SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 35, No. 8, pp. 2103–2110, 2016
© 2016 SETAC
Printed in the USA

MERCURY CORRELATIONS AMONG BLOOD, MUSCLE, AND HAIR OF NORTHERN ELEPHANT SEALS DURING THE BREEDING AND MOLTING FASTS

SARAH H. PETERSON,*† JOSHUA T. ACKERMAN,‡ and DANIEL P. COSTA†
†Department of Ecology and Evolutionary Biology, University of California, Santa Cruz, Santa Cruz, California, USA
‡Dixon Field Station, Western Ecological Research Center, US Geological Survey, Dixon, California, USA

(Submitted 14 October 2015; Returned for Revision 9 December 2015; Accepted 7 January 2016)

Abstract: Mercury (Hg) biomonitoring and toxicological risk assessments for marine mammals commonly sample different tissues, making comparisons with toxicity benchmarks and among species and regions difficult. Few studies have examined how life-history events, such as fasting, influence the relationship between total Hg (THg) concentrations in different tissues. The authors evaluated the relationships between THg concentrations in blood, muscle, and hair of female and male northern elephant seals (*Mirounga angustirostris*) at the start and end of the breeding and molting fasts. The relationships between tissues varied among tissue pairs and differed by sampling period and sex. Blood and muscle were generally related at all time periods; however, hair, an inert tissue, did not strongly represent the metabolically active tissues (blood and muscle) at all times of year. The strongest relationships between THg concentrations in hair and those in blood or muscle were observed during periods of active hair growth (end of the molting period) or during time periods when internal body conditions were similar to those when the hair was grown (end of the breeding fast). The results indicate that THg concentrations in blood or muscle can be translated to the other tissue type using the equations developed but that THg concentrations in hair were generally a poor index of internal THg concentrations except during the end of fasting periods. *Environ Toxicol Chem* 2016;35:2103–2110. © 2016 SETAC

Keywords: Bioaccumulation Tissue correlation Biomonitoring Marine mammal Pinniped

INTRODUCTION

Mercury (Hg) is of concern in the environment because it is widespread, bioaccumulates in organisms, and negatively affects a wide range of taxa [1,2]. Concentrations are increasing in ocean waters [3,4] and marine organisms [5,6]. Aquatic predators are at an elevated risk of Hg bioaccumulation, especially those at high trophic positions [7,8]. Consequently, contaminant monitoring programs for ocean environments often sample marine mammals [7,9]. Because marine mammals have relatively long life spans and smaller population sizes, contaminant monitoring is increasingly reliant on tissues that can be collected nonlethally from live animals. However, the relationships between Hg concentrations in different tissues have not been fully investigated, especially in regard to the breeding and molting time periods when many marine mammals experience substantial weight loss during fasting events [10]. Rapid fluctuations in animal mass in addition to rapid hair growth while molting, similar to feather growth in birds, complicate relationships between Hg concentrations among tissues [11,12]. Few studies on aquatic predators have examined the relationships between Hg in different tissues in the context of fluctuating Hg exposure, body condition, or varying life history events [12–15].

Among tissues, the circulating Hg in metabolically active tissue such as blood or muscle can directly influence the current toxicological risk to an animal [16,17]. In contrast, Hg in keratinous tissues, such as hair or feathers, is inert and no longer biologically available [15] but may indicate past toxicological risk to an animal when the tissue was grown [15,18]. To maximize the interpretation of tissue concentrations in the context of current and past toxicological risk, it is important to understand how concentrations of circulating Hg relate to Hg in inert tissues that are commonly sampled [19]. Having equations available to translate inert tissues, such as hair, into equivalent Hg concentrations in tissues that are directly related to the toxicological risk to the animal, such as blood or muscle, would help in interpreting previous exposure risk to the animal. Moreover, being able to translate Hg concentrations among internal tissues can further help define the toxicological risk to the animal because toxicity benchmarks have only been established for some of the commonly sampled tissues [17,20–22]. Having the capacity to translate toxicity benchmarks among tissues and thus directly compare Hg concentrations in studies that use different tissues would substantially improve the interpretability of contaminant monitoring in marine mammals.

Among marine mammals, pinnipeds (fur seals, sea lions, seals, and walrus) are ubiquitous in the world's oceans and commonly used to sample contaminants in marine environments [7,23,24]. Pinnipeds are upper–trophic level marine predators that forage at sea but depend on terrestrial haul-outs for annual life history events including breeding, lactation, and molting. All pinnipeds undergo an annual molt, during which time their hair is entirely replaced, although the duration of molt varies among species from weeks to months ([25]; R.G. Daniel, 2003, Master's thesis, University of British Columbia, Vancouver, Canada). Some pinniped species undergo extensive fasting periods during breeding and molting [10], whereas the remaining species continuously forage in between relatively brief haul-out periods on land. Among pinnipeds, the northern elephant seal (*Mirounga angustirostris*) is distinctive in that adults annually undergo 2 fasting periods in between extensive foraging migrations [26]. In addition, elephant seals undergo a catastrophic molt, which results in a full replacement of the

* Address correspondence to saepeter@ucsc.edu
Published online 12 January 2016 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/etc.3365

Table 1. Number of northern elephant seals (*Mirounga angustirostris*) with pairs of tissues (muscle and blood, hair and blood, and hair and muscle) analyzed for total mercury concentrations, separated by sex and sampling period[a]

Tissue pairs	Sex	Early breeding	Late breeding	Early molt	Late molt	Total
Muscle and blood	Female	41	53	47	43	184
	Male	13	12	8	7	40
Hair and blood	Female	44	58	52	48	202
	Male	22	16	13	13	64
Hair and muscle	Female	40	52	48	43	183
	Male	13	11	8	7	39

[a]See Figure 1 for sampling periods. Some individuals were sampled during multiple sampling periods, but no individuals were sampled multiple times during the same sampling period.

epidermis and hair, with the majority of growth occurring over just a couple of weeks [27]. Fluctuations in mass and differences in molting strategies among pinniped species can influence the relationship between Hg in different tissues and thus the interpretation of toxicological risk [12].

