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HOW ARE TRACE ELEMENTS MOBILIZED DURING THE POSTWEANING FAST IN NORTHERN ELEPHANT SEALS?

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Abstract—Northern elephant seal (*Mirounga angustirostris*) pups undergo a substantial intertissue reorganization of protein, minerals, and other cellular components during their postweaning development, which might entail the mobilization of associated contaminants. The authors investigated the changes in concentrations of 11 elements (Ca, Cd, Cr, Cu, Fe, Hg, Ni, Pb, Se, V, and Zn) in a longitudinal study on 22 northern elephant seal pups during the postweaning fast. Slight changes in most element concentrations were observed in blood throughout the fast. Circulating levels of Hg, Se, and Cu appeared less altered during the postweaning fast than during suckling (previously measured). Despite the considerable fat utilization, element concentrations in blubber remained stable throughout the fast (except Fe), which suggests that elements are mobilized from blubber as efficiently as lipids. As indicators of the placental transfer, concentrations in lanugo hair revealed the existence of maternal transfer and accumulation of all assayed trace elements during fetal development. In addition, the new pelage, rapidly produced after weaning, appeared to be an important elimination route for toxic metals like Hg, Cd, and Pb. The high mineral content detected in pup hair suggests that this species would be more exposed to trace elements than other phocids (except Cd and Pb). This statement needs nevertheless further monitoring and toxicological studies to determine better the exposition to trace elements and its potential impact on the northern elephant seal's health. Environ. Toxicol. Chem. 2012;9999:1–12. © 2012 SETAC

Keywords—*Mirounga angustirostris* Trace element Postweaning fast Blood Hair

INTRODUCTION

Northern elephant seals (*Mirounga angustirostris*) are top marine predators from the north Pacific Ocean. They have a striking physiology, able to undergo natural extended periods of complete food and water abstinence. They fast for up to three months during the terrestrial phase of their life cycle, that is, during reproduction, molting, as well as postweaning development of pups. Mothers give birth to a single black-coated pup, which they suckle for approximately 25 d with a lipid-rich milk [1]. During the nursing period, the pups gainapproximately90 kg [2], with a daily average of 2 kg of adipose and 1 kg of lean tissue [3]. At weaning, the fat mass averages 38% in healthy pups [4]. Pups are weaned abruptly when the mother returns to sea and, incapable of effectively diving and swimming, fast on land for up totwo and half months [1]. During this time, they continue neonatal development and acquire the motor skills necessary for making their first trip to sea [5]. They molt their black natal coat (also called "lanugo") for a new pelage soon after weaning [5]. Weaned pups rely on reserves accumulated during lactation, and this entails substantial intertissue reorganization of protein, minerals, and other cellular components. Seals can selectively utilize reserves from different parts of the body (i.e., core proteins or fats vs blubber tissues) during different stages of the fast [6]. They are facedwitha dual challenge of providing energy during the fast and maintaining a sufficient blubber layer for thermal insulation when they enter the ocean at the conclusion of the fast [6]. Elephant seal pups lost an average of 0.9 kg mass/d during the first two weeks of fasting and approximately 0.5 kg/d for the remainder of the fast [7]. In terms relative to initial body compartment masses, northern elephant seal weaned pups lost approximately 26% of initial lipid stores (i.e., lipid content at weaning) and approximately 30% of initial protein stores over the postweaning fast [4]. Because northern elephant seal pups undergo a complete molt soon after weaning, mobilization of protein from body stores would be also required for the production of new pelage [4]. According to estimations from Noren et al. [4], proteins mobilized for the molt represent approximately 18% of initial protein reserves (or ~60% of lost protein mass).

The substantial tissue reorganization can involve the mobilization of contaminants potentially associated with energy reserves (lipids and proteins). Contaminants such as organ chlorines [8–10] and trace elements [11–13] concentrate in marine organisms at higher levels than those measured in their surrounding environment. Some studies focused on anthropogenic organic chemicals in pups of northern [9] and southern [14,15] elephant seals. It appears that pups accumulate contaminants through maternal transfer via transplacental and lactational routes and that concentrations of organochlorine contaminants generally increase from pups to juveniles to adults [14,15]. Debier et al. [9] also showed that the mobilization of lipids during the postweaning fast entailed the increase of organochlorine concentrations in pup blubber and serum. These phenomena may increase the risk of adverse health effects on the developing endocrine or immune system. In contrast, little is known about levels of trace elements in relation to the development of pinnipeds. Trace elements occur naturally on earth.

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However, anthropogenic releases associated withurban and industrial activities in coastal regions, as well as withoffshore waste disposal, increase the input of trace elements in marine ecosystems [16]. Trace elements can be either essential or nonessential for living organisms. The essential trace elements Zn, Fe, Cu, and Se are important as components of protein complexes (metalloproteins). They are required for enzymatic activities and can play structural roles in connective tissue and cell membranes [17]. The essentiality of other trace elements, like Ni, V, and Cr, has also been well established for various biological functions; however, information on their optimal and deficient concentrations is limited [18–20]. Although beneficial at low concentrations for living organisms, essential trace elements can become toxic at higher concentrations above a certain threshold. In contrast, Hg, Cd, and Pb are not required for any physiological processes. These nonessential trace elements are considered toxic even at very low levels [21,22]. Lead binds tightly to both Ca and Zn sites in proteins and alters their activity [23]. Calcium was thus included in the present study to assess the potential interactions between Pb and Ca, although it is an essential macroelement.

Few data exist about the degree to which northern elephant seals are contaminated by trace elements of toxicological concern in high trophic–level wildlife. Only levels of Hg and Se were analyzed in northern elephant seal females and their pups during lactation(reaching 0.3 and 1.1 mg/kg wet wt, respectively, in blood of females from early lactation [13]). That study showed that fasting associated withmilk production in lactating females, as well as suckling in pups, induced rapidly significant changes in Hg and Se blood levels over the lactation period. Therefore, we can wonder how trace element levels varyduring the postweaning development of pups.

Northern elephant seals have life-history characteristics and a relative ease of handling that make them a good "model" in which to study fasting physiologyin marine mammals. We investigated the changes in levels of 11 elements (Hg and Se, but also Ca, Cd, Cr, Cu, Fe, Ni, Pb, V, and Zn) in a longitudinal study on 22 northern elephant seal pups during the postweaning fast period. The main objective was to determine the changes in element levels in blood and blubber during fasting under the constraints of development. Moreover, hair analysis enabledthe assessment of contamination levels in northern elephant sealsas well ascomparison with other pinniped species.

MATERIALS AND METHODS

Field techniques

The present study was performedat Año Nuevo State Reserve, California, USA (37°06′30″N, 122°20′10″W), after the 2010 breeding season (February–April). Pups were monitored and dates of weaning were recorded by daily observations of the breeding areas. Twenty-twopups were captured three times throughout the postweaning fast. Captures occurred weeks1, 4, and 7 of the postweaning fast. Blood and blubber samples were collected at each capture. Lanugo and new hair were collected at the first and second captures, respectively. Fourteen of the 22 weaned pups were still presentat the rookery at week 9 of the postweaning fast; additional blood samples from these 14 pups were thus collected once moreat this time.

