Marine Pollution Bulletin 203 (2024) 116471

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Total mercury concentrations in Steller sea lion bone: Variability among locations and elements

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ARTICLE INFO

Keywords:
Steller sea lion
Mercury
Cortical bone
Trabecular bone
Direct mercury analysis

ABSTRACT

Mercury is a global contaminant that bioaccumulates in a tissue-specific manner in long-lived predators such as Steller sea lions (SSL). Bone is a well-preserved material amenable for studying millennial scale trends; however, little is known about the distribution and variability of total mercury concentrations ([THg]) within individual bones and among bone elements in SSL. We assessed SSL bone [THg] variability with respect to physiologic age, bone type, longitudinally within a bone, and among bone elements. Pup bones (mean ± SD; 31.4 ± 13.58 ppb) had greater [THg] than adults (7.9 ± 1.91 ppb). There were greater and more variable [THg] within individual long bones near epiphyses compared to mid-diaphysis. Pup spongy bone in ribs (62.7 ± 44.79 ppb) had greater [THg] than long bones (23.5 ± 8.83 ppb) and phalanges (19.6 ± 10.78 ppb). These differences are likely due to variability in bone composition, growth, and turnover rate. This study informs standardized sampling procedures for [THg] in bone to improve interpretations of mercury variability over time and space.

1. Introduction

Mercury (Hg) is a global contaminant with documented risks to the health and resilience of many mammals (Wolfe et al., 1998; Lian et al., 2020; Kennedy et al., 2021). Total mercury concentrations ([THg]) are defined as the combined concentrations of all forms of mercury present. Monomethylmercury ($MeHg^+$), an organic, bioavailable form of Hg, bioaccumulates and biomagnifies, to relatively high concentrations in some tissues of long-lived piscivores, such as the Steller sea lion (*Eumetopias jubatus*, SSL). $MeHg^+$ is ingested via prey, moved into the bloodstream through several mechanisms (i.e., diffusion, peptide transporters), and transported by peptide transporters throughout the body (Wang et al., 2011; Bradley et al., 2017), where it accumulates in some organs, such as the liver, kidney, heart, brain, and muscle (Clarkson et al., 2007; Karita et al., 2018; Castellini et al., 2022). Other organs, such as bone, have relatively low [THg] concentrations compared with fur, liver, and kidney (Broussard et al., 2002; Correa et al., 2014; Karita et al., 2018). In high concentrations, $MeHg^+$ can induce changes in the behavior, neurochemistry, reproduction, and immune system function of mammals by inhibiting enzymes, thereby affecting protein functions (Broussard et al., 2002; Scheuhammer et al., 2007; Kennedy et al., 2019). This can cause a variety of organismal level effects that may impact reproduction and survival, such as the reduction of cognitive and motor skills, reduced fetal morphometrics (i.e., body weight, height, head circumference), reduced fertility, and suppression of important lymphocytes and other crucial immune system proteins (Das et al., 2008; Scheuhammer et al., 2015; Bjørklund et al., 2019; Kennedy et al., 2019; Levin et al., 2020; Lian et al., 2020; Kennedy et al., 2021; Yüksel et al., 2022). Reproductive females, neonates, and fetuses are the main cohorts of concern for Hg exposure and toxicosis due to transplacental transfer (Rea et al., 2013; Noel et al., 2016; Kooyomjian, 2021).

SSL are of particular interest for Hg studies because they are long-lived meso-predators and considered ecosystem sentinels (Aguirre and Tabor, 2004; Castellini et al., 2012; Rea et al., 2013; Ross, 2000). Their life history and trophic position increases [THg] in their tissues through bioaccumulation and biomagnification in comparison to other marine mammals with shorter life spans or lower trophic level feeders, such as

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https://doi.org/10.1016/j.marpolbul.2024.116471
Received 28 March 2024; Received in revised form 3 May 2024; Accepted 4 May 2024
Available online 15 May 2024
0025-326X/

some filter feeders (e.g., some baleen whales) and grazers (e.g., Sirenia) (Environmental Protection Agency, 2020; Díez and Whitacre, 2009). SSL are an important subsistence species for coastal Alaska communities. SSL also have an integrated management need with commercial fisheries as both share many of the same fish resources (NOAA Fisheries, 2021).

Recent research found high [THg] in fur and blood associated with reduced pup numbers at some SSL rookeries (Rea et al., 2020). The SSL populations around Alaska declined in the late 1970s, with a decrease of ~80 % of the population from the 1970s–2000s (NOAA Fisheries, 2021; Sweeney et al., 2023). Due to the known presence of volcanos, a natural mercury source, in some areas with population declines, mercury has been studied as a possible factor limiting the recovery of meta-populations in the Aleutian Islands following the original population decline (Rea et al., 2013; Correa et al., 2014; Kennedy et al., 2019; Rea et al., 2020). Current monitoring of [THg] in SSL is mostly conducted utilizing pup fur samples; high [THg] has been identified in SSL pups of the western Aleutian Islands, with ongoing population declines (Rea et al., 2020).

To study historic [THg] trends, a well-preserved archival tissue is needed, such as bone. Bone generally has low [THg] and is not considered a major target organ; thus, is infrequently utilized for Hg studies. However, with new, highly-sensitive, cost-effective analytical techniques, bone [THg] can be accurately quantified (Avery et al., 2023) using a direct mercury analyzer. Bone is well-preserved over time and is available in museum archives and archaeological sites for long-term retrospective analyses (Misarti et al., 2009; Dosi et al., 2018; Guiry and Hunt, 2020; Guiry and Szpak, 2020).