We examined total Hg (THg) concentrations in the blood, muscle, and hair of northern elephant seals during 4 distinct time periods (early breeding, late breeding, early molt, and late molt) and assessed the relationships between THg concentrations among tissues. We specifically sampled tissues at the start and end of each of the breeding and molting fasting sampling periods to evaluate the influence of body condition, molt, and sex on the relationships between THg concentrations in each tissue.

METHODS

We sampled blood, muscle, and hair from adult female and subadult 3, subadult 4, and adult male age classes (hereafter pooled and referred to as "adult males") [28] of northern elephant seals between 2011 and 2013 at the Año Nuevo colony (Año Nuevo State Reserve, CA, USA) at the start and end of each of the 2 annual fasting periods associated with breeding and molting (Table 1). During the breeding season, adult females were sampled 5 d after parturition (hereafter, "early breeding") and at approximately 23 d after parturition (hereafter, "late breeding"; Figure 1). Females typically gave birth within 1 wk of arriving at the colony. Males were sampled during the breeding season approximately 7 d to 14 d into the fast (early breeding) and at approximately 56 d to 70 d into the fast (late breeding). During the molting season, female seals were sampled within several days (2 ± 3 d on shore) of arriving to the colony (hereafter, "early molting") and when hair looked to be fully grown and seals appeared to be ready to depart the colony (hereafter, "late molting"). Males were sampled on arrival to the colony before showing any evidence of molting hair (early molting) and after all hair from the previous molt had been replaced by new hair (late molting), but the exact timing of individual seal arrival for the molt was not monitored as closely for males as for females. Some individuals were sampled during multiple sampling periods, but no individuals were sampled multiple times during the same sampling period. All procedures were approved by the University of California, Santa Cruz, Institutional Animal Care and Use Committee and conducted under National Marine Fisheries Service permit 14636.

We used standard protocols for the sedation of elephant seals [26,29] and the collection of tissue samples for Hg analysis [30,31]. In brief, we used a battery-operated, cordless clipper with a size 40 cryotech stainless steel blade to collect hair samples from the dorsal pelvic region, standardized across animals because Hg concentrations can vary among sampling locations on an animal [32]. We used a sterile 6-mm biopsy punch (Miltex) to collect a muscle biopsy from the lateral pelvic area of each seal (going through the blubber layer to the muscle). Whole blood (hereafter, "blood") was sampled from the extradural vein in 10-mL vacutainers containing the anticoagulant sodium heparin. Muscle and blood samples were stored on

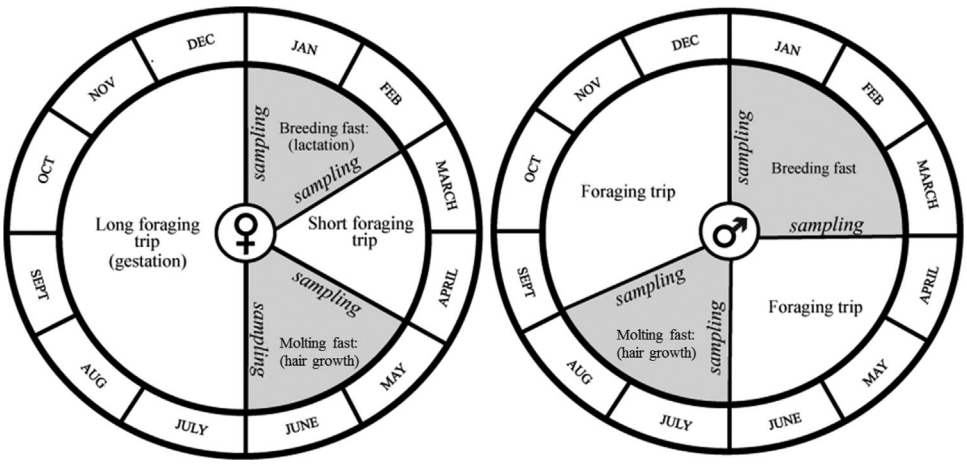

Figure 1. A year in the life of an adult northern elephant seal (*Mirounga angustirostris*; left panel = female, right panel = male). Paired blood, muscle, and hair samples were collected at the start and the end of the breeding and molting fasting periods. Each female seal fasts during the breeding season for approximately 4 wk to 5 wk and for a similar duration while molting. Males fast for a longer duration during the breeding season but approximately the same time while molting. Importantly, individual seals are not on land for the full duration shown in Figure 1 because individuals arrive to the colony at varying times for the start of both fasting periods.

ice in the field and then in polypropylene cryovials at –20 °C until analysis.

We prepared muscle and hair samples using standard protocols for Hg analysis [33]. In brief, muscle samples were thawed, rinsed with deionized water, and gently dried using Kimwipes (Kimberly-Clark). The wet mass of each muscle sample was obtained to the nearest 0.0001 g using an Adventurer Balance model AR0640 (Ohaus). Muscle samples were then dried for 48 h at 50 °C or until completely dried, and samples were reweighed after drying. We cleaned hair by sonicating samples in a 1% mild detergent solution (Alconox), followed by 2 rounds of sonication and rinses with deionized water. Hair samples were then dried for 24 h to 48 h at 50 °C.

We used a DMA-80 Direct Mercury Analyzer (Milestone) to analyze tissue samples for THg at the US Geological Survey Dixon Field Station Mercury Lab, following US Environmental Protection Agency method 7473. We analyzed blood, muscle, and hair samples for THg because the vast majority of Hg in these tissues of predators is methylmercury [6,34–37]. Quality-assurance measures during each batch included reference materials certified by the National Research Council of Canada, Ottawa, Canada (DORM-3, DOLT-3, DOLT-4, or TORT-3), continuing calibration verifications, system and method blanks, and duplicate samples. Recoveries (mean ± standard error) of certified reference materials were 102.4 ± 0.5% ($n = 46$), and recoveries of calibration verifications were 101.4 ± 0.9% ($n = 67$). Duplicate samples had a mean absolute relative percentage difference of 4.1 ± 0.8% ($n = 53$). Concentrations of THg (micrograms per gram) are reported as dry weight for hair and muscle and as wet weight for blood.