Weaned pups were immobilized with an intramuscular injection of tiletamine HCl and zolazepam HCl (Telazol, 1 ml/100 kg of estimated bodymass), and immobilizationwas maintained with intravenous injections of ketamine (Ketaset; Fort Dodge Animal Health). Blood samples were collected from the extradural vein. Whole blood samples were collected in 6-mlroyal blue Vacutainer plastic tubes with clot activator (Fisher Scientific) and certified for trace-element analyses (throughout the text, "blood" refers to whole blood). A blubber biopsy was taken at each capture from the lateral pelvic area after a subcutaneous injection of local anesthetic (lidocaine, 1.5 ml). A small part of the anesthetizedarea was cleaned with alcohol. A small incision was then made, and a blubber biopsy, extending the full depth of the blubber layer, was taken with a 6-mm biopsy punch (Acu-Punch; Acuderm). Blubber biopsies were stored in plastic tubes. Lanugo was simply plucked from the dorsal midline region at the first capture when pupswere molting. At the second capture, a patch of new hair (~15 × 15 cm, ~3 g) was shaved from the dorsal midline region using a "one-use" stainless-steel blade. Lanugo and new hair were placed in a polyethylene bag. At each capture, length and axial girth of pups were measured using a measuring tape, and pups were weighed using a scale (capacity 500 ± 0.2 kg) suspended from a tripod. The sex was also determined. Biometric data on the weaned pups are summarized in Table 1.

Weaned pupswere individually identified at the first capture with a hair dye mark (Clairol) to aid recapture. After each procedure, pups were released and monitored until they had regained mobility. All samples were kept on ice in the field (at 4°C), then stored at −20°C in the laboratory until analysis.

Sample preparation

Prior to analysis, bloodsamples were freeze-dried and ground with a mortar and pestle into powder. Water content was on average 74.5%. After thawing, lanugo and new hair werewashed ultrasonically with reagent-grade acetone (acetone for analysis, EMSURE; Merck) and rinsed repeatedlywith 18.2 MΩ-cm deionized water to remove exogenous contaminants, according to the method recommended by the International Atomic Energy Agency [24]. Lanugo and new hair samples were then freeze-dried for 24 h. Blubber biopsies were large enough to cut in two equal parts, separating the inner and outer blubber layers.

Elementanalyses

Concentrations of chemical elements (Cd, Cr, Cu, Fe, Ni, Pb, Se, V, Zn, and Ca) were[Q1] measured in blood, blubber, lanugo,

Table 1. Biometry of northern elephant seals[a] throughout the postweaning fast period[b]

	n	Postweaning fast day	Mass (kg)	Standard length (cm)	Axial girth (cm)
Week 1	22	1 ± 1 (0–4)	128 ± 13 (92–148)	146 ± 7 (131–156)	135 ± 6 (119–145)
Week 4	22	23 ± 1 (22–25)	112 ± 11 (77–129)	147 ± 8 (130–159)	125 ± 6 (107–137)
Week 7	22	46 ± 2 (44–50)	96 ± 11 (66–115)	151 ± 7 (132–160)	118 ± 6 (101–126)
Week 9	14	61 ± 2 (58–66)	—	—	—

[a] Sex ratio (female:male): 50:50.
[b] mean ± SD (range).

Table 2. Certified reference material recoveries ($n = 10$) and instrumental quantification limits (IQL; ppb) for element analyses

			Certified reference material recovery (%)		
Element	Method	IQL	Seronorm L-3[a]	DOLT-3[b]	NIES-13[c]
Ca	ICP-MS	1.220	101	NC	92
Cd	ICP-MS	0.008	129	93	117
Cr	ICP-MS	0.007	115	71	NC
Cu	ICP-MS	0.013	99	99	97
Fe	ICP-MS	0.180	102	96	90
Ni	ICP-MS	0.013	ND	92	NC
Pb	ICP-MS	0.009	98	106	97
Se	ICP-MS	0.067	122	87	ND
V	ICP-MS	0.002	89	NC	73
Zn	ICP-MS	0.146	95	94	88
Hg	AAS	0.898	100	ND	92

[a] Seronorm level 3, trace elements whole blood (Sero).
[b] DOLT-3, dogfish liver (National Research Council of Canada).
[c] NIES-13, human hair no.13 (National Institute for Environmental Studies, NIES).
ICP-MS = inductively coupled plasma mass spectroscopy; AAS = ; NC = not certified value; ND = not determined.

and new hair of 22 weaned pups. Approximately 0.2 g of freeze-dried bloodand 0.25 g of washed and freeze-dried lanugo or new hair were weighed; the mass was recorded to the nearest 0.0001 g. Thawed blubber subsamples (~0.2 g) were weighed. All these samples were subjected to microwave-assisted digestion in Teflonvessels with 4 ml HNO_3 (65%), 1 ml H_2O_2 (30%), and 3 ml 18.2 MΩ-cm deionized water. After cooling, samples were diluted to 50 ml with 18.2 MΩ-cm deionized water in a volumetric flask. Cadmium, Cr, Cu, Fe, Ni, Pb, Se, V, Zn, and Caconcentrationswere determined by inductively coupled plasma mass spectroscopy (ICP-MS; PerkinElmer; Sciex, DCR 2). Multiple-element (^{74}Ge, ^{103}Rh, ^{209}Bi, ^{69}Ga) internal standards (CertiPUR; Merck) were added to each sample and calibration standard solutions. Quality control and quality assurance for ICP-MS included field blanks, method blanks, and certified reference materials—Seronorm L-3, DOLT-3, and NIES-13. Certified reference material recovery (%) and the instrumental quantification limits for each element are listed in Table 2. Reported[Q2] concentrations for all elements in blood and blubber are expressed on a wet weight basis in milligrams per kilogram, whereas concentrations for lanugo and hair are expressed on a dry-weight basis in milligrams per kilogram.

Total Hg analysis

Approximately 30 to 50 mg of freeze-dried blood and 1 to 4 mg of lanugo and new hair were accurately weighed and loaded into quartz boats. Masses were recorded to the nearest 0.01 mg. Total Hg (THg) concentrations were determined by atomic absorption spectroscopy (DMA-80, Direct Mercury Analyzer; Milestone). The method has been validated for solid samples using U.S. Environmental Protection Agency(U.S. EPA) method 7473. Quality-assurance methods included evaluating by measuring blanks, duplicates, and certified reference materials(Seronorm L-3 and NIES-13) with every 10 samples (Table 2).

Statistical analyses

A Kolmogorov-Smirnov test was used to determine whether data departed from normality. The variables were not normally distributed, and nonparametric tests were used for statistical analyses. To evaluate changes in element concentrations during the postweaning fast in tissues, Wilcoxon signed-rank tests were used to compare means at different sampling times. Spearman's rank correlation coefficient was used to test correlations between two variables. Statistical analysis of the data was performed using Statistica software (Statsoft, Version 10), and $p < 0.05$ was considered significant (with $\alpha = 0.05$). Results are presented as mean (median) ± standard deviation (SD), range.

RESULTS

Results of elements in blood, blubber, and hair of northern elephant seals at different stages of the postweaning fast are summarized in Table 3. To easily compare values with other species, mean concentrations in the whole biopsy (Table 3) were also calculated from concentrations in blubber subsamples (inner and outer layers). Calcium, Fe, Zn, Cu, and Ni were detected and quantified in the different tissues. Cadmium was not detected in blood and blubber. Selenium and Pb were below the limit of quantification in all blubber samples. Vanadium and Cr were below the limit of quantification in some blood and blubber samples (13 and6% of assayed samples, respectively). Mercury could not be determined in blubber samples because concentrations were below the limit of quantification after the microwave-assisted digestion and the successive dilution (THg < 0.225 mg/kg wet wtblubber). Data below quantifiable limits were not subjected to further statistical analyses. Element concentrations in blood, blubber, and hair did not differ significantly between males and females (for all $p > 0.05$, Mann-Whitney test). Therefore, we combined the sexes in the following analyses.

