<0.001 for each year; Fig. 3). There was no effect of year on hair [THg] of pups that stranded in counties outside of the San Francisco Bay area ($F_{4,123}$=0.58, P=0.678), whereas temporal differences for stranded seals in San Francisco and surrounding counties was primarily due to higher concentrations in 2008 compared with all other years ($F_{4,66}$=6.95, P<0.001; Tukey's honest signifi-

TABLE 2. The mean ± SD and range of total mercury concentrations in blood and hair of live-stranded pinnipeds admitted to The Marine Mammal Center (Sausalito, California, USA), including California sea lions (*Zalophus californianus*) in 2013, harbor seals (*Phoca vitulina*) in 2008 and 2012–15, and northern elephant seals (*Mirounga angustirostris*) in 2013. For each matrix, the point estimate of the prevalence of individuals with values exceeding selected lower and upper toxicologic thresholds of concern are also shown with 95% confidence interval. Lower and upper thresholds of concern were 0.2 and 0.5 μg/g wet weight (ww; blood) and 20 and 30 μg/g dry weight (dw; hair), respectively.[a]

	California sea lion	Harbor seal	Northern elephant seal
Blood (μg/g ww)			
Mean ± SD	0.07±0.06 A	0.13±0.13 A	0.20±0.06 B
Range	<0.01–0.27	0.02–1.13	0.09–0.30
Lower threshold (%)	5.3 (0.3–24.6)	12.3 (8.3–18.0)	40.0 (24.6–57.7)
Upper threshold (%)	0 (<0.01–16.8)	2.8 (1.2–6.4)	0 (<0.01–11.4)
Hair (μg/g dw)			
Mean ± SD	1.69±0.26	10.85±10.62	—
Range	1.30–2.02	0.46–81.98	—
Lower threshold (%)	0 (0–43.4)	10.0 (6.6–15.0)	—
Upper threshold (%)	0 (0–43.4)	4.0 (2.1–7.7)	—

[a] Uppercase letters indicate significant differences among species ($P<0.050$).

cant difference $P<0.010$ for all comparisons). There were no temporal or spatial differences in blood [THg] for 2012–15 (year, $F_{3,171}=1.88$, $P=0.135$; location, $F_{1,171}=1.15$, $P=0.285$). Pups that stranded in San Francisco Bay and surrounding counties in 2008 had significantly greater [THg] than wild-caught pups sampled in the same year and geographic region (25.62 vs. 13.89 μg/g, $t=2.32$, $P=0.040$).

DISCUSSION

We used samples collected from stranded pinnipeds admitted to TMMC as a follow-up to studies documenting Hg exposure at levels of concern, with a focus on the relationships among [THg] with different sampling methods (Hansen et al. 2014) and tissue matrices (Peterson et al. 2016) and identification of target individuals or groups for risk assessment. There was a wide range of [THg] in blood and hair of stranded pinnipeds during our study years, with some values exceeding thresholds of concern. Although many of the individuals in our study were underweight at the time of admittance, a common occurrence in stranded animals that can influence contaminant concentrations in some tissues (Fuglei et al. 2007; Peterson et al. 2018), our results were broadly consistent with studies on wild-caught individuals from California with respect to species ranges and interspecific differences in [THg] (Brookens et al. 2007; McHuron et al. 2014, 2016; Peterson et al. 2015). Although the value of stranded animals in adverse risk and outcome studies is not contingent on their representation of wild populations, it suggests that the findings from future studies involving stranded animals in this region will be directly applicable to wild populations.

Methodological comparisons

The use of FP has shown promise as an alternative field sampling and storage method for measurements of blood THg (Hansen et al. 2014), which is particularly useful for researchers working in remote areas with limited access to electricity or liquid nitrogen for sample processing and storage. Our study extended this initial effort, and although there were slight differences in [THg] between blood and FP, these differences were largely unimportant with respect to toxicology thresholds. The effect of year on the agreement in [THg] between these two methods did not appear to be a consequence of annual variation in [THg] and instead may have been

