PROCEEDINGS B

Research

Author for correspondence:
Sarah H. Peterson
e-mail: saepeter@ucsc.edu

THE ROYAL SOCIETY
PUBLISHING

Foraging and fasting can influence contaminant concentrations in animals: an example with mercury contamination in a free-ranging marine mammal

Sarah H. Peterson[1,2,3], Joshua T. Ackerman[3], Daniel E. Crocker[4] and Daniel P. Costa[1]

[1]Department of Ecology and Evolutionary Biology, and [2]Institute of Marine Science, University of California, Santa Cruz, Santa Cruz, CA, USA
[3]US Geological Survey, Western Ecological Research Center, Dixon Field Station, Dixon, CA, USA
[4]Department of Biology, Sonoma State University, Rohnert Park, CA, USA

(iD) SHP, 0000-0003-2773-3901

Large fluctuations in animal body mass in relation to life-history events can influence contaminant concentrations and toxicological risk. We quantified mercury concentrations in adult northern elephant seals (*Mirounga angustirostris*) before and after lengthy at sea foraging trips ($n = 89$) or fasting periods on land ($n = 27$), and showed that mercury concentrations in blood and muscle changed in response to these events. The highest blood mercury concentrations were observed after the breeding fast, whereas the highest muscle mercury concentrations were observed when seals returned to land to moult. Mean female blood mercury concentrations decreased by 30% across each of the two annual foraging trips, demonstrating a foraging-associated dilution of mercury concentrations as seals gained mass. Blood mercury concentrations increased by 103% and 24% across the breeding and moulting fasts, respectively, demonstrating a fasting-associated concentration of mercury as seals lost mass. In contrast to blood, mercury concentrations in female's muscle increased by 19% during the post-breeding foraging trip and did not change during the post-moulting foraging trip. While fasting, female muscle mercury concentrations increased 26% during breeding, but decreased 14% during moulting. Consequently, regardless of exposure, an animal's contaminant concentration can be markedly influenced by their annual life-history events.

1. Introduction

Mercury is a protein-bound contaminant that is widely distributed in the ocean [1] and bioaccumulates in top predators [2,3]. Mercury exposure in animals can cause overt and subclinical health effects [4,5]. Marine vertebrates that occupy upper trophic levels within oceanic food webs are especially vulnerable to bioaccumulation of mercury, and these species accumulate higher concentrations of mercury in their tissues than their terrestrially foraging counterparts [6,7]. Pacific Ocean mercury concentrations are predicted to increase until they reach equilibration with current levels of atmospheric mercury [8].

The concentration of mercury in animal tissues varies in response to environmental exposure. Individuals may bioaccumulate higher quantities of methylmercury (MeHg), the form of mercury that biomagnifies and is most toxic to biological organisms, when foraging in certain habitats [9,10]. For example, MeHg concentrations in fish tissues increase with ocean depth such that animals foraging deeper in the mesopelagic (200–1000 m) region accumulate higher concentrations of mercury than epipelagic (0–200 m) species [11].

2

Consequently, changes in an individual's foraging habitat or diet over time can alter their mercury exposure [12,13].

Whereas most studies of mercury contamination have focused on the likelihood of mercury exposure through ingestion, the life history of animals and associated changes in physiology can also influence contaminant concentrations [14–18]. For example, rapid growth in juvenile birds caused a decrease in mercury concentrations, despite continued consumption of fish containing relatively high levels of mercury [14]. Similarly, blood mercury concentrations in northern elephant seal pups dramatically decreased while they were nursing and undergoing a period of rapid growth [15,19]. In addition, faster growing Atlantic salmon (*Salmo salar*) had lower mercury concentrations than their slower-growing conspecifics [17]. These examples illustrate the large influence that growth in body mass can have on mercury concentrations. By contrast, adults of many marine and terrestrial species, including birds, fishes, turtles, bears, cetaceans and pinnipeds, undergo significant decreases in body mass as a result of extensive fasting periods [20–24] or lengthy migrations with limited food availability [25–28]. Significant proportional changes in body mass for small- or large-bodied species as a result of these life-history events can dramatically influence contaminant concentrations and toxicological risk within individuals [14–18,29].

The northern elephant seal (*Mirounga angustirostris*) undergoes extreme fluctuations in body mass while fasting on the beach during annual breeding and moulting periods, which can each last several weeks to months depending on the individual and sex [20,30,31]. Conversely, seals rapidly accumulate body mass at sea during the post-breeding foraging trip (approx. 75 days for females and 117 days for males) and the post-moulting foraging trip (approx. 219 days for females and 112 days for males) [32,33]. These dramatic variations in body mass associated with foraging and fasting periods can be used to assess the influence of animal physiology on contaminant concentrations. We studied changes in mercury concentrations in blood, muscle and hair of adult northern elephant seals across the time periods associated with the two annual fasting periods on land (breeding and moulting) and the two annual foraging migrations at sea (post-breeding and post-moulting). Northern elephant seals are a relatively tractable study system that can illuminate risk for species undergoing similar physiological conditions and fluctuations in body mass.

2. Material and methods

(a) Animal sampling

To quantify the changes in mercury (Hg) concentrations of whole blood, muscle and hair associated with foraging and fasting by northern elephant seals, we collected samples from the same adult seals. We refer to males falling into the subadult 3, subadult 4 and adult-age classes [34] as adult males. From 2011 to 2014, we used standard protocols to chemically immobilize seals at the Año Nuevo colony (Año Nuevo State Reserve, San Mateo County, CA, USA) [32,33,35] while we collected tissue samples and morphometric measurements, including standard length and mass, although male elephant seals were not weighed owing to their large body size. To decrease the variability associated with sampling specific body locations [36], we standardized the sampling location of hair and muscle across all animals. We used cordless clippers to sample hair from the dorsal pelvic

region, and we used a sterile 6 mm biopsy punch (Miltex, Inc., York, PA, USA) to collect a muscle biopsy from the lateral pelvic area. We collected whole blood (hereafter blood) from the extradural vein into sodium heparin treated vacutainers. Blood and muscle samples were stored at −20°C until analysis. These samples are a subset of tissue concentrations reported previously to describe correlations among total Hg (THg) concentrations in different tissues [29].