Concentrations of trace elements in weaned pups

The concentration of elements in the blood ofnorthern elephant seal weaned pupsat week 1 decreased according to the following pattern: Fe > Ca > Zn > Se > Cu > Hg > Pb > Ni > Cr > V (Table 3). Mercury had the highest concentration of the toxic metals measured in blood, with 0.066 mg/kg wet weightat week 1. Individual variability in blood concentrations differed according to element. Variability between individuals was the lowest for Ca, Fe, and Zn (6–10%) and the greatest for Cr and V (21–52%)(Table 3). Blood concentrations of elements varied significantly during thepostweaning fast. Blood concentrations of Ca and Cr decreased from week 4 untilthe end of the fast (by −13 and −15% of the initial value, respectively; Fig. 1). In contrast, blood concentrations of other elements increased during this period, especially for Fe, Hg, and V (up to +22, +38, and +880%, respectively; Fig. 1). The blood concentration of Pb increased highly at the beginning (up to +36%), then decreased progressively during the postweaning fast (Fig. 1). Only the blood concentration of Ni did not vary, or varied very little, during the period (Fig. 1).

Element concentrations in whole blubber followed the sequence Fe > Ca > Zn > Cu > Cr > Ni > V (Table 3). Individual variability in metal concentrations was greater in blubber than in blood. Concentrations of Ca and V in blubber showed a very high variability among animals of the present study (up to 68 and 108%, respectively)(Table 3). Concentrations of Ni, Cr, and V in blubber were greater than those in blood (Table 3). Results in the blubber subsamples—inner and outer blubber layers—are summarized for each capture in Supplemental Data, Table S1 (detailed mean concentrations and statistical results). Overall, we observed that concentrations differed between inner and outer blubber for Fe, Cr, and Ni. At any captures (weeks1, 4, or 7), Fe concentration was greater in inner

Table 3. Element concentrations (in mg/kg wet wt) in northern elephant seals at different stages of the postweaning fast: mean (median) ± SD (range), coefficient of variation

		n	Ca	Fe	Zn	Cu	Se	Hg	V	Cr	Ni	Cd	Pb
Blood	Week 1	22	62 (61) ± 4 (54–68) 7%	610 (605) ± 46 (547–699) 8%	2.7 (2.8) ± 0.2 (2.4–3.0) 7%	0.98 (0.95) ± 0.11 (0.85–1.33) 12%	1.5 (1.5) ± 0.3 (0.9–2.1) 19%	0.066 (0.065) ± 0.015 (0.037–0.097) 23%	0.002 (0.001) ± 0.001 (<0.002–0.003) 47%	0.004 (0.004) ± 0.002 (<0.002–0.010) 52%	0.016 (0.015) ± 0.003 (0.013–0.026) 18%	<LD	0.017 (0.016) ± 0.004 (0.011–0.030)[b] 25%
	Week 4	22	62 (62) ± 5 (50–72) 8%	686 (680) ± 44 (611–769) 6%	2.8 (2.8) ± 0.2 (2.4–3.2) 8%	1.06 (1.03) ± 0.10 (0.96–1.44) 10%	1.7 (1.8) ± 0.4 (0.8–2.4) 22%	0.062 (0.062) ± 0.017 (0.028–0.099) 28%	0.005 (0.005) ± 0.002 (0.002–0.009) 43%	0.003 (0.003) ± 0.001 (<0.002–0.006) 26%	0.016 (0.016) ± 0.003 (0.013–0.026) 16%	<LD	0.022 (0.021) ± 0.005 (0.015–0.032) 23%
	Week 7	22	55 (55) ± 4 (48–61) 6%	680 (660) ± 48 (595–753) 7%	2.9 (2.8) ± 0.2 (2.4–3.4) 8%	1.01 (1.01) ± 0.08 (0.90–1.26) 8%	1.6 (1.7) ± 0.4 (0.8–2.7) 26%	0.075 (0.076) ± 0.021 (0.035–0.126) 29%	0.008 (0.007) ± 0.003 (0.004–0.016) 37%	0.003 (0.003) ± 0.001 (<0.002–0.004) 21%	0.015 (0.015) ± 0.001 (0.013–0.017) 8%	<LD	0.019 (0.019) ± 0.004 (0.015–0.028) 19%
	Week 9	14	53 (52) ± 5 (47–63) 10%	745 (763) ± 67 (581–835) 9%	3.1 (3.1) ± 0.2 (2.7–3.4) 8%	1.08 (1.02) ± 0.16 (0.96–1.59) 15%	1.8 (1.8) ± 0.4 (1.0–2.5) 22%	0.092 (0.094) ± 0.029 (0.040–0.154) 31%	0.010 (0.010) ± 0.004 (0.005–0.020) 38%	0.003 (0.003) ± 1.0 (<0.002–0.005) 31%	0.016 (0.016) ± 0.002 (0.013–0.019) 10%	<LD	0.017 (0.018) ± 0.001 (0.015–0.019) 7%
Blubber	Week 1	22	35 (33) ± 19 (14–85)[b] 52%	53 (46) ± 20 (25–91)[b] 37%	1.6 (1.5) ± 0.4 (1.3–2.8)[b] 24%	0.4 (0.4) ± 0.1 (0.2–0.7)[b] 29%	<0.18	ND	0.052 (0.022) ± 0.055 (<0.004–0.189)[b] 106%	0.183 (0.184) ± 0.067 (0.091–0.353)[b] 36%	0.144 (0.140) ± 0.036 (0.087–0.213)[b] 25%	<LD	<0.021
	Week 4	22	26 (23) ± 11 (13–59)[c] 44%	62 (57) ± 25 (23–118)[c] 41%	1.7 (1.6) ± 0.4 (1.2–2.7)[c] 25%	0.3 (0.2) ± 0.1 (0.2–0.5)[c] 32%	<0.18	ND	0.021 (0.013) ± 0.022 (<0.004–0.091)[c] 105%	0.134 (0.139) ± 0.034 (0.076–0.208)[c] 26%	0.122 (0.125) ± 0.020 (0.079–0.149)[c] 17%	<LD	<0.021
	Week 7	22	35 (22) ± 24 (14–83)[c] 68%	85 (85) ± 27 (39–136)[c] 32%	1.9 (1.7) ± 0.6 (1.4–3.8)[c] 30%	0.4 (0.4) ± 0.1 (0.3–0.6)[c] 21%	<0.18	ND	0.028 (0.014) ± 0.030 (<0.004–0.097)[c] 108%	0.153 (0.149) ± 0.055 (0.080–0.270)[c] 36%	0.141 (0.131) ± 0.035 (0.096–0.203)[c] 25%	<LD	<0.021
Lanugo[a]	Week 1	22	1,927 (1,607) ± 1,357 (1,014–7,743) 70%	517 (475) ± 423 (136–2,194) 46%	249 (250) ± 21 (208–288) 9%	4.0 (3.7) ± 0.9 (3.0–6.9) 22%	4.7 (4.7) ± 0.6 (3.3–5.6) 14%	19.0 (19.4) ± 4.3 (8.2–28.9) 22%	1.9 (1.6) ± 1.5 (0.5–7.9) 80%	1.5 (1.4) ± 1.4 (0.2–6.7) 92%	0.98 (0.76) ± 0.74 (0.28–3.63) 76%	0.28 (0.20) ± 0.23 (0.11–1.00) 81%	0.27 (0.23) ± 0.16 (0.10–0.88) 60%
Hair[a]	Week 4	22	1,257 (1,098) ± 545 (596–2,783) 43%	494 (451) ± 310 (50–1,335) 63%	305 (310) ± 20 (254–338) 7%	7.6 (7.6) ± 0.9 (5.6–9.6) 12%	6.0 (6.0) ± 1.0 (3.2–7.5) 17%	13.8 (12.9) ± 4.7 (5.2–28.0) 34%	1.6 (1.4) ± 0.9 (0.2–3.2) 55%	1.4 (1.3) ± 0.9 (0.2–3.6) 65%	0.75 (0.66) ± 0.44 (0.12–2.17) 58%	0.14 (0.14) ± 0.06 (0.03–0.28) 44%	0.26 (0.24) ± 0.14 (0.05–0.65) 54%

[a] Values expressed on a dry weight basis.
[b] $n = 21$.
[c] $n = 20$ due to outliers.
LD = limit of detection; ND = not determined.