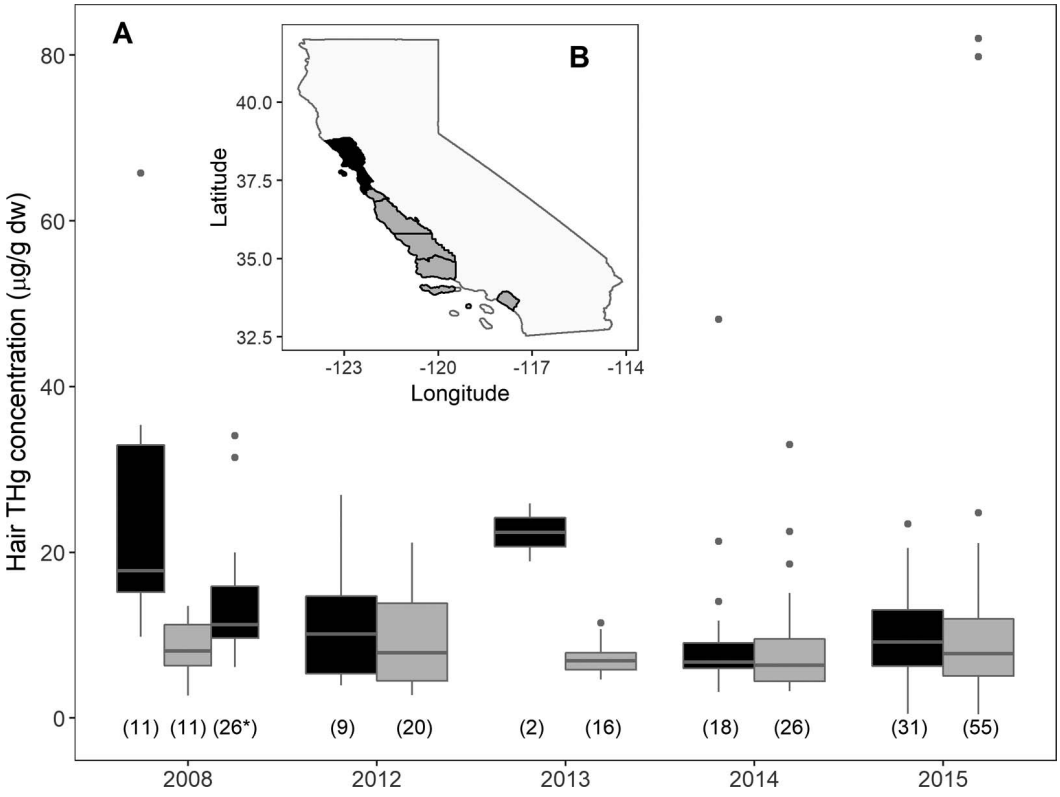

FIGURE 3. Total mercury (THg) concentrations in hair of stranded and wild-caught harbor seal (*Phoca vitulina*) pups. (A) Strandings by year and location. Sample sizes shown in parentheses below each boxplot, with the asterisk denoting wild-caught seals. (B) Stranding locations were binned according to whether (black) or not (gray) a seal stranded in a county that bordered San Francisco Bay. Wild-caught animals were captured in San Francisco Bay and Tomales Bay in 2008.

due to initial user inexperience during sample analysis that improved in later study years. User inexperience also may explain why there was less variability in [THg] between the two methods in our study compared with initial efforts by Hansen et al. (2014), despite our use of assumed species-specific blood water content to convert FP [THg] to a wet weight basis. The relationship between [THg] in blood and FP was not different among species, suggesting this method can be used across a variety of species without further validation, assuming that [THg] values fall within our reliably measured ranges.

Tissue matrix comparisons

The discrete nature of hair growth in pinnipeds can result in a temporal disconnect between blood and hair [THg], particularly for neonates where [THg] in these two tissues are influenced by different pathways at varying developmental stages (i.e., via gestation vs. lactation). Despite this potential disconnect, the strength of the relationship between [THg] in blood and hair of HS pups was relatively strong, with similar predictive power to adult and juvenile HS (Peterson et al. 2016). The use of both tissues may therefore be unnecessary in studies focused on exposure at the population level; however, several individuals displayed discrepancies in [THg] between the two tissues that affected classification with respect to toxicology thresholds. These discrepancies, although likely unimportant for population-level studies, emphasize the importance of sampling blood and hair in adverse effects studies where accurate

assessment of Hg exposure is crucial for study outcomes.