To quantify the relationship of THg concentrations between the 3 different seal tissues, we used mixed effects models (R package *nlme*) in the statistical program R, Ver 3.0.2 [38] and *F* tests (R package *afex*) to determine significance. We examined the relationship between pairs of tissues (muscle vs blood, hair vs blood, and hair vs muscle) in adult females and males at the start and end of each of the breeding and molting fasting time periods (4 distinct sampling periods; Figure 1). Paired THg in hair and blood has been presented elsewhere, as part of an overall assessment of hair as a tool for Hg-monitoring programs in pinnipeds [12].

We ran a global model for each pair of tissue THg concentrations with sex and sampling period (early breeding fast, late breeding fast, early molt fast, and late molt fast) as factors and included the following interactions: predictor tissue THg concentration × sex, predictor tissue THg concentration × sampling period, and sex × sampling period. If any of the interactions were significant, we conducted subsequent analyses for each group separately. To account for repeated sampling of the same individual at different sampling periods, individual seal was included as a random effect in all analyses. If subsequent analyses were conducted on separate sampling periods, individuals were no longer repeated and we used general linear models without individual seal as a random effect. To generate predictive equations for the significant relationships between tissues, we removed any remaining nonsignificant variables from the final model. All THg concentrations were natural log–transformed prior to analysis to meet the normality and homogenous variance assumptions of general linear models.

RESULTS

In total, we obtained 224 paired samples of muscle and blood, 266 paired samples of hair and blood, and 222 paired samples of hair and muscle from adult female and male northern elephant seals ($n = 103$ and 49 unique individuals, respectively) during 4 different sampling periods (Table 1). Total Hg concentrations ranged from 0.18 μg/g to 1.16 μg/g wet weight for females and from 0.22 μg/g to 1.27 μg/g wet weight for males in blood, from 1.21 μg/g to 10.15 μg/g dry weight for females and from 2.48 μg/g to 14.79 μg/g dry weight for males in muscle, and from 2.83 μg/g to 32.43 μg/g dry weight for females and from 3.52 μg/g to 75.23 μg/g dry weight for males in hair (Figure 2).

Global models with females and males for all sampling periods

The interaction between sex and sampling period was significant for each of the tissue pairs (muscle to blood $F_{3,210} = 4.31, p = 0.006$; hair to blood $F_{3,228} = 15.35, p < 0.001$; hair to muscle $F_{3,195} = 16.08$, $p < 0.001$), indicating that the relationship between THg concentrations in tissues differed among sampling periods by sex. Thus, we conducted separate statistical analyses for each sampling period.

Mercury relationships between muscle and blood

There was no interaction between THg concentrations in blood and sex in any of the muscle THg concentration models ($p \geq 0.41$); thus, we removed this interaction from all models. Total Hg concentrations in muscle were positively related to THg concentrations in blood during each sampling period (early breeding $F_{1,51} = 66.46$, $p < 0.001$; late breeding $F_{1,62} = 32.29$, $p < 0.001$; early molting $F_{1,52} = 66.02$, $p < 0.001$; late molting $F_{1,47} = 40.30$, $p < 0.001$), while accounting for the effect of sex (early breeding $F_{1,51} = 0.03$, $p = 0.87$; late breeding $F_{1,62} = 10.02$, $p = 0.002$; early molting $F_{1,52} = 13.34$, $p < 0.001$; late molting $F_{1,47} = 0.05$, $p = 0.82$; Figure 2A–D). The significant sex effect during late breeding and early molting showed that the intercept differed between the sexes and was greater for males than females, indicating that the same THg concentration in blood corresponded to greater THg concentrations in the muscle of males than females. In contrast, there was not a significant sex effect during early breeding and late molting, indicating that similar THg concentrations in blood corresponded to similar THg concentrations in muscle for both males and females at these sampling periods. For all sampling periods, the slopes of the regressions were <1, indicating that higher THg concentrations in muscle corresponded to proportionally lower THg concentrations in blood. The specific equations to predict THg concentrations in muscle from THg concentrations in blood were as follows (Equations 1–6; Figure 2A–D)

$$\text{Early breeding female and male } (R^2 = 0.61):$$
$$\ln\left(\text{Muscle THg}\,\frac{\mu g}{g}\,\text{dry wt}\right) = 0.7723 \times \ln\left(\text{Blood THg}\,\frac{\mu g}{g}\,\text{wet wt}\right) + 2.3422 \quad \text{(Figure 2A)} \tag{1}$$

$$\text{Late breeding female } (R^2 = 0.30):$$
$$\ln\left(\text{Muscle THg}\,\frac{\mu g}{g}\,\text{dry wt}\right) = 0.6851 \times \ln\left(\text{Blood THg}\,\frac{\mu g}{g}\,\text{wet wt}\right) + 1.8836 \quad \text{(Figure 2B)} \tag{2}$$