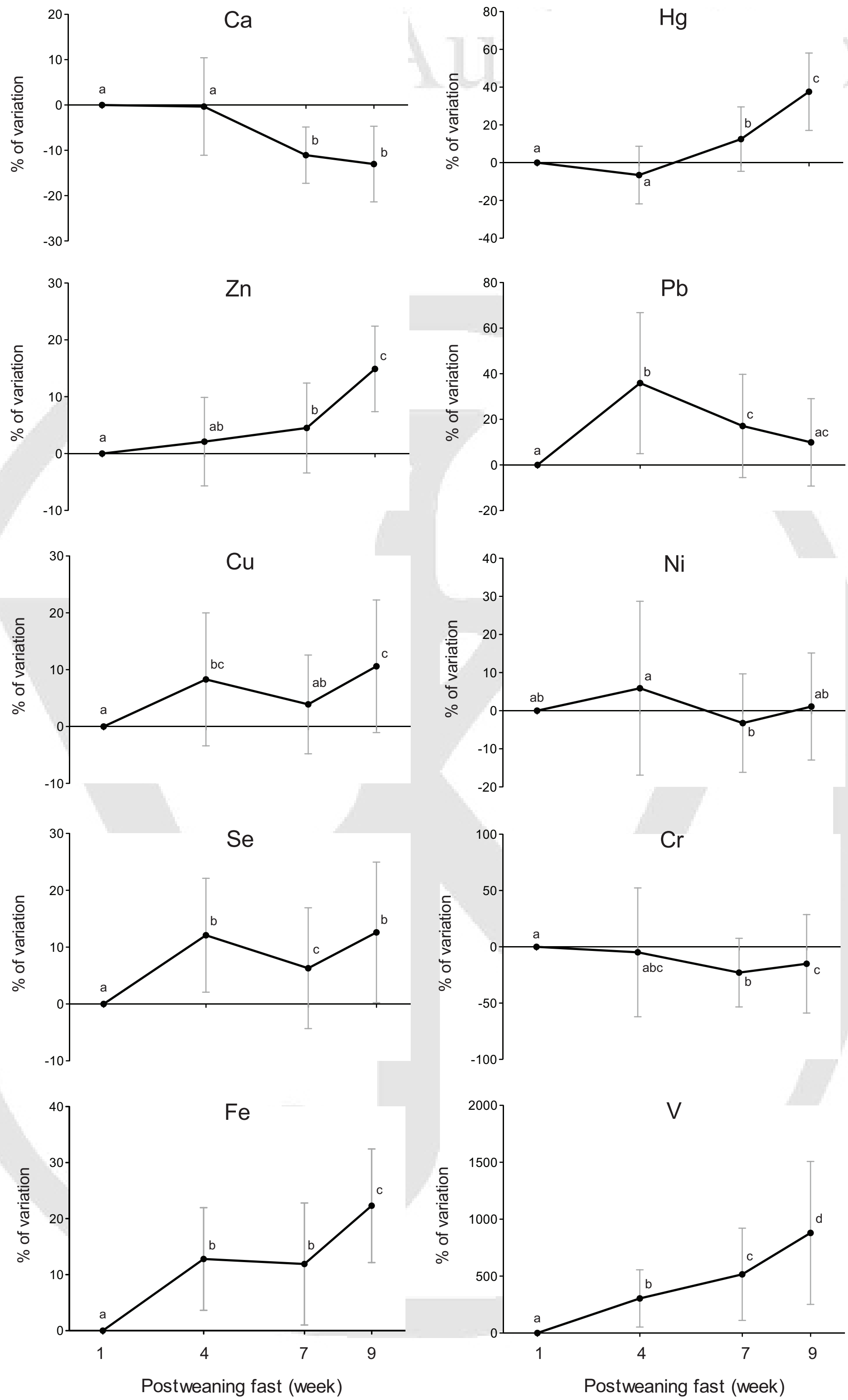

Fig. 1. Blood concentrations of elements (as percentage of initial concentration) as a function of time throughout the postweaning fast in northern elephant seals (ratio ± SD; [100 ×(week *x*)/(week 1)–100]). Different letters indicate significant difference in values between weeks1, 4, 7, and 9 (Wilcoxon signed-rank tests).

blubber (at any stage, $p < 0.01$, Wilcoxon signed-rank tests), while Cr and Ni concentrations were greater in outer blubber (at any stage and for both elements, $p < 0.001$, Wilcoxon signed-rank tests). For other elements, concentrations were quite similar between inner and outer blubber. Element concentrations in blubber layers varied very little throughout the postweaning fast. Inboth layers, Ca, Ni, Cr, and V concentrations did not vary significantly or varied weakly during the fast (Fig. 2). The concentration of Zn increased in inner blubber at week 7, while the Cu concentration decreased in outer