In general, all adult seals are on land to breed, after which they take a post-breeding foraging trip. Males and females then return to land to moult but not at the same time, after which they take a post-moulting foraging trip. The post-breeding foraging trip is substantially shorter than the post-moulting trip for females, although the trip lengths are similar for males. At the end of the post-moulting trip, seals are back on land to breed (electronic supplementary material, figure S1).

Before and after foraging, we collected paired samples from 83 adult female seals. We collected 82 paired samples of blood and hair ($n = 51$ post-breeding trip and 31 post-moulting trip) and 69 paired samples of muscle ($n = 42$ post-breeding trip and 27 post-moulting trip). Female seals were sampled a mean 80 ± 13 days apart for their shorter, post-breeding foraging trip (mean mass gain $= 53 \pm 33$ kg) and 240 ± 12 days apart for their longer, post-moulting foraging trip (mean mass gain $= 168 \pm 34$ kg). Additionally, we collected paired samples of blood and hair from six male elephant seals before and after a foraging trip ($n = 4$ post-breeding trip and 2 post-moulting trip), with a mean 131 ± 7 days in between sample collection for the post-breeding foraging trip and 151 ± 24 days in between sample collection for the post-moulting trip.

For the breeding and moulting fasting periods on land, we collected paired early and late fasting samples of blood and hair from 19 adult females ($n = 10$ breeding fast and 9 moulting fast) and muscle from 18 of those females ($n = 9$ each fast). Female seals were sampled a mean 18 ± 1 days apart during the breeding fast (mean mass loss $= 142 \pm 19$ kg) and a mean 36 ± 3 days apart during the moulting fast (mean mass loss $= 113 \pm 14$ kg). During the breeding season, we collected paired early and late fasting samples from male elephant seals of blood ($n = 7$), muscle ($n = 5$) and hair ($n = 8$). Males were on land for longer during the breeding fast than females, and tissues samples were taken 50 ± 8 days apart. Males were not sampled across the moulting fast.

(b) Mercury analysis

We cleaned hair by sonicating samples in a mild detergent [37], rinsed the samples multiple times in deionized water and then dried samples for 24–48 h at 50°C. Muscle samples were thawed, rinsed, gently blotted dry, weighed to obtain a wet mass and then dried for 48 h at 50°C to obtain a final dry weight (dw). Hair, muscle and blood samples were weighed to the nearest 0.0001 g prior to analysis (Mettler Toledo XS105, Columbus, OH, USA). All samples were analysed for THg, because the Hg in these three tissues types is almost entirely MeHg [38–42].

We followed US Environmental Protection Agency Method 7473 and analysed tissue samples for THg using a Milestone DMA-80 Direct Mercury Analyzer (Milestone, Shelton, CT, USA) at the US Geological Survey Dixon Field Station Environmental Mercury Laboratory. Each run of samples included certified reference materials (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 for quality assurance. Recoveries (mean ± standard error) of certified reference materials were $102.4 \pm 0.5\%$ ($n = 46$), recoveries of calibration verifications were $101.4 \pm 0.9\%$ ($n = 67$) and duplicate samples had a mean

absolute relative percentage difference of $4.1 \pm 0.8\%$ ($n = 53$), as reported previously [29]. Concentrations of THg ($\mu g\,g^{-1}$) are reported as dw for hair and muscle and as wet weight (ww) for blood.

(c) Statistical analysis

For females, we used mixed-effects models to examine how tissue concentrations varied within individuals between the start and end of each of the two foraging trips and two fasting periods. We analysed foraging trips separately from fasting periods. For foraging, the sampling period (before versus after foraging), foraging trip (post-breeding versus post-moulting trip) and a sampling period × foraging trip interaction were fixed effects. For fasting, the sampling period (early versus late fasting), fast (breeding versus moulting fast) and a sampling period × fast interaction were fixed effects. Individual seal was included as a random effect in the models to statistically nest samples from the same individual. We determined type III tests of significance with F-statistics using the *afex* package, and degrees of freedom were calculated using the Kenward–Roger method [43]. If the interaction was not significant, we removed it and reran the analysis with type II tests of significance. We conducted post hoc pairwise comparisons using least squares mean estimates in the *lsmeans* R package [44]. We report back-transformed least squares mean estimates and standard errors derived using the delta method. To demonstrate the effect size, we calculated the percentage change in THg concentrations between sampling periods using least squares mean estimates for each sampling period.

For males, we conducted paired t-tests to test for changes in THg concentrations across foraging trips (with both foraging trips combined for analyses of blood and hair THg concentrations) and across the breeding fast.

Secondly, we used general linear models to examine if the proportional change in mass of adult female elephant seals explained the significant differences observed in blood or muscle THg concentrations across foraging and fasting periods. For each individual female, we calculated a proportional change in mass ($Mass_{Final}/Mass_{Initial}$) and a proportional change in tissue THg concentrations ($[THg]_{Final}/[THg]_{Initial}$) across each foraging trip or fasting period. Note that the change in mass is between sampling events and does not represent absolute mass gain while foraging. We ran separate analyses for THg concentrations in blood and muscle across each fasting and foraging period. In addition to proportional mass loss, we examined if the THg concentrations within individuals at the first sampling period influenced the proportional change in THg concentrations across the following fasting or foraging periods. To test this, we used likelihood ratio tests to determine if the addition of the initial THg concentration, from the beginning of the fasting or foraging period, improved the model fit. If the proportional change in THg concentrations was significantly related to the initial THg concentrations, we tested an additional null hypothesis ($\beta = -1$) to verify that the relationship was not a statistical artefact because the initial THg concentration was both a predictor variable and in the denominator of the response variable.

All female THg concentrations and the proportional change in THg concentrations were natural log transformed prior to analysis to meet the assumptions of normality and homogeneous variance. Refer to the electronic supplementary material, table S1 for the sample sizes and summary THg concentrations for females and males at each sampling period and electronic supplementary material, table S2 for a summary of the change in THg concentration and the per cent change in THg concentration across fasting and foraging periods. Analyses were conducted in R v. 3.2.1 [45] and α was set at $p = 0.05$.