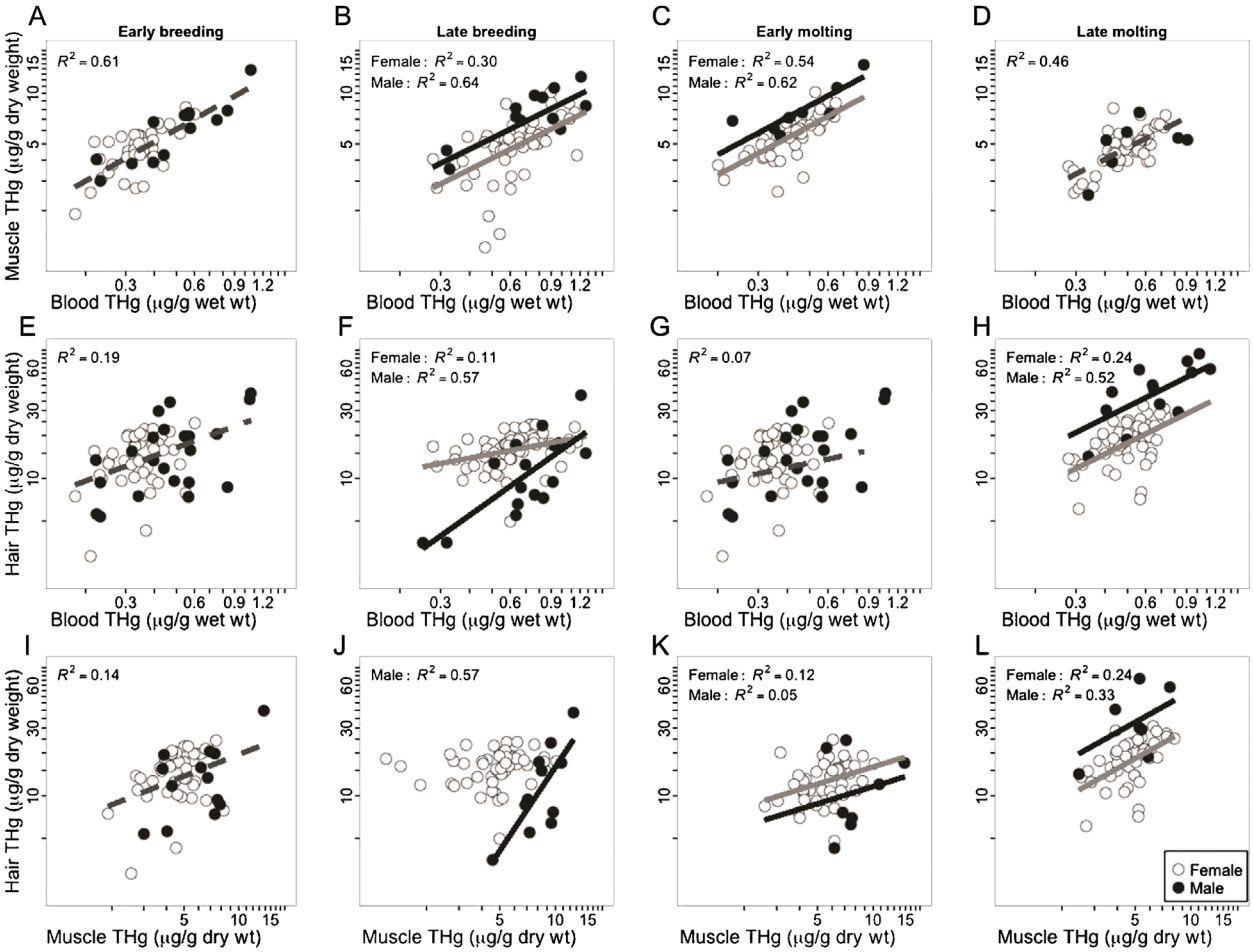

Figure 2. Relationships between total mercury (THg) concentrations in muscle and blood (**A–D**), hair and blood (**E–H**), and hair and muscle (**I–L**) of adult female and male northern elephant seals (*Mirounga angustirostris*). Regression lines indicate significant relationships ($p < 0.05$) between 2 tissues during a particular sampling period; if there was a significant difference in the slope or the intercept between females and males, the relationship and R^2 value for both sexes are shown. Males are solid circles and black lines, whereas females are open symbols and solid gray lines. Dashed lines represent the relationship for both males and females when they were not significantly different. Note that there was not a significant relationship between the tissues for female seals in (**J**).

$$\text{Late breeding male } (R^2 = 0.64):$$
$$\ln\left(\text{Muscle THg}\,\frac{\mu g}{g}\,\text{dry wt}\right) = 0.6851 \times \ln\left(\text{Blood THg}\,\frac{\mu g}{g}\,\text{wet wt}\right) + 2.2251 \quad \text{(Figure 2B)} \tag{3}$$

$$\text{Early molting female } (R^2 = 0.54):$$
$$\ln\left(\text{Muscle THg}\,\frac{\mu g}{g}\,\text{dry wt}\right) = 0.7357 \times \ln\left(\text{Blood THg}\,\frac{\mu g}{g}\,\text{wet wt}\right) + 2.3638 \quad \text{(Figure 2C)} \tag{4}$$

$$\text{Early molting male } (R^2 = 0.62):$$
$$\ln\left(\text{Muscle THg}\,\frac{\mu g}{g}\,\text{dry wt}\right) = 0.7357 \times \ln\left(\text{Blood THg}\,\frac{\mu g}{g}\,\text{wet wt}\right) + 2.6434 \quad \text{(Figure 2C)} \tag{5}$$

$$\text{Late molting female and male } (R^2 = 0.46):$$
$$\ln\left(\text{Muscle THg}\,\frac{\mu g}{g}\,\text{dry wt}\right) = 0.7075 \times \ln\left(\text{Blood THg}\,\frac{\mu g}{g}\,\text{wet wt}\right) + 2.0451 \quad \text{(Figure 2D)} \tag{6}$$

Mercury relationships between hair and blood

There was a significant interaction between THg concentrations in blood and sex for late breeding ($F_{1,70} = 13.66$, $p < 0.001$) but not for the other 3 sampling periods ($p \geq 0.22$); therefore, we conducted separate analyses for males and females for the late breeding sampling period and removed the interaction term for the other sampling periods. During late breeding, THg concentrations in hair were positively related to THg concentrations in blood for males ($F_{1,14} = 18.39$, $p < 0.001$) and females ($F_{1,56} = 7.31$, $p = 0.009$; Figure 2E–H), although the relationships were not as strong overall as those between muscle and blood. Total Hg concentrations in hair also were positively related to THg concentrations in blood during early breeding ($F_{1,63} = 15.87$, $p < 0.001$), early molting

($F_{1,62} = 6.75$, $p = 0.012$), and late molting ($F_{1,58} = 28.98$, $p < 0.001$), while accounting for the effect of sex (early breeding $F_{1,63} = 1.19$, $p = 0.28$; early molting $F_{1,62} = 3.75$, $p = 0.06$; late molting $F_{1,58} = 28.33$, $p < 0.001$; Figure 2E–H). The significant sex effect at late molting showed that the intercept differed between the sexes and was greater for males than females, indicating that greater THg concentrations in hair of males than females were predicted for the same THg concentrations in blood. In contrast, the lack of a significant sex effect during early breeding and early molting indicated that similar THg concentrations in hair were observed for both males and females at the same THg concentrations in blood. The slopes of the regressions were <1 for all sampling periods except for late breeding males, indicating that higher THg concentrations in blood corresponded to proportionally lower THg concentrations in hair. For late breeding males, the opposite was observed, with a slope >1, and greater THg concentrations in hair corresponded to lower THg concentrations in blood. The specific equations to predict THg concentrations in hair from THg concentrations in blood were as follows (Equations 7–12; Figure 2E–H)