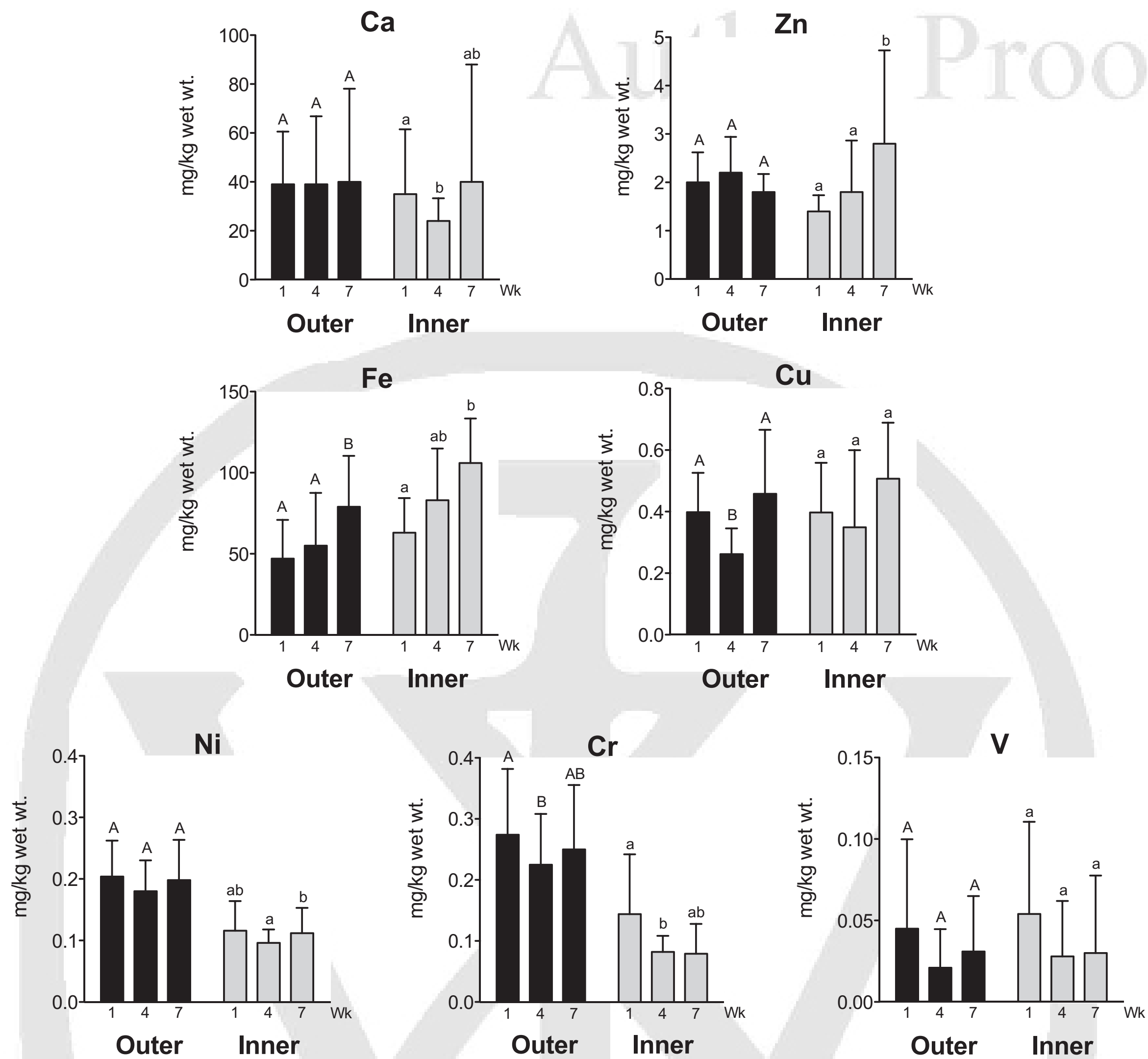

Fig. 2. Concentrations of Ca, Fe, Zn, Cu, Ni, Cr, and V (mean ± SD, in mg/kg wet wt) in outer (blackbars) and inner (graybars) layers of blubber at different stages of the postweaning fast (weeks 1, 4, and 7). Different letters (A, B, C for outer blubber and a, b, c for inner blubber) indicate significant difference in values between weeks 1, 4, and 7 (Wilcoxon signed-rank tests).

blubber at week 4 before coming back to its initial level (Fig. 2). Only the Fe concentration increased progressively in inner and outer blubber throughout thepostweaning fast (Fig. 2).

In contrast with blood and blubber, all element concentrations in lanugo and new hair were above the limit of quantification. Element concentrations decreased according to the following patterns: Ca > Fe > Zn > Hg > Se > Cu > V > Cr > Ni > Cd > Pb in lanugo and Ca > Fe > Zn > Hg > Cu > Se > V > Cr >Ni > Pb > Cd in new hair (Table 3). Concentrations of Ca, Zn, Cu, Se, Hg, and Cd differed significantly between lanugo and new hair. Concentrations of Ca, Hg, and Cd in new hair were 81, 73, and 72% of the values in lanugo, respectively (for all $p < 0.01$, Wilcoxon signed-rank tests; Fig. 3). In contrast, concentrations of Zn, Cu, and Se in new hair were 124, 197, and 131% of the values in lanugo, respectively (for all $p < 0.001$, Wilcoxon signed-rank tests; Fig. 3). Concentrations of Fe, V, Cr, Ni, and Pb were similar in lanugo and new hair (for all $p > 0.05$, Wilcoxon signed-rank tests; Fig. 3).

Relationships between elements, tissues, and biometric parameters

Surprisingly, element concentrations in blood and blubber were weakly correlated between the different stages of the postweaning fast(weeks 1, 4, 7, and 9). Only blood Se and Hg concentrations were highly correlated between weeks 1, 4, 7, and 9($r = 0.87$–0.99 for Se and $r = 0.73$–0.93 for Hg, $p < 0.001$ for both, Spearman's rank correlation coefficient); blood Zn concentrations were weakly correlated between weeks 1, 4, 7, and 9($r = 0.44$–0.63, $p < 0.05$, Spearman's rank correlation coefficient). No relationship was observed between concentrations in lanugo and concentrations in new hair (for all elements, $p > 0.05$, Spearman's rank correlation coefficient).

Blood concentrations of elements were correlated between tissues[Q3] (Table 4). However[Q4], the main relationship found during the entire fast was only between Fe and Zn concentrations in blood (at any stage, $r = 0.60$–0.78 and $p < 0.001$, Spearman's rank correlation coefficient). In blubber, the main relationships between elements occurred between Cr and Ni and between Ca and V in both blubber layers at weeks 1, 4, and 7 of the fast (Table 4). The relationship between Cr and V concentrations was also observed but only in inner blubber (Table 4). In contrast, numerous strong correlations between elements were found in lanugo and new hair (Table 4).

Very few correlationswere observed between tissues (blood, blubber, and lanugo/hair). Only positive relationships between blood and lanugo were observed for Se and Hg concentrations

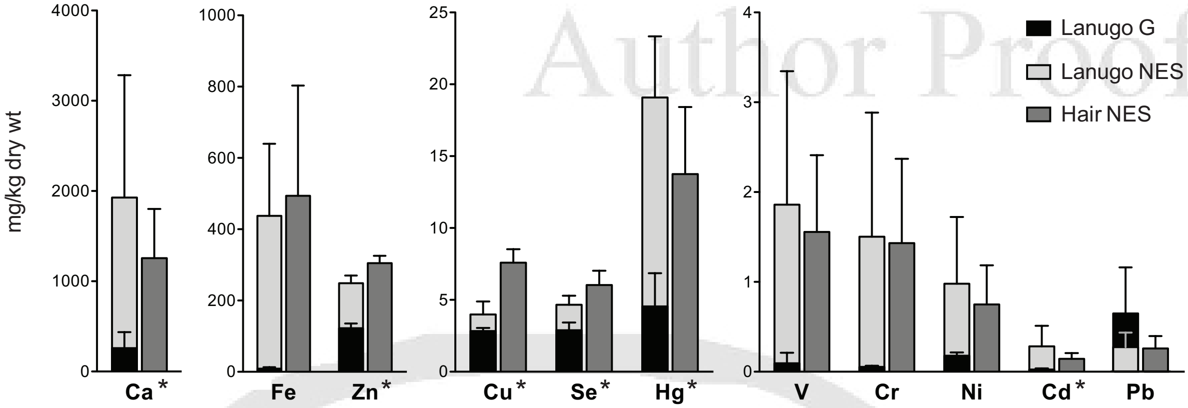

Fig. 3. Element concentrations (mean ± SD, in mg/kg dry wt) in lanugo (light gray bars) and new hair (dark gray bars) of northern elephant seal (NES). Concentrations in lanugo of gray seals (GS, *Halichoerus grypus*; black bars) are also included for comparison [26]. * Significant difference in values between lanugo and new hair of NES (Wilcoxon signed-rank tests).

(at any stage, $r = 0.52$–0.63 and $p < 0.01$, Spearman's rank correlation coefficient). No relationship was observed between blubber and lanugo/hair orbetween blood and blubber (for all $p > 0.05$, Spearman's rank correlation coefficient). Biometric parameters (i.e., mass, length, and axial girth) did not show any interesting relationship with element concentrations in the different tissues of northern elephant seal weaned pups (for all $p > 0.05$, Spearman's rank correlation coefficient).