3. Results

(a) Mercury concentrations across foraging and fasting periods

(i) Foraging females

THg concentrations in the three tissues did not change in the same manner over the course of the two foraging trips (figure 1; electronic supplementary material, table S2). We did not observe a significant interaction between foraging trip × sampling period (before versus after foraging) on THg concentrations in blood ($F_{1,81.5} = 0.16$, $p = 0.69$) or hair ($F_{1,81.6} = 1.07$, $p = 0.30$), but we observed a significant interaction between foraging trip × sampling period on THg concentrations in muscle ($F_{1,67.9} = 4.72$, $p = 0.03$). Thus, we removed the foraging trip × sampling period interaction for blood and hair. For muscle, we did not test the main effects, but ran pairwise tests on the least squares means for all four sampling periods.

Blood THg concentrations declined while foraging ($F_{1,135.3} = 19.73$, $p < 0.001$; figure 1), with mean blood THg concentrations 31% and 32% lower at the end of the post-breeding and post-moulting foraging trips, respectively. Additionally, mean blood THg concentrations were higher in females at both the start and end of the shorter, post-breeding trip than at the start and end of the longer, post-moulting trip ($F_{1,82.5} = 307.46$, $p < 0.001$; figure 1). We were able to sample eight seals over at least four consecutive time periods. This unique time series demonstrates the same trends in figure 1 but within an individual (figure 2; electronic supplementary material, table S3). Similar to blood, hair THg concentrations declined across each foraging trip ($F_{1,82.6} = 65.83$, $p < 0.001$). Specifically, mean hair THg concentrations declined by 15% and 17% from the start to the end of the post-breeding and post-moulting trips, respectively. Unlike blood, overall hair THg concentrations did not differ between the two foraging trips ($F_{1,124.1} = 1.82$, $p = 0.18$).

In contrast to blood and hair, mean muscle THg concentrations increased by 16% across the post-breeding foraging trip ($t = 2.93$, $p = 0.005$), but were not different between the start and end of the post-moulting foraging trip ($t = 0.44$, $p = 0.66$; figure 1). Additionally, mean THg concentrations in muscle at the start of the two foraging trips were not different ($t = 0.23$, $p = 0.82$), although THg concentrations were 25% higher at the end of the post-breeding trip than at the end of the post-moulting trip ($t = 2.58$, $p = 0.01$).

(ii) Foraging males

Mean blood THg concentrations in males did not significantly decrease from the start to the end of their foraging trips ($t = 2.18$, $p = 0.08$; electronic supplementary material, table S1 and figure S2), although our sample size was limited to only six individuals and conducting a two-tailed t-test resulted in more conservative results. Unlike females, hair THg concentrations did not decrease over the foraging trip ($t = 1.03$, $p = 0.35$).

(iii) Fasting females

THg concentrations changed differently across the two fasting periods, as indicated by the significant interactions we observed between the sampling period (early versus late fast) × fast (breeding versus moulting) for each of the three

3

rspb.royalsocietypublishing.org   Proc. R. Soc. B 285: 20172782
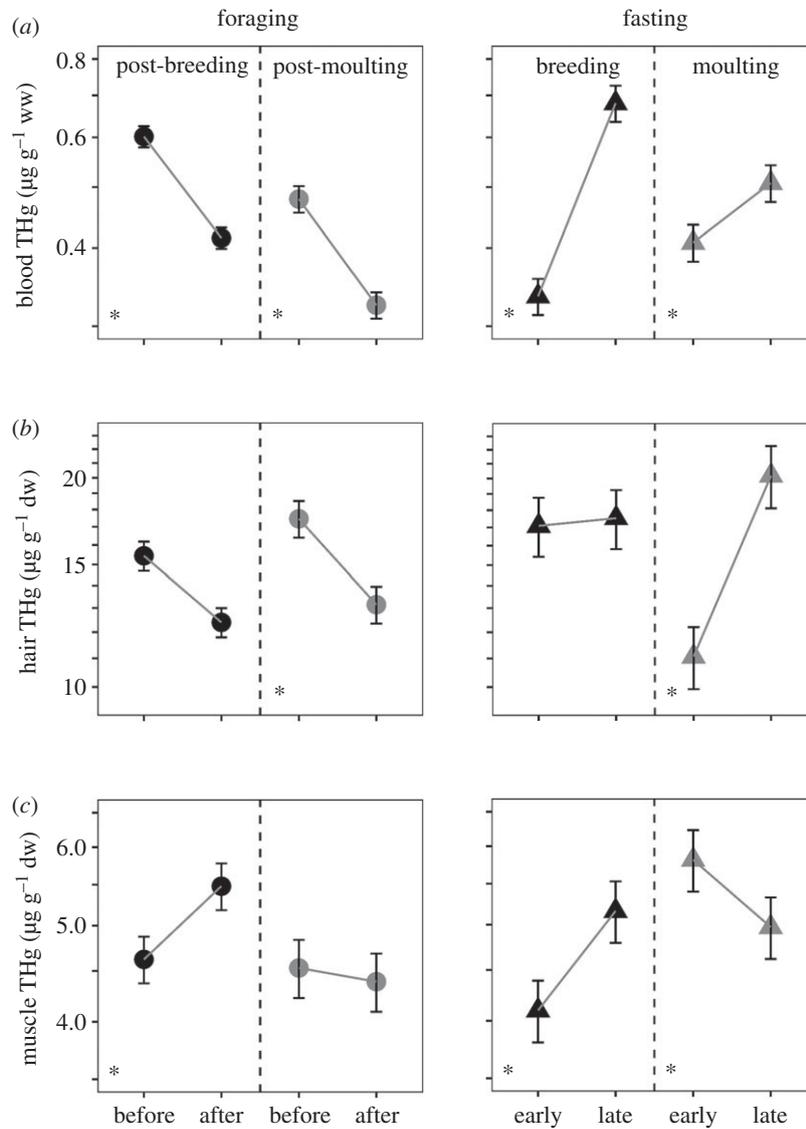
Figure 1. Blood (*a*), hair (*b*) and muscle (*c*) samples were collected from the same adult female northern elephant seals (*M. angustirostris*) before and after one of the two annual foraging trips (the short, post-breeding trip and the long, post-moulting trip) or early and late during one of the two annual fasting periods (breeding and moulting fasts) and analysed for total mercury (THg) concentrations. Model-generated, back-transformed least squares mean $\pm$ standard error THg concentrations are reported in ww for blood and dw for hair and muscle. Standard error was calculated using the delta method. Asterisks indicate significant differences ($p \leq 0.05$) between the tissues collected before and after a foraging trip or early and late in a fasting period. Refer to the electronic supplementary material, tables S1 and S2 for sample sizes.