$$\text{Early breeding female and male } (R^2 = 0.19): \ln\left(\text{Hair THg}\frac{\mu g}{g}\text{dry wt}\right) = 0.5884 \times \ln\left(\text{Blood THg}\frac{\mu g}{g}\text{wet wt}\right) + 3.206 \text{ (Figure 2E)} \quad (7)$$

$$\text{Late breeding female } (R^2 = 0.11): \ln\left(\text{Hair THg}\frac{\mu g}{g}\text{dry wt}\right) = 0.3062 \times \ln\left(\text{Blood THg}\frac{\mu g}{g}\text{wet wt}\right) + 2.910 \text{ (Figure 2F)} \quad (8)$$

$$\text{Late breeding male } (R^2 = 0.57): \ln\left(\text{Hair THg}\frac{\mu g}{g}\text{dry wt}\right) = 1.1615 \times \ln\left(\text{Blood THg}\frac{\mu g}{g}\text{wet wt}\right) + 2.768 \text{ (Figure 2F)} \quad (9)$$

$$\text{Early molting female and male } (R^2 = 0.07): \ln\left(\text{Hair THg}\frac{\mu g}{g}\text{dry wt}\right) = 0.3424 \times \ln\left(\text{Blood THg}\frac{\mu g}{g}\text{wet wt}\right) + 2.788 \text{ (Figure 2G)} \quad (10)$$

$$\text{Late molting female } (R^2 = 0.24): \ln\left(\text{Hair THg}\frac{\mu g}{g}\text{dry wt}\right) = 0.8127 \times \ln\left(\text{Blood THg}\frac{\mu g}{g}\text{wet wt}\right) + 3.437 \text{ (Figure 2H)} \quad (11)$$

$$\text{Late molting male } (R^2 = 0.52): \ln\left(\text{Hair THg}\frac{\mu g}{g}\text{dry wt}\right) = 0.8127 \times \ln\left(\text{Blood THg}\frac{\mu g}{g}\text{wet wt}\right) + 4.023 \text{ (Figure 2H)} \quad (12)$$

Mercury relationships between hair and muscle

There was a significant interaction between THg concentrations in muscle and sex for late breeding ($F_{1,59} = 22.20$, $p < 0.001$) but not for the other 3 sampling periods ($p \geq 0.59$); therefore, we conducted separate analyses for males and females during late breeding and removed the interaction term for the other sampling periods. During late breeding, THg concentrations in hair were positively related to THg concentrations in muscle for males ($F_{1,9} = 12.10$, $p = 0.007$) but not for females ($F_{1,50} = 1.06$, $p = 0.31$), in contrast to the same time period for hair and blood. Total Hg concentrations in hair were positively related to THg concentrations in muscle during early breeding ($F_{1,50} = 10.65$, $p = 0.002$), early molting ($F_{1,53} = 5.57$, $p = 0.022$), and late molting ($F_{1,47} = 17.07$, $p < 0.001$), while accounting for the effect of sex (early breeding $F_{1,50} = 2.64$, $p = 0.11$; early molting $F_{1,53} = 4.36$, $p = 0.042$; late molting $F_{1,47} = 17.14$, $p < 0.001$; Figure 2I–L). The significant sex effect differed between early and late molting, indicating that THg concentrations in hair were greater for females than males early in the molt but greater for males than females late in the molt at similar THg concentrations in muscle. There was no significant effect of sex during early breeding. Similar to the relationships between THg in hair and blood, the slopes of the regressions between THg in hair and muscle were <1 for all sampling periods except for late breeding males, indicating that higher THg concentrations in muscle corresponded to proportionally lower THg concentrations in hair. In contrast, the regression for the late breeding males had a slope >1, indicating that higher THg concentrations in hair corresponded to proportionally lower THg concentrations in muscle. The specific equations to predict THg concentrations in hair from THg concentrations in muscle were as follows (Equations 13–18; Figure 2I–L)

$$\text{Early breeding female and male } (R^2 = 0.14): \ln\left(\text{Hair THg}\frac{\mu g}{g}\text{dry wt}\right) = 0.5048 \times \ln\left(\text{Muscle THg}\frac{\mu g}{g}\text{dry wt}\right) + 1.806 \text{ (Figure 2I)} \quad (13)$$

$$\text{Late breeding male } (R^2 = 0.57): \ln\left(\text{Hair THg}\frac{\mu g}{g}\text{dry wt}\right) = 1.9595 \times \ln\left(\text{Muscle THg}\frac{\mu g}{g}\text{dry wt}\right) - 1.744 \text{ (Figure 2J)} \quad (14)$$

$$\text{Early molting female } (R^2 = 0.12): \ln\left(\text{Hair THg}\frac{\mu g}{g}\text{dry wt}\right) = 0.4006 \times \ln\left(\text{Muscle THg}\frac{\mu g}{g}\text{dry wt}\right) + 1.847 \text{ (Figure 2K)} \quad (15)$$

$$\text{Early molting male } (R^2 = 0.05): \ln\left(\text{Hair THg}\frac{\mu g}{g}\text{dry wt}\right) = 0.4006 \times \ln\left(\text{Muscle THg}\frac{\mu g}{g}\text{dry wt}\right) + 1.527 \text{ (Figure 2K)} \quad (16)$$

$$\text{Late molting female } (R^2 = 0.24):$$
$$\ln\left(\text{Hair THg}\frac{\mu g}{g}\text{dry wt}\right) = 0.7269 \times \ln\left(\text{Muscle THg}\frac{\mu g}{g}\text{dry wt}\right) + 1.735 \text{ (Figure 2L)} \quad (17)$$

$$\text{Late molting male } (R^2 = 0.33):$$
$$\ln\left(\text{Hair THg}\frac{\mu g}{g}\text{dry wt}\right) = 0.7269 \times \ln\left(\text{Muscle THg}\frac{\mu g}{g}\text{dry wt}\right) + 2.324 \text{ (Figure 2L)} \quad (18)$$

DISCUSSION

We found strong relationships between THg concentrations in several nonlethally sampled tissues from elephant seals, but the relationships often differed by sampling period and sex. Total Hg concentrations in muscle and blood were strongly related at all sampling periods ($0.30 \leq R^2 \leq 0.64$), whereas THg concentrations in hair and blood as well as hair and muscle were not as strongly related and the strength of the relationship was more variable ($0.02 \leq R^2 \leq 0.57$). This indicates that THg concentrations in the metabolically active tissues closely tracked each other throughout the year, whereas THg concentrations in hair, the inert tissue, did not strongly represent the THg concentrations in the metabolically active tissues at all sampling periods.