DISCUSSION

Changes in blood concentrations

As elements involved in many biological functions, Ca, Fe, Zn, and Cu appear more tightly regulated in blood than the other elements, including Se, Hg, V, Cr, Ni, and Pb. The coefficients of variation were around 9 ± 2% for the first group of elements and around 26 ± 11% for the second group. Blood concentrations of elements found in northern elephant seal pups were comparable to those reported in studies of other phocid species [25–28]. Concentrations of elements(Ca, Cr, Cu, Ni, Pb, Se, and Zn) in blood of northern elephant seals slightly varied throughout the postweaning fast: concentrations at week 9 were between 85 and 115% of their initial value at weaning. Nevertheless, Fe, Hg, and V showed greater changes in concentrations, reaching at the end of the fast 122, 138, and 980%, respectively, of their initial value at weaning. The main part of blood Fe and Hg is bound to hemoglobin in the red blood cells [29–31]. Changes in hemoglobin concentration (or in hematocrit) can thus affect Fe and Hg concentrations measured in whole blood. In phocids, these hematological parameters, related to the oxygen storage and transport capacity, increase throughout postnatal development, to assure diving capability-from pups to juveniles [32–34]. Therefore, an increase of hemoglobin concentration contributes to the increase of Fe and Hg levels in the blood. Interestingly, blood concentrations of Hg, Se, and Cu in weaned pups of the present study were totally in continuity with those previously determined in northern elephant seal suckling pups [13]. Values in the early postweaning fast were very close to values in late lactation (respectively, 0.066 and 0.077 mg/kg wet wtfor Hg, 1.5 and 1.3 mg/kg wet wtfor Se, and 0.98 and 0.93 mg/kg for Cu) in following the increasing or decreasing trend observed during lactation (Fig. 4). In comparison with changes in concentrations during lactation, Hg, Se, and Cu concentrations seem progressively to level off at weaning (Fig. 4). We expected to observe greater variations during the fast due to the important utilization of lipids and proteins(26 and 30%, respectively [4]) to provide energy for metabolism and new hair. Nevertheless, it appears that suckling affects more the circulating levels of these elements than the postweaning fast in northern elephant seal pups. This highlights the role of biomagnification associated to the input pathway for trace elements via the milk, in contrast with the postweaning fast period when no input is available. Similar longitudinal changes in blood concentrations during lactation and fasting for Hg, Se, and Cu and during fasting only for Ca and Zn have been observed in blood of gray seal pups, *Halichoerus grypus* [27]. A strong relationship was observed only between blood Fe and Zn concentrations in northern elephant seals, like in gray seals [27].

Changes in blubber concentrations

Blubber of seals is stratified in three chemically distinct layers, each having a different function: (1) the outer layer is primarily structural and thermoregulatory, (2) the inner layer is metabolically active with a fatty acid composition that is strongly affected by lipid mobilization/deposition, and (3) the middle layer is a storage site [35]. These different blubber layers show changes in fatty acid composition [35]. The structural and chemical composition of the blubber mightalso lead to differences in the mineral content according to the layers of this tissue. In the present study, concentrations of the investigated elements differed between the inner and outer blubber layers only for Fe, Cr, and Ni. These differences between inner and outer layers were kept throughout the postweaning fast (at weeks 1, 4, and 7). In lactating gray seal females, element distribution in blubber layers was different fromthat in northern elephant seal weaned pups: Ca, Fe, Zn, and Cu concentrations were greater in inner blubber than in outer blubber in late lactation, while concentrations of other elements were similar in the twolayers [27]. Like for the fatty acid composition of blubber layers [35,36], diverse factors such as age, individual reproductive status, and nutritional status might influence the mineral content of the blubber layers.

During the postweaning fast, northern elephant seal pups fast and rely on their body fat, mainly from the blubber, to maintain their metabolism. Approximately 26% of body fat content is used during this period in weaned pups [4]. Few longitudinal changes in concentrations of elements were observed in north-

Table 4. Correlations between elements in the different tissues of northern elephant seals[a]

	Blood				Blubber			Lanugo	New hair
	Week 1	Week 4	Week 7	Week 9	Week 1	Week 4	Week 7	Week 1	Week 4
Ca		−Fe	−Fe, −Zn, −V, −Cr	−Fe, −Zn, −Hg	+Zn^{Out}, +Cu^{Out}, +V$^{In, Out}$	+Zn^{In}, +V$^{In, Out}$	+V$^{In, Out}$	+Fe, −Zn, +V, +Cr, +Ni, +Cd, +Pb	+Fe, −Zn, −Cu, +V, +Cr, +Ni, +Cd
Fe	+Zn	−Ca, +Zn, +V, +Hg	−Ca, +Zn, +Cu, +Cr, +Ni	−Ca, +Zn, +Hg	+Zn^{In}, +Cu^{In}, +V$^{In, Out}$, +Cr^{In}	+Zn^{In}, +Cu^{Out},		+Ca, −Zn, +V, +Cr, +Ni, +Cd, +Pb	+Ca, −Zn, −Cu, +V, +Cr, +Ni, +Cd, +Pb
Zn	+Fe	+Fe, +Cu, +Hg	−Ca, +Fe, +Cr, +Ni, +Hg	−Ca, +Fe, +Hg, +V	+Ca^{Out}, +Fe^{In}	+Ca^{In}, +Fe^{In}, +Cu$^{In, Out}$		−Ca, −Fe, −V, −Cr, −Pb	−Ca, −Fe, +Cu, −V, −Cr, −Ni, +Hg
Cu		+Zn	+Fe	−Se	+Ca^{Out}, +Fe^{In}, +Cr^{In}	+Fe^{Out}, +Zn$^{In, Out}$	+Cr^{Out}	+V, +Cr, +Ni, +Cd, +Pb, +Ni	−Ca, −Fe, +Zn, −V, −Ni, −Cd
Se				−Cu					
V	+Pb	+Fe, +Pb	−Ca, +Hg	+Zn	+Ca$^{In, Out}$, +Cr^{In}, +Fe$^{In, Out}$, +Ni^{In}	+Ca$^{In, Out}$, +Cr^{In}	+Ca$^{In, Out}$, +Cr$^{In}_{In}$, +Ni	+Ca, +Fe, −Zn, +Cu, +Cr, +Ni, +Cd, +Pb	+Ca, +Fe, −Zn, −Cu, +Cr, +Ni, +Cd, +Pb
Cr		+Ni	−Ca, +Fe, +Zn, +Ni		+Fe^{In}, +Cu$^{In}_{Out}$, +V^{In}, +Ni^{In,}	+V^{In}, +Ni$^{In, Out}$	+V$^{In}_{Out}$, +Ni^{In,}, +Cu^{Out}	+Ca, +Fe, −Zn, +Cu, +V, +Ni, +Cd, +Pb	+Ca, +Fe, −Zn, +V, +Ni, +Pb
Ni	−Hg, +Pb	+Cr	+Fe, +Zn, +Cr		+V^{In}, +Cr$^{In, Out}$	+Cr$^{In, Out}$	+Cr$^{In, Out}$, +V^{In}	+Ca, +Fe, +Cu, +Se, +V, +Cr, +Cd, +Pb	+Ca, +Fe, −Zn, −Cu, +V, +Cr, +Cd, +Pb
Cd								+Ca, +Fe, +Cu, +V, +Ni, +Cr, +Pb	+Ca, +Fe, −Cu, +V, +Ni, +Pb
Pb	+V, +Ni	+V						+Ca, +Fe, −Zn, +Cu, +V, +Ni, +Cr, +Cd	+Fe, +V, +Ni, +Cr, +Cd
Hg	−Ni	+Fe, +Zn	+Zn, +V	−Ca, +Fe, +Zn					+Zn

[a] Double underlined = $p < 0.001$; underlined = $p < 0.01$; other = $p < 0.05$; Spearman's rank correlation.