tissues (blood: $F_{1,19.0} = 87.72$, $p < 0.001$; muscle: $F_{1,18.2} = 15.84$, $p < 0.001$; hair: $F_{1,19.5} = 12.31$, $p = 0.002$; figure 1). THg concentrations in blood increased across both the breeding fast ($t = 19.66$, $p < 0.001$) and moulting fast ($t = 5.75$, $p < 0.001$), but they increased more across the breeding fast (by 103%: $0.25-0.64$ μg g^{-1} ww) than the moulting fast (by 24%: $0.04-0.17$ μg g^{-1} ww). Additionally, mean THg concentrations in blood were 22% higher at the start of the moulting fast compared with the start of the breeding fast ($t = 3.80$, $p = 0.001$), but 25% lower at the end of the moulting fast than at the end of the breeding fast ($t = 5.63$, $p < 0.001$). In muscle, mean THg concentrations increased by 26% across the breeding fast ($t = 3.36$, $p = 0.004$), but decreased by 14% across the moulting fast ($t = 2.27$, $p = 0.035$). In hair, THg concentrations did not change across the breeding fast ($t = 0.23$, $p = 0.82$). However, mean hair THg concentrations increased by 82% across the moulting fast ($t = 5.05$, $p < 0.001$), indicating that almost fully grown new hair had higher THg concentrations than old hair that had been grown during the previous moult.

(iv) Fasting males

Across the breeding fast, THg concentrations in blood increased ($t = 2.47$, $p = 0.048$), whereas THg concentrations in hair decreased ($t = 3.73$, $p = 0.007$; range: $1.0-7.3$ μg g^{-1} dw; electronic supplementary material, figure S2). For muscle THg concentrations, we observed a small increase across the breeding fast ($t = 2.05$, $p = 0.11$), with a sample size of only five paired individuals.

(b) Change in mass related to change in mercury concentration

(i) Foraging females

Females increased their body mass by a mean $17 \pm 10\%$ during the shorter, post-breeding foraging trip, whereas females increased their body mass by $55 \pm 13\%$ during the longer, post-moulting foraging trip (electronic supplementary material, table S2). Individual females that gained proportionally more mass also had a greater proportional decrease in
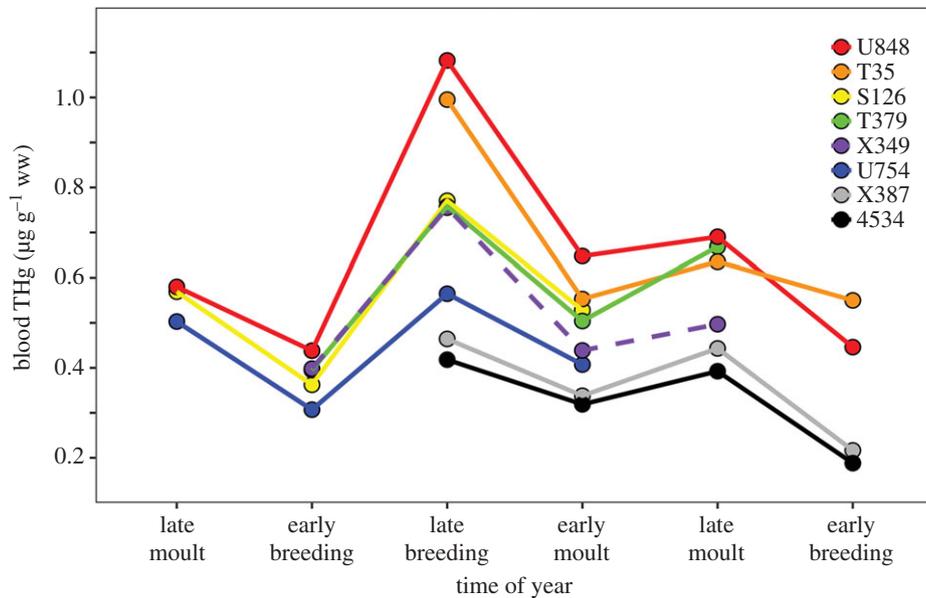
Figure 2. A subset of eight adult female northern elephant seals (*M. angustirostris*) that were sampled for total mercury (THg) in blood during at least four consecutive time periods from 2012 to 2014. Blood THg concentrations were quantified as $\mu g \, g^{-1}$ ww. The first samples were collected late in the 2012 moult and the last samples were collected early in the 2014 breeding fast, with the exception of T35, who was sampled from late breeding 2011 to early breeding 2012. (Online version in colour.)

blood THg concentrations during the post-breeding foraging trip ($F_{1,49} = 35.04$, $p < 0.001$, $R^2 = 0.42$) and during the post-moulting foraging trips ($F_{1,29} = 5.02$, $p = 0.03$, $R^2 = 0.15$; figure 3).

The proportional change in muscle THg concentrations was related to the proportional change in mass during the post-moulting trip ($F_{1,25} = 6.47$, $p = 0.02$, $R^2 = 0.21$) but not during the post-breeding foraging trip ($F_{1,40} = 0.86$, $p = 0.36$; figure 3). All seals gained mass during the post-moulting trip; however, seals that gained proportionally more mass decreased their muscle THg concentrations, whereas seals that gained proportionally less mass increased their muscle THg concentrations.

(ii) Fasting females

Between sampling events, fasting females lost a mean $30 \pm 4\%$ of their body mass during the breeding season and lost $28 \pm 3\%$ of their body mass during the moulting fast (electronic supplementary material, table S2). In contrast to foraging, the proportional change in blood THg concentrations did not relate to that in mass during either the breeding fast ($F_{1,8} = 1.34$, $p = 0.28$) or the moulting fast ($F_{1,7} = 0.28$, $p = 0.61$; figure 3). Furthermore, for muscle, the proportional change in THg concentrations did not relate to that in seal mass during either the breeding fast ($F_{1,7} < 0.01$, $p = 0.97$) or the moulting fast ($F_{1,7} = 0.09$, $p = 0.78$; figure 3).