Blood and muscle are both metabolically active tissues, although THg concentrations in these tissues may have been influenced differently by physiological processes associated with the sampling periods and variability in the proportional loss or gain of protein versus fat during these life history phases. At the early breeding sampling period, when animals had just returned from an extensive foraging trip, we did not observe a significant effect of sex on the slope or intercept of the relationship between THg in these tissues. Without the nutritional stress associated with fasting, it is probable that THg concentrations in blood and muscle for both sexes were at relative equilibrium during early breeding, similar to observations in nonfasting birds [33]. The concentrations of THg in blood and muscle likely represented a combination of the THg gained while foraging and any redistribution of THg within internal tissues. During late breeding, the relationships between THg concentrations in tissues consistently differed between sexes. This difference in intercept between sexes at the end of the breeding fast may be attributed in part to sex differences in fasting duration [39,40] and the allocation of blubber versus muscle stores during the fast [41,42]. Across the breeding fast, elephant seal females lose approximately 40% of their total mass [39,41] and male elephant seals lose approximately 36% of their total mass [40]; however, they do not lose the same proportion of fat and lean tissue. Protein accounts for approximately 15% of total mass lost for females [41] but only 9% of total mass lost for males [42], likely because of the relatively high (10%) protein content of milk produced while fasting [43]. Significant mass loss can result in increased THg concentrations in blood [31], although comparable longitudinal studies have not been conducted on THg concentrations in muscle. Thus, it is probable that the increased catabolism of protein stores by females over males resulted in an increased relative mobilization of THg from muscle into blood of females. Compared with early in the breeding season, seals had relatively more THg in their blood than muscle at the end of the breeding fast, and this difference was more pronounced in females than males (Figure 2A,B).

Even after an extensive foraging trip following the breeding season, THg concentrations in muscle during early molt remained elevated in males relative to females at similar THg concentrations in blood. This may have been a legacy effect from the same muscle to blood relationship that was observed at the end of the breeding period. Whereas both sexes arrived for the molting period with muscle to blood relationships similar to when they left at the end of breeding (relatively higher THg in male muscle vs female muscle for the same blood THg concentration), the male versus female difference disappeared by the end of the molt (Figure 2C,D). While molting, females and males can integrate THg into hair [15,44], serving as the only real offloading mechanism for males and 1 of 2 annual offloading pathways for females (in addition to maternal transfer either during lactation or through the placenta during gestation [31]).

The inert nature of THg in hair and the dynamic nature of THg in blood and muscle likely caused the weaker relationships between these tissue pairs. The strength of the relationship between hair THg concentrations and blood or muscle THg concentrations was relatively weak ($R^2 \leq 0.19$), except during late molting and for late breeding males. As keratinous tissues grow, they incorporate THg from blood and thus tend to reflect blood THg concentrations at the time of growth [11,15,18] or with a time lag [44]. The catastrophic molt of elephant seals results in a short time period of growth for the entire epidermis and hair layer [27], which may allow THg to be rapidly depurated into hair and result in a stronger relationship with internal tissues at the time of hair growth (late molt) but a weak relationship with internal tissues at any other time of the year. In the present study, females were sampled when hair growth was likely completed, whereas males were sampled slightly earlier during molt when hair growth may not have been fully finished. Slight differences between the sexes in the timing of sampling during molt may have resulted in the improved relationships between hair and blood or muscle observed for males versus females and highlight that THg concentrations in hair of northern elephant seals may only represent internal tissue THg concentrations during a very short time period (days) when hair is actively growing. In males, THg concentrations in hair were related to blood and muscle at the end of the breeding fast as well. This suggests that internal conditions in male elephant seals during late breeding had returned to similar conditions as when the hair was grown during the previous molt. It is unclear why female elephant seals did not demonstrate the same relationship during late breeding.

The present study's results demonstrate how life history events can decouple the relationship between THg concentrations in animal tissues. Total Hg concentrations in hair were weakly related to THg concentrations in metabolically active tissues, except immediately after hair growth at the end of the molt and when male blood and muscle THg concentrations were elevated at the end of the breeding fast. For animals with similar life history strategies to the northern elephant seal, THg concentrations in hair may not be suitable as an index of current toxicological risk, except for when hair is actively being grown or internal conditions are similar to conditions when hair was grown. On the other hand, the present study's results indicate that blood is a suitable matrix for sampling because it represents

THg levels in other internal tissues throughout the year, is more commonly and less invasively collected than muscle, and is representative of current ecotoxicological risk.

The utility of the observed relationships between THg concentrations in different tissues is that they can be used to estimate THg concentrations in one tissue from another tissue. In general, THg concentrations in muscle are typically well correlated to THg concentrations in liver and kidney [30,33,45], where Hg concentrations have been more associated with toxicity benchmarks [17]. We found that THg concentrations in muscle were well related to THg concentrations in blood, suggesting that either of these tissues can be sampled to represent circulating Hg in seals. For example, marine mammals strand and die on beaches, at which time it is no longer feasible to sample blood. However, with a predictable relationship between these tissues, muscle samples could be used to estimate THg concentrations in blood, the metabolically active tissue with the most established toxicity benchmarks [22]. As such, the equations presented in the present study represent options for predictive equations to use, and researchers can select the most appropriate equation on a case-by-case basis. For example, the relationship between THg in blood and muscle late in fasting may be similar to that of stranded and nutritionally stressed animals, whereas otherwise healthy animals that died from other causes, such as predation or entanglement, would be better represented by an early fasting relationship. The utility of these equations can be improved by quantifying these same relationships in other marine mammal species to ascertain similarity among species. Although it is critical that estimation of Hg for one tissue versus another for any species be done with caution and transparency, the increased ability to directly compare Hg concentrations in studies that sampled different tissues will improve the framework for Hg monitoring in marine mammals and predictions of toxicological risk.