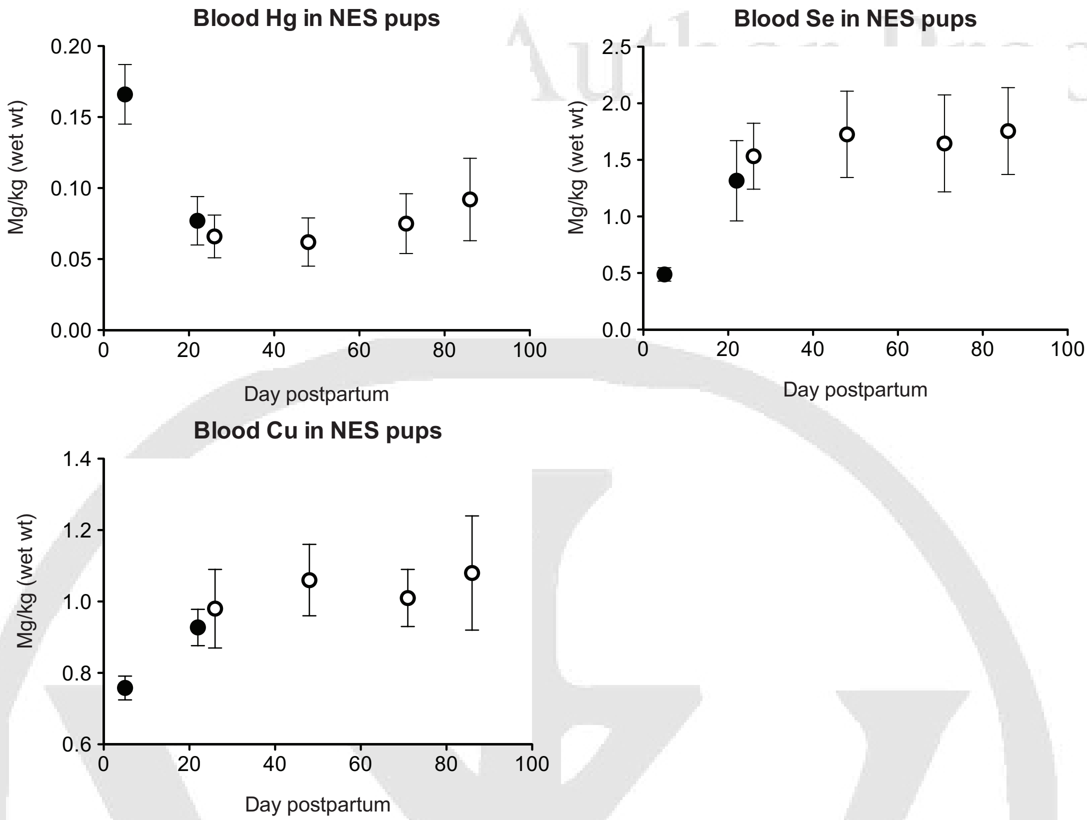

Fig. 4. Blood concentrations of Hg, Se, and Cu (mean ± SD, in mg/kg wet wt) in northern elephant seal (NES) pups during neonatal development: suckling pups (black circles)([13]; Cu concentrations not published) and weaned pups (open circles)(the presentstudy).

ern elephant seal blubber throughout the postweaning fast (except for Fe, which increased). This may suggest that elements are mobilized from the blubber as efficiently as triglycerides, the dominant lipid type [37]. Similar results were observed in gray seal weaned pups [27]. Nevertheless, we could expect greater changes, like in lactating gray seal females, in which all element concentrations in the blubber increased highly between the beginning and the end of lactation [27]. Indeed, both periods (i.e., lactation and postweaning fast) involve lipid mobilization to insure metabolism of fasting seals. In contrast with weaned pups, a much greater proportion of body fat content (~60%) is used during lactation in gray seal females [38]. Lactating and fasting females must meet both their own metabolic requirements and the nutrient requirements for milk production. The lower utilization of fat by weaned pups compared to that of lactating females might explain the absence of changes in element concentrations in pup blubber. Blubber does not appear to bethe best indicator of exposure to most trace elements. Only 0.5 to 3.5% of the total burdens of Zn, Cu, Se, Cd, and Hg are distributed in that tissue [39]. Nevertheless, 11.5, 26, and 45% of total burdens of V, Cr, and Pb, respectively, are found in the blubber [39]. Besides, it allows work on alive, free-ranging animals, unlike other tissues such as liver, kidney, or muscle.

Concentrations in hair and assessment of the contamination

In contrast with blood, which reflects the current exposure to trace elements (or remobilization), hairis an episodic indicator of exposure and enables monitoring of annual fluctuations by repeated sampling of molt hair over successive seasons. Several studies detail trace-element concentrations in pinniped hair/fur [28,40–46]. The usefulness of hair for trace-element analysis in pinnipeds, including its advantages and limitations, was already very well discussed in the study of Gray et al. [44]. These aspects thus will not be described here.

Hair and lanugo are inert tissues in which trace-elementlevels represent circulating levels in the blood during the period of hair growth [43]. Seal hair grows rapidly and not continuously [47]. These periods of hair growth in northern elephant sealsoccur, according to each age class, during fetal development (pup's lanugo), at the end of suckling, early in the postweaning fast (weaned pup's hair), or during the annual molting period (hair of juveniles and adults). In addition to the deposition into growing hair from circulating elements in the blood, trace elements in hair are derived from external deposition onto the surfaces of hair in pinnipeds [43]. Hair collected soon after growing (or "new" hair) will reflect thus the most recent exposure to trace elements, while hair collected just before molting (or "molt" hair) will reflect an extra exogenous contamination by the ambient environment [43]. Moreover, a potential loss of certain metals due to the depigmentation of hair over time may occur [48]. Consequently, it is paramount that the type of hair collected is noted at the time of sampling to enable meaningful comparisons of trace-element concentrations in future studies [44].

Lanugo in pups reflects the incorporation of elements during the fetal period. In the present study, all assayed elements were quantified in lanugo (including Cd), which means that all elements were accumulated in northern elephant seal mothers and transferred to offspring through the placenta during gestation. This resultis consistent with observations made in the lanugo of gray seals [27]. The mineral content of lanugo was, however, much greater in northern elephant seals than in gray seals, except for Pb (0.27 mg/kg dry wtin northern elephant

Table 5. Comparison of the mean (arithmetic or geometric according to the studies) concentrations (mg/kg dry wt) of elements in hair of phocid seals