### (iii) Influence of initial tissue mercury concentrations

We found that the animal's THg concentration at the start of a foraging trip or a fasting period was an additional variable that helped explain the proportional change in THg concentrations in specific cases (electronic supplementary material, figure S3). For blood, initial THg concentrations improved model fit for the post-breeding foraging trip ($\chi_1^2 = 8.35$, $p = 0.004$) but not for the post-moulting foraging trip ($\chi_1^2 = 0.39$, $p = 0.53$), breeding fast ($\chi_1^2 = 0.23$, $p = 0.63$) or moulting fast ($\chi_1^2 = 2.99$, $p = 0.08$). For muscle, initial THg

concentrations improved the model fit for both foraging trips (post-breeding: $\chi_1^2 = 39.97$, $p < 0.001$; post-moulting: $\chi_1^2 = 4.79$, $p = 0.03$) and the moulting fast ($\chi_1^2 = 7.21$, $p = 0.007$). For muscle, the initial THg concentration improved the model fit for the breeding fast ($\chi_1^2 = 8.22$, $p = 0.004$), but this relationship may have been a statistical artefact because we failed to reject the second null hypothesis ($p = 0.27$). For these four of eight foraging and fasting time periods, females with lower starting THg concentrations gained relatively more (or decreased less) in THg concentrations than females with higher starting THg concentrations.

4. Discussion

THg concentrations in adult northern elephant seals changed substantially based on their annual life-history events, regardless of whether or not they were still acquiring Hg through their diet. Specifically, we demonstrated that blood Hg concentrations increased by as much as 153% over 18 days during the breeding fast, despite the fact that seals were not acquiring any Hg through their diet at that time. Furthermore, we showed that blood Hg concentrations declined by as much as 55% over the approximately 75 day post-breeding foraging period as seals rapidly increased in mass and diluted the concentration of Hg in their blood, probably through an increase in blood volume and muscle. Sampling the same individuals at different points in their annual life cycle illustrated the profound influence of physiology on THg concentrations and the resulting toxicity risk.

Blood and muscle THg concentrations, notably, did not fluctuate in parallel, which indicates that different mechanisms influenced the changes in THg concentrations in these tissues while animals were foraging and fasting. For example, muscle THg concentrations increased during the post-breeding foraging trip, while blood THg concentrations decreased during the same foraging trip. Furthermore, we observed the
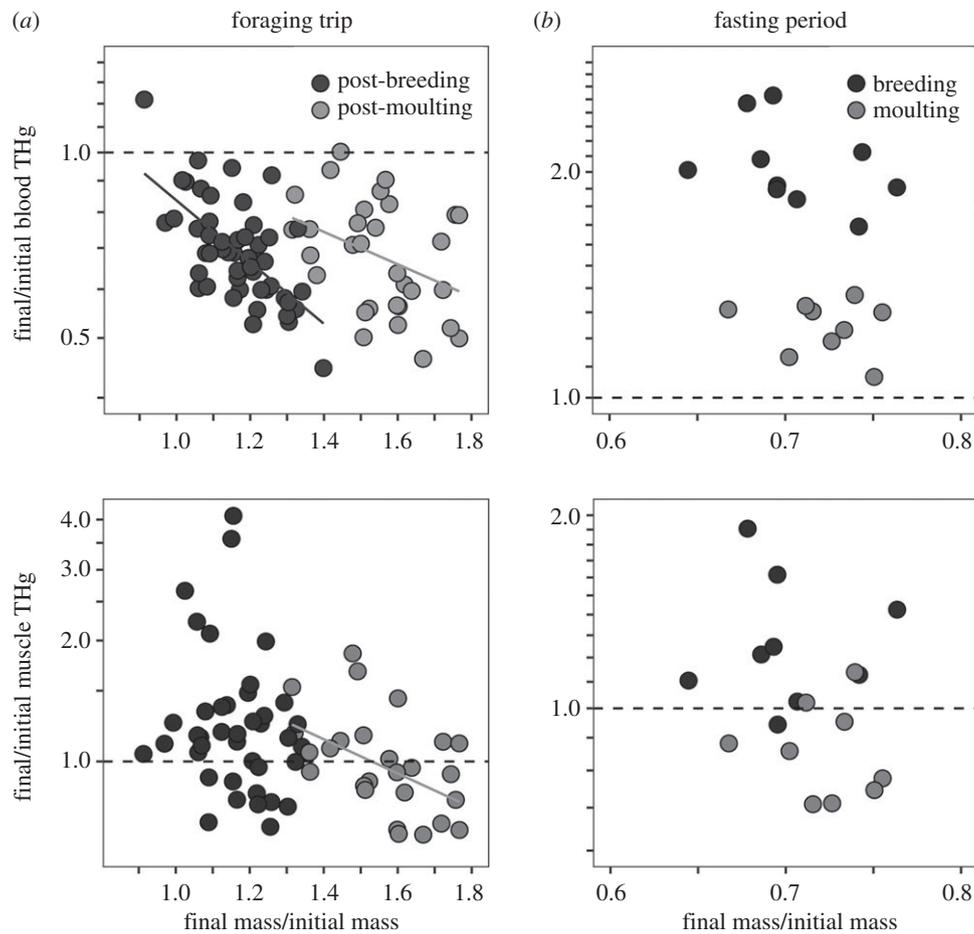
Figure 3. The proportional change in blood and muscle THg concentrations in adult female northern elephant seals (*Mirounga angustirostris*) related to the proportional change in mass while foraging (*a*) and fasting (*b*). Seals were sampled at the start and end of the short, post-breeding foraging trip (*n* = 52) and the long, post-moulting foraging trip (*n* = 31), or early and late in the breeding (*n* = 10) or moulting fast (*n* = 9). The dotted line indicates the separation of seals that increased in THg concentrations from those that decreased in THg concentrations. Refer to the electronic supplementary material, tables S1 and S2 for tissue-specific sample sizes.