Acknowledgment—We are appreciative of the hard work of many people who supported the present study, especially D. Crocker, P. Robinson, C. Goetsch, L. Hückstädt, L. McHuron, M. Tift, M. Peterson, X. Rojas-Rocha, P. Morris, R. Condit, and the docents and rangers at Año Nuevo State Reserve. We thank R. Keister, T. Watts, and M. Herzog for help in the Hg laboratory. Funding was provided to D.P. Costa by grants N00014-13-1-0134 and N00014-10-1-0356 from ONR; to S.H. Peterson by a NRS Mathias Grant, the Rebecca and Steve Sooy Graduate Fellowship, the ARCS Foundation, Friends of Long Marine Laboratory, and the Myers Oceanographic Trust; and to J.T. Ackerman from the US Geological Survey Western Ecological Research Center.

Disclaimer—We declare no conflict of interest. The use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the US government.

Data availability—Data can be made available on request to Sarah H. Peterson (saepeter@ucsc.edu).

REFERENCES

1. Boening DW. 2000. Ecological effects, transport, and fate of mercury: A general review. *Chemosphere* 40:1335–1351.
2. Scheuhammer AM, Meyer MW, Sandheinrich MB, Murray MW. 2007. Effects of environmental methylmercury on the health of wild birds, mammals, and fish. *Ambio* 36:12–19.
3. Sunderland EM, Krabbenhoft DP, Moreau JW, Strode SA, Landing WM. 2009. Mercury sources, distribution, and bioavailability in the north Pacific Ocean: Insights from data and models. *Global Biogeochem Cycles* 23:GB2010. DOI: 10.1029/2008GB003425
4. Streets DG, Devane MK, Lu Z, Bond TC, Sunderland EM, Jacob DJ. 2011. All-time releases of mercury to the atmosphere from human activities. *Environ Sci Technol* 45:10485–10491.
5. Monteiro LR, Furness RW. 1997. Accelerated increase in mercury contamination in north Atlantic mesopelagic food chains as indicated by time series of seabird feathers. *Environ Toxicol Chem* 16: 2489–2493.
6. Dietz R, Born EW, Rigét F, Aubail A, Sonne C, Drimmie R, Basu N. 2011. Temporal trends and future predictions of mercury concentrations in northwest Greenland polar bear (*Ursus maritimus*) hair. *Environ Sci Technol* 45:1458–1465.
7. Braune B, Chételat J, Amyot M, Brown T, Claydon M, Evans M, Fisk A, Gaden A, Girard C, Hare A, Kirk J, Lehnherr I, Letcher R, Loseto L, Macdonald R, Mann E, McMeans B, Muir D, O'Driscoll N, Poulain A, Reimer K, Stern G. 2015. Mercury in the marine environment of the Canadian Arctic: Review of recent findings. *Sci Total Environ* 509–510:67–90.
8. McGrew AK, Ballweber LR, Moses SK, Stricker CA, Beckmen KB, Salman MD, O'Hara TM. 2014. Mercury in gray wolves (*Canis lupus*) in Alaska: Increased exposure through consumption of marine prey. *Sci Total Environ* 468:609–613.
9. Bossart GD. 2011. Marine mammals as sentinel species for oceans and human health. *Vet Pathol Online* 48:676–690.
10. Champagne CD, Crocker DE, Fowler MA, Houser DS. 2012. Fasting physiology of the pinnipeds: The challenges of fasting while maintaining high energy expenditure and nutrient delivery for lactation. In McCue MD, ed, *Comparative Physiology of Fasting, Starvation, and Food Limitation*. Springer, New York, pp 309–336.
11. Ackerman JT, Eagles-Smith CA, Herzog MP. 2011. Bird mercury concentrations change rapidly as chicks age: Toxicological risk is highest at hatching and fledging. *Environ Sci Technol* 45:5418–5425.
12. Peterson SH, McHuron EA, Kennedy SN, Ackerman JT, Rea LD, Castellini JM, O'Hara TM, Costa DP. 2015. Evaluating hair as a predictor of blood mercury: The influence of ontogenetic phase and life history in pinnipeds. *Arch Environ Contam Toxicol* 70:28–45.
13. Hobson KA, Riget FF, Outridge PM, Dietz R, Born E. 2004. Baleen as a biomonitor of mercury content and dietary history of north Atlantic minke whales (*Balaenopetra acutorostrata*): Combining elemental and stable isotope approaches. *Sci Total Environ* 331:69–82.
14. Lavoie RA, Baird CJ, King LE, Kyser TK, Friesen VL, Campbell LM. 2014. Contamination of mercury during the wintering period influences concentrations at breeding sites in two migratory piscivorous birds. *Environ Sci Technol* 48:13694–13702.
15. Wang W, Evans RD, Hickie BE, Rouvinen-Watt K, Evans HE. 2014. Methylmercury accumulation and elimination in mink (*Neovison vison*) hair and blood: Results of a controlled feeding experiment using stable isotope tracers. *Environ Toxicol Chem* 33:2873–2880.
16. Aschner M, Aschner JL. 1990. Mercury neurotoxicity: Mechanisms of blood-brain barrier transport. *Neurosci Biobehav Rev* 14:169–176.
17. Dietz R, Sonne C, Basu N, Braune B, O'Hara T, Letcher RJ, Scheuhammer T, Andersen M, Andreasen C, Andriashek D. 2013. What are the toxicological effects of mercury in Arctic biota? *Sci Total Environ* 443:775–790.
18. Lewis SA, Furness RW. 1991. Mercury accumulation and excretion in laboratory reared black-headed gull *Larus ridibundus* chicks. *Arch Environ Contam Toxicol* 21:316–320.
19. Hartman CA, Ackerman JT, Herring G, Isanhart J, Herzog M. 2013. Marsh wrens as bioindicators of mercury in wetlands of Great Salt Lake: Do blood and feathers reflect site-specific exposure risk to bird reproduction? *Environ Sci Technol* 47:6597–6605.
20. Basu N, Scheuhammer AM, Sonne C, Letcher RJ, Born EW, Dietz R. 2009. Is dietary mercury of neurotoxicological concern to wild polar bears (*Ursus maritimus*)? *Environ Toxicol Chem* 28:133–140.
21. Rea LD, Castellini JM, Correa L, Fadely BS, O'Hara TM, Hara TMO. 2013. Maternal Steller sea lion diets elevate fetal mercury concentrations in an area of population decline. *Sci Total Environ* 454:277–282.
22. Clarkson TW, Magos L. 2006. The toxicology of mercury and its chemical compounds. *Crit Rev Toxicol* 36:609–662.
23. Peterson SH, Ackerman JT, Costa DP. 2015. Marine foraging ecology influences mercury bioaccumulation in deep-diving northern elephant seals. *Proc R Soc Lond B Biol Sci* 282:20150710.
24. Castellini JM, Rea LD, Lieske CL, Beckmen KB, Fadely BS, Maniscalco JM, O'Hara TM. 2012. Mercury concentrations in hair from neonatal and juvenile Steller sea lions (*Eumetopias jubatus*): Implications based on age and region in this northern Pacific marine sentinel piscivore. *Ecohealth* 9:267–277.
25. Worthy GAJ, Morris PA, Costa DP, Le Boeuf BJ. 1992. Moult energetics of the northern elephant seal (*Mirounga angustirostris*). *J Zool* 227:257–265.
26. Robinson PW, Costa DP, Crocker DE, Gallo-Reynoso JP, Champagne CD, Fowler MA, Goetsch C, Goetz KT, Hassrick JL, Hückstädt LA, Kuhn CE, Maresh JL, Maxwell SM, McDonald BI, Peterson SH,