Species	Location	Ca	Fe	Zn	Cu	Se	Hg	V	Cr	Ni	Cd	Pb	Reference
Caspian seal (*Pusa caspica*)	Caspian Sea, RU	–	–	98	33.8	2.3	1.6	0.7	1.20	–	0.39	3.53	[43]
Baikal seal (*Pusa sibirica*)	Lake Baikal, RU	–	–	105	5.4	2.3	3.6	1.0	0.94	–	0.09	13.40	[43]
Baikal seal (*Pusa sibirica*)	Lake Baikal, RU	–	–	–	–	–	4.5	–	–	–	–	–	[46]
Bearded seal (*Erignathus barbatus*)	White Sea, RU	–	–	146	5.7	–	0.8	–	–	3.11	1.30	1.42	[41]
Ringed seal (*Pusa hispida hispida*)	White Sea, RU	–	–	178	14.1	–	4.3	–	–	2.32	1.45	1.58	[41]
Ringed seal (*Pusa hispida ladogensis*)	Lake Ladoga, RU	–	–	324	22.5	–	17.5	–	–	4.11	0.96	6.34	[41]
Saimaa ringed seal (*Pusa hispida saimensis*)	Lake Saimaa, FI	–	–	–	–	–	12.1	–	0.92	5.7	0.62	5.52	[55]
Mediterranean monk seal (*Monachus monachus*)	Greece	–	–	129	12.6	–	22.4	–	–	–	0.21	0.78	[56]
Harp seal (*Phoca groenlandica*)	Canada	–	27	124	3.7	1.8	4.0	0.7	0.28	–	0.38	0.40	[57]
Harbor seal (*Pusa vitulina*)	Netherlands	–	–	133	5.0	1.5	17.0	0.6	0.40	–	0.17	1.44	[39]
Harbor seal (*Pusa vitulina*)	Germany	–	–	–	–	–	33.5	–	–	–	0.12	0.60	[42]
Harbor seal (*Pusa vitulina*)	Denmark	–	–	–	–	–	7.8	–	–	–	–	–	[45]
Gray seal (*Halichoerus grypus*)	Denmark	–	–	–	–	–	10.1	–	–	–	–	–	[45]
Gray seal (*Halichoerus grypus*)[a]	Scotland	259	9	122	2.8	2.9	4.5	0.1	0.05	0.18	0.02	0.64	[27]
Gray seal (*Halichoerus grypus*)[b]	Scotland	1936	87	101	4.2	4.1	7.7	2.4	0.24	1.29	0.27	2.24	[27]
Harbor seal (*Pusa vitulina*)[c]	California	–	–	–	–	–	8.2	–	–	–	–	–	[28]
Harbor seal (*Pusa vitulina*)[d]	California	–	–	–	–	–	9.9	–	–	–	–	–	[28]
Harbor seal (*Pusa vitulina*)[b]	California	–	–	–	–	–	15.1	–	–	–	–	–	[28]
Northern elephant seal (*Mirounga angustirostris*)[a]	California	1927	438	249	4.0	4.7	19.1	1.9	1.50	0.98	0.28	0.27	This study
Northern elephant seal (*Mirounga angustirostris*)[c]	California	1257	494	305	7.6	6.0	13.8	1.6	1.43	0.75	0.14	0.25	This study
Southern elephant seal (*Mirounga leonina*)[d]	Antarctica	–	–	164	11.2	–	–	–	0.24	0.47	0.08	ND	[52]
Southern elephant seal (*Mirounga leonina*)[b]	Antarctica	–	–	168	12.7	–	–	–	0.37	1.02	0.38	ND	[52]
Weddell seal (*Leptonychotes weddellii*)	Antarctica	–	–	99	4.4	–	0.7	–	–	–	0.53	ND	[40]
Weddell seal (*Leptonychotes weddellii*)[b]	Antarctica	604	74	137	15.1	3.1	5.6	4.2	5.87	3.52	2.81	1.29	[44]
Leopard seal (*Hydrurga leptonyx*)[e]	Antarctica	896	73	103	3.4	2.9	3.1	1.8	3.81	1.35	1.12	0.06	[44]
Leopard seal (*Hydrurga leptonyx*)[f]	Antarctica	563	77	128	3.7	4.1	4.6	0.9	4.12	0.76	0.31	0.01	[44]

[a] Pups (lanugo).
[b] Adult females.
[c] Pups (first pelage).
[d] Juveniles.
[e] Molt hair of adults.
[f] New hair of adults.
ND = not detected.

seals vs 0.64 mg/kg dry wtin gray seals; Fig. 3). As reported in gray seals [27], lanugo seems to be an important site of accumulation for trace elements from early stages of development. This indicates that gestation represents a significant elimination route for trace elements in adult females. Lanugo is an interesting tissue to assess easily the maternal transfer and to compare it between pinniped species. Concentrations of trace elements are high, facilitating their detection.

Concentrations of most elements (i.e., Fe, V, Cr, Ni, and Pb) in new hair were similar to concentrations in lanugo, but some elements (Zn, Cu, and Se) showed greaterconcentrations in new hair, while others (Ca, Hg, and Cd) showed lower concentrations than those in lanugo (Fig. 3). This would suggest that circulating blood concentrations of Ca, Hg, and Cd were greater during the fetal period than during the early postweaning fast. Overall, results in lanugo and new pelage indicate that significant transfers of trace elements occur from mother to offspring. Toxic metal exposure affects thus the offspring during its most sensitive period of development. For instance, Hg, known to pass through the placenta [49,50], can impact on normal neuronal development and the immune system of offspring [51], potentially having consequences to future growth and fitness. Further toxicological studies are needed to understand the adverse effects that these trace element levels may exert on the offspring's health. Moreover, northern elephant sealweaned pups contain other pollutants such as organochlorines in their tissues [9], generating likely other toxic effects related to chemical mixtures. Detecting effects in marine mammals is, however, particularly challenging. Usually, indicators of exposure (e.g., tissue levels, biomarkers) are more readily available and provide information on where to anticipate biotic responses [22]. In any case, the successive hair productions in pups (i.e., lanugo and new pelage) appear to be important elimination routes for toxic metals like Hg, Cd, and Pb. These processes enablepinnipeds to reduce significantly the body burdens of trace elements in pups, unlike cetaceans or sirenians.

Although hair type was often different according to studies and species, the concentrations in hair of northern elephant seal pups seem to be comparable to those of other phocids (Table 5). Nevertheless, northern elephant seal pups showed greater concentrations of Hg in hair than harbor seal pups, *Pusa vitulina*, from California (13.8 mg/kg dry wtin NES vs 8.2 mg/kg dry wtin harbor seals [28]) and greater concentrations of Zn, Ni, Cr, Cd, and Pb in hair than southern elephant seal juveniles, *Mirounga leonina* [52](Table 5). We can expect that northern elephant seal adults have greater levels of trace elements than their pups since concentrations in hair appear to increase with age [27,28,41]. Therefore, hair concentrations of almost all elements in northern elephant seals would be in the highest range of values found in phocids (except Cd and Pb; Table 5). Although northern elephant seals are long-lived top predators in the trophic network, this would be surprising given the feeding habits of northern elephant seal adult females. They usually forage pelagically in the open northeastern Pacific Ocean on prey in the deep scattering layer and spend only a limited amount of time in coastal areas near the contamination sources [53]. Nevertheless, it appears that a few of them spend some time foraging near the continental shelf, like males [53,54]. Therefore, further monitoring studies would be interesting to determine better the exposition to trace elements in northern elephant seals in relation totheir individual foraging areas.

SUPPLEMENTAL DATA

Table S1 Element concentrations (mean [median] ± SD [range] in mg/kg wet wt) in the inner and outer blubber during the postweaning fast period. (53 KB DOC).

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Q1: Author: Please check: only 10 listed here. Add Hg as listed above? See below in this paragraph also.

Q2: Author: Define AAS is Table 2.

Q3: Author: As meant: “between tissues”? Original wording (“between them”) was unclear.

Q4: Author: Please clarify what « other » is referring to in the Table 4 note.

Q5: Author: Please add publication number.

Q6: Author: Ref 24 : Please check source added (as found online).

Q7: Author: Ref 26: Please update. If not yet accepted for publication by the proof stage of this article, please delete from ref list and cite in text as “unpublished data” and remove from the reference section. Work that has not been accepted for publication should not be listed in a ref section.