lowest blood THg concentrations when female seals were heaviest, at the start of the breeding fast, having just returned from the longer, post-moulting foraging trip at sea. We observed the highest blood THg concentrations at the end of the breeding fast when females had been fasting and were about to wean their pup to depart on the short, post-breeding foraging trip. By contrast, muscle THg concentrations in females were highest at the start of the moulting fast, when seals were in good body condition and had just returned from the post-breeding foraging trip. For hair, THg concentrations were lowest in the old hair that was sampled at the start of the moult when the old hair was about to be shed, whereas THg concentrations were highest towards the end of the moulting period when the new hair had not fully finished growing and the sampling was biased towards the 'tips' of the hair that were grown first during moult. Owing to abrasion of the tips of the hair in between sampling periods, the old hair samples may have been biased more towards the base of the hair and thus, hair that was grown later in moult when body Hg concentrations would have been lower. Abrasion of the tips may also explain the decrease in male hair THg concentrations across the breeding fast, when males were on land for 2.7 times longer than females.

Generally, we observed a strong foraging-associated dilution effect on THg concentrations in blood that was partially explained by the proportional amount of mass gained

while foraging. Elephant seal females increased their body mass by a mean 17% during the post-breeding foraging trip and 55% during the post-moulting foraging trip, and these mass gains at sea corresponded to a decline in their blood THg concentrations. Elephant seal blood comprises approximately 20% of their body mass and their blood volume is tightly correlated with their body mass at the start and end of foraging trips [46]; thus, an increase in body mass during their foraging trips corresponds to a similar increase in blood volume. The dilution of blood THg concentrations in female and male elephant seals while foraging is most likely explained by an increase in body mass and blood volume that outpaces the ingestion of Hg through their diet, similar to growth dilution of THg concentrations in juvenile birds [14].

The foraging-associated dilution effect, however, did not apply to muscle tissue. Instead, mean muscle THg concentrations increased during the post-breeding foraging trip and did not change during the post-moulting trip. Nonetheless, THg concentrations in muscle declined proportionally more in those individuals that gained proportionally more mass during the post-moulting foraging trip. The increase in mean muscle THg concentrations across the post-breeding foraging trip indicated that, for many seals, sequestration of Hg into muscle probably outpaced any potential effect of mass dilution as a result of increasing body mass and muscle stores during this foraging trip. Foraging elephant seals may sequester Hg

in protein-rich tissues like muscle while they actively rebuild their protein and fat reserves while foraging at sea after they had relied on those tissue compartments to fuel their extensive fasting periods on land during both the breeding season and the moulting period [20,30]. Notably, during both at sea foraging trips, seals with lower initial muscle THg concentrations increased their muscle THg concentrations relatively more during foraging trips than those with higher initial muscle THg concentrations. Specifically, seals that started foraging with lower muscle THg concentrations increased their THg concentration during foraging trips, whereas seals that started with the highest muscle THg concentrations decreased their THg concentrations while foraging. Consequently, this suggests that the mechanisms of Hg deposition into and mobilization out of muscle tissue may be concentration-dependent.

Several reasons may account for the decline in blood and increase in muscle THg concentrations during the post-breeding foraging trip. Seals starting the post-breeding foraging trip had the highest intra-annual blood THg concentrations and then went to sea where they foraged on highly contaminated mesopelagic prey [10], which probably provided a reservoir of Hg in blood to be deposited in muscle tissue that was actively being regenerated as body mass increased by a mean 17% while at sea (between sampling periods). Within the mesopelagic zone, methylation of inorganic Hg provides a source of MeHg to deep-ocean food webs [47], and moreover, fishes in the mesopelagic zone contain higher THg concentrations than those from the epipelagic zone [11]. Furthermore, there is little opportunity for elephant seals to offload Hg during the post-breeding foraging trip, because seals are not undergoing gestation or hair growth. By contrast, gestation during the post-moulting foraging trip allows trans-placental transfer of Hg from mother to pup [15,48] and may partially explain the lack of an overall increase in muscle THg concentrations between the start and the end of this longer, post-moulting foraging trip at sea.

Female and male elephant seals markedly increased in blood THg concentrations while fasting, supporting a fasting-associated concentration of blood THg, although the proportional decrease in body mass while fasting did not relate to these changes during breeding or moulting. While on land for breeding and moulting, seals are fasting and, consequently, THg concentrations should increase [15,18], despite the fact that they are no longer ingesting Hg-contaminated prey. However, concurrent with fasting, seals are offloading some Hg into their offspring during the breeding-associated fast or into their growing hair during the moulting-associated fast. These offloading mechanisms may have countered any concentration of THg associated with the decline in body mass (24–36%) while fasting. Extensive fasting periods occur in other taxonomic groups and are well documented for bears, birds and cetaceans [22,49]; thus, the fasting dynamics of other species may result in similar fluctuations in contaminant concentrations.

The proportional change in blood THg concentrations was markedly higher during the breeding fast than the moulting fast, which is probably attributable to differences between the Hg offloading mechanisms. During breeding, multiple seal species and humans transfer a portion of their THg burden to their offspring via milk [15,18,48,50]. For elephant seals, although Hg is transferred to offspring through milk, the mammary gland acts as a filter to reduce maternal transfer, and THg concentrations in milk are not correlated

with the THg concentrations in maternal blood [15]. During moult, elephant seals completely shed their old hair along with the epidermis and grow an entirely new epidermis and hair before returning to the ocean to forage [51]. Circulating Hg in blood is incorporated into these protein-rich tissues (skin and hair) in mammals [52] in a manner similar to how Hg is offloaded into avian feathers [53], which can substantially decrease the burden of Hg in blood and reduce the fasting-associated concentration of contaminants. Therefore, the smaller increase in blood THg concentrations during the moulting fast compared with the breeding fast is probably attributed to the offloading mechanisms of Hg into skin and hair during the moulting fast. Moulting of hair is ubiquitous among mammals, although the frequency and duration can vary widely among species [54]. Variability in moult characteristics among mammals may influence the capacity of hair to serve as an offloading mechanism of Hg.