The presence of lanugo had no influence on the relationship between blood and hair [THg], potentially indicating that exposure to Hg from the dam's diet is relatively constant throughout gestation. Consistent exposure may reflect the fact that adult females in many regions, including California, are nonmigratory and have relatively small home ranges (Lowry et al. 2001; Sharples et al. 2012; Womble and Gende 2013). Further investigation of changes in Hg exposure during gestation are warranted, though, particularly because Noël et al. (2016) documented a large increase in [THg] in whiskers of HS pups between mid and late gestation that was attributed to increased fetal blood flow.

Spatial and temporal variability

We detected higher [THg] in hair but not in blood of HS pups that stranded in 2008 in the San Francisco Bay area, a region that suffers from Hg contamination from historic mining activities. The presence of spatial differences in hair but not blood may have reflected the confounding influence of pup health and body condition on circulating [THg]; these factors should not have influenced concentrations in hair because they reflected in utero exposure from maternal diet. The diet of HS in San Francisco Bay varies temporally and spatially (Gibble and Harvey 2015), and higher dependence on estuarine species has been associated with higher [THg] in seals from this location (McHuron et al. 2014). Temporal, spatial, and species-specific differences in [THg] have been detected in forage fish and attributed to variation in fish movements, habitat use, and net production of $MeHg^{+}$ (Greenfield and Jahn 2010; Greenfield et al. 2013). Spatial differences in HS pup exposure for just a single year could thus be due to variation in fish [THg] or variation in maternal behavior that affected dietary exposure, either because of actual interannual dietary variation or perceived variation (i.e., from sampling effects). The relatively consistent [THg] in stranded HS pups from other locations may have resulted from low levels of environmental Hg contamination. The fact that we were able to detect similar spatial patterns in hair [THg] of stranded HS pups as have been found in wild populations (McHuron et al. 2014), at least in one year, suggests that hair samples collected from stranded pinnipeds may indeed have utility in addressing spatial and potentially temporal trends in Hg exposure. The utility of this approach deserves further exploration, because assessment of temporal and spatial trends can be logistically challenging to accomplish with wild-caught animals. Stranding location did not exclude HS pups from having [THg] exceeding toxicologic thresholds, indicating that stranding location alone should not be used to identify high-risk individuals with respect to Hg exposure.

Stranded vs. wild comparisons

Stranded HS pups had higher hair [THg] than wild-caught seals from the same geographic region and year, providing preliminary evidence that samples collected from stranded animals may have reflected the range but not the average THg exposure in wild populations. These differences were surprising given concentrations should have been unaffected by pup health at the time of stranding, and further study is needed to determine potential causes and whether this finding can be generalized to other years and regions.

Conclusions

Phocids from California continue to be exposed to relatively large amounts of Hg on the basis of published toxicology thresholds. Total Hg concentrations in hair and blood of stranded animals were within the range documented in free-ranging animals from California, although future studies addressing the factors that influence Hg exposure in stranded animals (e.g., disease, maternal behavior) are needed to better assess how representative samples collected from stranded animals are of the broader population. The presence of a large number of NES with

blood [THg] exceeding thresholds is concerning, but their utility in adverse effects studies may be limited because of the challenges in quantifying gestational Hg exposure (e.g., elephant seal pups often molt their lanugo coat before stranding). In contrast, stranded HS pups are a promising candidate species because they are a key cohort of concern and exhibit a wide range of [THg], which represents a potential "dose response" scenario for assessing adverse outcomes that span published THg thresholds of interest. The finding that stranded HS pups had higher hair [THg] than wild-caught pups further underscores the utility of using this group in adverse effects studies, because they are the individuals most likely to demonstrate the effects of exposure. Indeed, stranding per se could potentially be a component of an effect. An added benefit of using stranded animals is that comprehensive health assessments are routine at TMMC, allowing researchers to account for the multitude of interacting factors that determine an individual's immune status, body condition, and other health metrics. Intensive clinical assessment is necessary to determine the role of Hg in stranding and rehabilitation outcomes of stranded HS pups, and our group is following up on this study by using improved and taxonomically relevant neurologic examinations, as well as high-quality clinical and pathologic assessment.

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