Simmons SE, Teutschel NM, Villegas-Amtmann S, Yoda K. 2012. Foraging behavior and success of a mesopelagic predator in the northeast Pacific Ocean: Insights from a data rich species, the northern elephant seal. *PLoS One* 7:e36728.
27. Ling JK. 2012. The skin and hair of the southern elephant seal, *Mirounga leonina* (Linn.). IV. Annual cycle of pelage follicle activity and moult. *Aust J Zool* 60:259–271.
28. Le Boeuf BJ. 1974. Male-male competition and reproductive success in elephant seals. *Am Zool* 14:163–176.
29. Le Boeuf BJ, Crocker DE, Costa DP, Blackwell SB, Webb PM, Houser DS. 2000. Foraging ecology of northern elephant seals. *Ecol Monogr* 70:353–382.
30. Brookens TJ, O'Hara TM, Taylor RJ, Bratton GR, Harvey JT. 2008. Total mercury body burden in Pacific harbor seal, *Phoca vitulina richardii*, pups from central California. *Mar Pollut Bull* 56:27–41.
31. Habran S, Debier C, Crocker DE, Houser DS, Das K. 2011. Blood dynamics of mercury and selenium in northern elephant seals during the lactation period. *Environ Pollut* 159:2523–2529.
32. McHuron EA, Harvey JT, Castellini JM, O'Hara TM. 2012. Hair sampling location in harbor seals (*Phoca vitulina*) affects selenium and mercury concentrations: Implications for study design of trace element determination in pinnipeds. *Mar Pollut Bull* 64: 2519–2522.
33. Eagles-Smith CA, Ackerman JT, Adelsbach TL, Takekawa JY, Miles AK, Keister RA. 2008. Mercury correlations among six tissues for four waterbird species breeding in San Francisco Bay, California, USA. *Environ Toxicol Chem* 27:2136–2153.
34. Wagemann R, Trebacz E, Hunt R, Boila G. 1997. Percent methylmercury and organic mercury in tissues of marine mammals and fish using different experimental and calculation methods. *Environ Toxicol Chem* 16:1859–1866.
35. Woshner V, Knott K, Wells R, Willetto C, Swor R, O'Hara T. 2008. Mercury and selenium in blood and epidermis of bottlenose dolphins (*Tursiops truncatus*) from Sarasota Bay, FL: Interaction and relevance to life history and hematologic parameters. *Ecohealth* 5:360–370.
36. Voegborlo RB, Matsuyama A, Adimado AA, Akagi H. 2010. Head hair total mercury and methylmercury levels in some Ghanaian individuals for the estimation of their exposure to mercury: Preliminary studies. *Bull Environ Contam Toxicol* 84:34–38.
37. Kehrig HA, Malm O, Akagi H, Guimarães JRD, Torres JPM. 1998. Methylmercury in fish and hair samples from the Balbina Feservoir, Brazilian Amazon. *Environ Res* 77:84–90.
38. R Development Core Team. 2012. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. [cited 2015 June 1]. Available from: http://www.R-project.org
39. Costa DP, Le Boeuf BJ, Huntley AC, Ortiz CL. 1986. The energetics of lactation in the northern elephant seal, *Mirounga angustirostris*. *J Zool* 209:21–33.
40. Deutsch CJ, Haley MP, Le Boeuf BJ. 1990. Reproductive effort of male northern elephant seals: Estimates from mass loss. *Can J Zool* 68:2580–2593.
41. Crocker DE, Williams JD, Costa DP, J LBB. 2001. Maternal traits and reproductive effort in northern elephant seals. *Ecology* 82:3541–3555.
42. Crocker DE, Houser DS, Webb PM. 2012. Impact of body reserves on energy expenditure, water flux, and mating success in breeding male northern elephant seals. *Physiol Biochem Zool* 85:11–20.
43. Davis TA, Nguyen HV, Costa DP, Reeds PJ. 1995. Amino acid composition of pinniped milk. *Comp Biochem Physiol B Biochem Mol Biol* 110:633–639.
44. Lieske CL, Moses SK, Castellini JM, Klejka J, Hueffer K, O'Hara TM. 2011. Toxicokinetics of mercury in blood compartments and hair of fish-fed sled dogs. *Acta Vet Scand* 53:66.
45. Ikemoto T, Kunito T, Watanabe I, Yasunaga G, Baba N, Miyazaki N, Petrov EA, Tanabe S. 2004. Comparison of trace element accumulation in Baikal seals (*Pusa sibirica*), Caspian seals (*Pusa caspica*) and northern fur seals (*Callorhinus ursinus*). *Environ Pollut* 127:83–97.