For muscle THg concentrations, breeding and moulting resulted in changing THg concentrations that went in opposite directions. The lower proportion of protein stores catabolized during breeding than during moulting [20,30] in conjunction with different offloading mechanisms may partially explain differences observed between the two fasting periods. During the breeding fast, muscle THg concentrations increased, similar to blood THg concentrations, suggesting that Hg was concentrated in the remaining muscle tissue. By contrast, muscle THg concentrations decreased during the moult, suggesting that Hg was mobilized out of muscle tissue faster than seals lost mass, and potentially this mobilized Hg was deposited in the newly grown epidermis and hair during moult.

Numerous marine and terrestrial species undergo large proportional changes in body mass as a result of growth, migration, breeding, fasting, hibernation and other life-history events [22–28,55], and adult northern elephant seals demonstrated that life-history events corresponding with substantial changes in body mass can significantly influence contaminant concentrations. If toxicological risk is driven by tissue contaminant concentrations, then there are probably specific times during the year that small- or large-bodied animals may be more vulnerable to contaminants, regardless of current exposure. Additionally, intra-annual changes in tissue contaminant concentrations and toxicological risk have important implications for the design of biomonitoring programmes. For comparisons of Hg exposure to be relevant, sampling should occur when animals are in a similar condition. Furthermore, biomonitoring for toxicological risk may be most effective if samples are collected during the time of year when animals will have the highest contaminant concentrations or during sensitive time periods such as breeding. In conclusion, our study highlights the importance of life-history events and physiological changes on the influence of contaminant concentrations and toxicity risk.

7

rspb.royalsocietypublishing.org Proc. R. Soc. B 285: 20172782

References

1. Lamborg CH et al. 2014 A global ocean inventory of anthropogenic mercury based on water column measurements. Nature 512, 65–68. (doi:10.1038/nature13563)

2. Campbell LM, Norstrom RJ, Hobson KA, Muir DCG, Backus S, Fisk AT. 2005 Mercury and other trace elements in a pelagic arctic marine food web (Northwater Polynya, Baffin Bay). Sci. Total Environ. 351–352, 247–263. (doi:10.1016/j.scitotenv.2005.02.043)

3. Atwell L, Hobson KA, Welch HE. 1998 Biomagnification and bioaccumulation of mercury in an arctic marine food web: insights from stable nitrogen isotope analysis. Can. J. Fish. Aquat. Sci. 55, 1114–1121. (doi:10.1139/f98-001)

4. 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. (doi:10.1897/08-251.1)

5. Harada M. 1995 Minamata disease: methylmercury poisoning in Japan caused by environmental pollution. CRC Crit. Rev. Toxicol. 25, 1–24. (doi:10.3109/10408449509089885)

6. Ackerman JT et al. 2016 Avian mercury exposure and toxicological risk across western North America: a synthesis. Sci. Total Environ. 568, 749–769. (doi:10.1016/j.scitotenv.2016.03.071)

7. 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. (doi:10.1016/j.scitotenv.2013.08.045)

8. Sunderland EM, Mason RP. 2007 Human impacts on open ocean mercury concentrations. Glob. Biogeochem. Cycles 21, GB4022. (doi:10.1029/2006gb002876)

9. Eagles-Smith CA, Ackerman JT. 2014 Mercury bioaccumulation in estuarine wetland fishes: evaluating habitats and risk to coastal wildlife. Environ. Pollut. 193, 147–155. (doi:10.1016/j.envpol.2014.06.015)

10. Peterson SH, Ackerman JT, Costa DP. 2015 Marine foraging ecology influences mercury bioaccumulation in deep-diving northern elephant seals. Proc. R. Soc. B 282, 20150710. (doi:10.1098/rspb.2015.0710)

11. Choy CA, Popp BN, Kaneko JJ, Drazen JC. 2009 The influence of depth on mercury levels in pelagic fishes and their prey. Proc. Natl Acad. Sci. USA 106, 13 865–13 869. (doi:10.1073/pnas.0900711106)

12. Fort J, Robertson GJ, Grémillet D, Traisnel G, Bustamante P. 2014 Spatial ecotoxicology: migratory arctic seabirds are exposed to mercury contamination while overwintering in the Northwest Atlantic. Environ. Sci. Technol. 48, 11 560–11 567. (doi:10.1021/es504045g)

13. 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, 13 694–13 702. (doi:10.1021/es502746z)

14. 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. (doi:10.1021/es200647g)

15. 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. (doi:10.1016/j.envpol.2011.06.019)

16. Seewagen CL, Cristol DA, Gerson AR. 2016 Mobilization of mercury from lean tissues during simulated migratory fasting in a model songbird. Sci. Rep. 6, 25762. (doi:10.1038/srep25762)

17. Ward DM, Nislow KH, Chen CY, Folt CL. 2010 Rapid, efficient growth reduces mercury concentrations in stream-dwelling Atlantic salmon. Trans. Am. Fish. Soc. 139, 1–10. (doi:10.1577/T09-032.1)

18. Habran S, Pomeroy PP, Debier C, Das K. 2013 Changes in trace elements during lactation in a marine top predator, the grey seal. Aquat. Toxicol. 126, 455–466. (doi:10.1016/j.aquatox.2012.08.011)

19. Peterson SH, McHuron EA, Kennedy SN, Ackerman JT, Rea LD, Castellini JM, O'Hara TM, Costa DP. 2016 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. (doi:10.1007/s00244-015-0174-3)

20. Worthy GAJ, Morris PA, Costa DP, Boeuf BJL. 1992 Moult energetics of the northern elephant seal (Mirounga angustirostris). J. Zool. 227, 257–265. (doi:10.1111/j.1469-7998.1992.tb04821.x)

21. 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 Comparative physiology of fasting, starvation, and food limitation (ed MD McCue), pp. 309–336. Berlin, Germany: Springer.

22. Oftedal OT. 2000 Use of maternal reserves as a lactation strategy in large mammals. Proc. Nutr. Soc. 59, 99–106. (doi:10.1017/S0029665100000124)

23. Polischuk SC, Norstrom RJ, Ramsay MA. 2002 Body burdens and tissue concentrations of organochlorines in polar bears (Ursus maritimus) vary during seasonal fasts. Environ. Pollut. 118, 29–39. (doi:10.1016/S0269-7491(01)00278-0)

24. Hays G, Broderick A. 2002 Change in body mass associated with long-term fasting in a marine reptile: the case of green turtles (Chelonia mydas) at Ascension Island. Can. J. 1302, 1299–1302. (doi:10.1139/Z02-110)

25. Nerini M. 1984 A review of gray whale feeding ecology. In The gray whale Eschrichtius robustus (eds ML Jones, SL Swartz, S Leatherwood), pp. 543–559. New York, NY: Academic Press.

26. McWilliams SR, Guglielmo C, Pierce B, Klaassen M. 2004 Flying, fasting, and feeding in birds during migration: a nutritional and physiological ecology perspective. J. Avian Biol. 35, 377–393. (doi:10.1111/j.0908-8857.2004.03378.x)

27. Mommsen TP. 2004 Salmon spawning migration and muscle protein metabolism: the August Krogh principle at work. Comp. Biochem. Physiol. B 139, 383–400. (doi:10.1016/j.cbpc.2004.09.018)

28. Jonsson N, Jonsson B, Hansen LP. 1997 Changes in proximate composition and estimates of energetic costs during upstream migration and spawning in Atlantic salmon Salmo salar. J. Anim. Ecol. 66, 425–436. (doi:10.2307/5987)

29. Peterson SH, Ackerman JT, Costa DP. 2016 Mercury correlations among blood, muscle, and hair of northern elephant seals during the breeding and molting fasts. Environ. Toxicol. Chem. 35, 2103–2110. (doi:10.1002/etc.3365)

30. 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. (doi:10.1111/j.1469-7998.1986. tb03563.x)

31. 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. (doi:10.1139/z90-360)

32. 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. (doi:10.1890/0012-9615(2000)070[0353:FEONES]2.0. CO;2)

33. Robinson PW *et al.* 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. (doi:10.1371/journal.pone.0036728)

34. Le Boeuf BJ. 1974 Male-male competition and reproductive success in elephant seals. *Am. Zool.* **14**, 163–176. (doi:10.1093/icb/14.1.163)

35. Simmons SE, Crocker DE, Hassrick JL, Kuhn CE, Robinson PW, Tremblay Y, Costa D. 2010 Climate-scale hydrographic features related to foraging success in a capital breeder, the northern elephant seal *Mirounga angustirostris*. *Endanger. Species Res.* **10**, 233–243. (doi:10.3354/esr00254)

36. 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. (doi:10.1016/j. marpolbul.2012.08.027)

37. Ackerman JT, Eagles-Smith CA, Takekawa JY, Bluso JD, Adelsbach TL. 2008 Mercury concentrations in blood and feathers of prebreeding Forster's terns in relation to space use of San Francisco Bay, California, USA, habitats. *Environ. Toxicol. Chem.* **27**, 897–908. (doi:10.1897/07-230.1)

38. 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. (doi:10.1002/etc. 5620160914)

39. 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. (doi:10.1007/ s10393-008-0164-2)

40. 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. (doi:10. 1021/es1028734)

41. Kehrig HA, Malm O, Akagi H, Guimarães JRD, Torres JPM. 1998 Methylmercury in fish and hair samples from the Balbina Reservoir, Brazilian Amazon. *Environ. Res.* **77**, 84–90. (doi:10.1006/enrs.1998. 3836)

42. 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. (doi:10.1007/s00128-009-9901-7)

43. Singmann H, Bolker B, Westfall J. 2015 *Afex: analysis of factorial experiments. R package v.0.14-2.* Vienna, Austria: R Foundation for Statistical Computing. See http://CRAN.R-project.org/ package=afex (accessed 28 July 2015).

44. Lenth R, Herv M. 2015 *Least-squares means. R package v.2.20-23.* Vienna, Austria: R Foundation for Statistical Computing. See http://CRAN.R-project. org/package=lsmeans (accessed 1 August 2016).

45. R Core Team. 2015 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. See http:// www.R-project.org/.

46. Hassrick JL, Crocker DE, Teutschel NM, McDonald BI, Robinson PW, Simmons SE, Costa DP. 2010 Condition and mass impact oxygen stores and dive duration in adult female northern elephant seals. *J. Exp. Biol.* **213**, 585–592. (doi:10.1242/jeb. 037168)

47. Blum JD, Popp BN, Drazen JC, Choy CA, Johnson MW. 2013 Methylmercury production below the mixed layer in the North Pacific Ocean. *Nat. Geosci.* **6**, 879–884. (doi:10.1038/ngeo1918)

48. Noël M, Jeffries S, Lambourn D, Telmer K, Macdonald R, Ross P. 2016 Mercury accumulation in harbour seals from the northeastern Pacific Ocean: the role of transplacental transfer, lactation, age and location. *Arch. Environ. Contam. Toxicol.* **70**, 56–66. (doi:10.1007/s00244-015-0193-0)

49. Jenni-Eiermann S, Jenni L. 2012 Fasting in birds: general patterns and the special case of endurance flight. In *Comparative physiology of fasting, starvation, and food limitation* (ed. MD McCue), pp. 171–192. Berlin, Germany: Springer.

50. Dorea JG. 2004 Mercury and lead during breast-feeding. *Br. J. Nutr.* **92**, 21–40. (doi:10.1079/ BJN20041163)

51. 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. (doi:10.1071/zo12049)

52. 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. (doi:10.1002/etc.2762)

53. 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. (doi:10.1007/BF01055352)

54. Ling JK. 1970 Pelage and molting in wild mammals. *Q. Rev. Biol.* **45**, 16–54. (doi:10.1086/ 406361)

55. Groscolas AR, Cherel Y. 1992 How to molt while fasting in the cold: the metabolic and hormonal adaptations of emperor and king penguins. *Ornis Scand.* **23**, 328–334. (doi:10.2307/3676657)

56. Peterson SH, Ackerman JT, Crocker DE, Costa DP. 2018 Data from: Foraging and fasting can influence contaminant concentrations in animals: an example with mercury contamination in a free-ranging marine mammal. Dryad Digital Repository. (http:// dx.doi.org/10.5061/dryad.q0t6b)