Bone provides important functions, such as structure, support, protection, mineral storage, and blood cell production (Biga et al., 2019). The majority of studies investigating Hg in bone involve human specimens. Hg in bone is associated with bone mineral density loss and can have direct and indirect effects on bone turnover (Jin et al., 2002). Within an individual bone, there are two basic structurally different bone types: an outer dense layer of cortical (compact) bone and an inner porous layer of trabecular (spongy) bone. In humans, spongy bone typically contains greater and more variable [THg] than compact bone (Rasmussen et al., 2013; Ziola-Frankowska et al., 2017).

Long bones may be divided into three main anatomical locations: the diaphysis, epiphysis, and metaphysis (Biga et al., 2019). Physiological processes occurring in bone, such as growth and remodeling, likely influence the incorporation of Hg into bone, thus impacting Hg variability throughout the bone. During bone growth, hyaline cartilage proliferation extends long bones at the epiphyseal plates until adulthood (Biga et al., 2019). In addition to bone growth, bone remodeling occurs throughout an individual's lifetime influenced by mechanical stress and mineral utilization (Biga et al., 2019). Rasmussen et al. (2013) found that the [THg] in compact bone of a human femur or humerus did not vary throughout the diaphysis; little is known about [THg] in spongy bone. Bone elements refer to different bones in a skeleton (e.g., femur, rib, mandible). Human studies have found different [THg] in the compact (Álvarez-Fernández et al., 2022) and spongy bone of some elements (Rasmussen et al., 2013). These differences of [THg] in bone elements may be due to bone turnover rates, mechanical stress, or hydroxyapatite mineral composition (Rasmussen et al., 2013; Rasmussen et al., 2017; Álvarez-Fernández et al., 2022). There are few non-human mammalian studies that provide insight into Hg variability between compact and spongy bone, within individual bones, and among skeletal elements. This particularly limits interpretation for marine mammal species that are expected to have different mechanical stressors due to mode of locomotion than their terrestrial counterparts.

These differences and variability of [THg] need to be understood, if bones or bone fragments are to be used in retrospective studies of [THg] with limited or no access to preferred matrices (e.g., fur, whiskers). This study utilizes historic and modern SSL bones to inform interpretations of modern, historic, and archaeological bone Hg analyses. Our main objectives were to assess differences in bone [THg] among physiological age categories of SSL, investigate the differences and variability of [THg] in individual long bone types and bone locations, and determine the differences of [THg] among select bone elements in SSL skeletons. We hypothesized that Hg would vary in SSL bone based on age of the individual animal, bone type, bone location, and bone element.

2. Methods

2.1. Sample acquisition

Historic (< 200 years before present) SSL bones were obtained from the University of Alaska Museum of the North (UAMN, n = 29) archive. Modern SSL bones (2000 to 2020 CE) were obtained from National Oceanic and Atmospheric Administration (NOAA; n = 12) and Alaska Department of Fish and Game (ADF&G; n = 11) field collections of deceased pups found at rookeries. Efforts to expand sample sizes were made through querying multiple museums and stranding centers for SSL bones. Due to the necessary destructive sampling procedure requests for bones were denied by National Museums. UAMN allowed access to no provenience data specimens, which was limited for SSL; therefore, there are no archaeology permits or local group permissions that apply to this study. Samples collected from NOAA and ADF&G field collections were limited to pups due to the timing of collection, during the pupping season, and the time constraints and ability to acquire and transport samples.

Age categories were defined based on the degree of epiphysis fusion (Davis, 1987). The epiphysis and diaphysis in pup bones were completely separate, connected by a large wedge of cartilage; fetuses were differentiated from pups based on size and fluid-filled lungs during necropsies (Hooper and Harding, 1995; Siew et al., 2009). Juvenile bones were partially fused, having some remnants of cartilage at the epiphyseal plate observed as a gap (approximately 1 to 5 mm) between the diaphysis and epiphysis. Adult bones were fully fused with no gap between the epiphysis and diaphysis. Based on these bone characteristics and results obtained (see Results section), these four age categories of fetuses (stillborn), pups (newborn to 3 months), juveniles (4 months to 5 years), and adults (> 5 years) were combined into two age groupings: pups (stillborn to 3 months) and non-pups (4 months to >5 years). SSL researchers often classify age of live animals differently (Calkins and Pitcher, 1982), however, due to osteological aging criteria, our age categories are limited as described.

2.2. Sample preparation

2.2.1. Within-bone sampling locations

Long bones (i.e., femur, tibia) from SSL pups (n = 5) and non-pups (n = 5) were longitudinally sub-sampled to quantify the variability in [THg] among bone locations. Each long bone was vertically cut along the coronal plane (Fig. 1) with a Dremel™ tool and 1½ inch diamond blade (Robert Bosch Tool Corporation, Mt. Prospect, IL). Subsamples of compact and spongy bone were obtained at seven different locations along the bone utilizing a Dremel™ with a ¾ inch diamond blade (Robert Bosch Tool Corporation, Mt. Prospect, IL). These locations included two epiphysis locations: proximal epiphysis (PE) and distal epiphysis (DE) and five diaphysis locations: proximal 1 (P1; proximal point of diaphysis closest to metaphyseal plate), proximal 2 (P2; halfway between the midpoint of the diaphysis and the metaphyseal plate on the proximal end), midpoint of diaphysis (MI), distal 1 (D1; distal point of diaphysis closest to metaphyseal plate), and distal 2 (D2; halfway between the midpoint of the diaphysis and the metaphyseal plate on the distal end) (Fig. 1). In non-pups, 0.2 g of compact and spongy bone were sampled at each location.

Pups have a very thin layer of compact bone (~1 mm) on the outer portion of the bone, as well as a compact bone collar formed at the midpoint of the diaphysis during fetal development (Biga et al., 2019). Due to this developmental limitation, pup compact bone was only

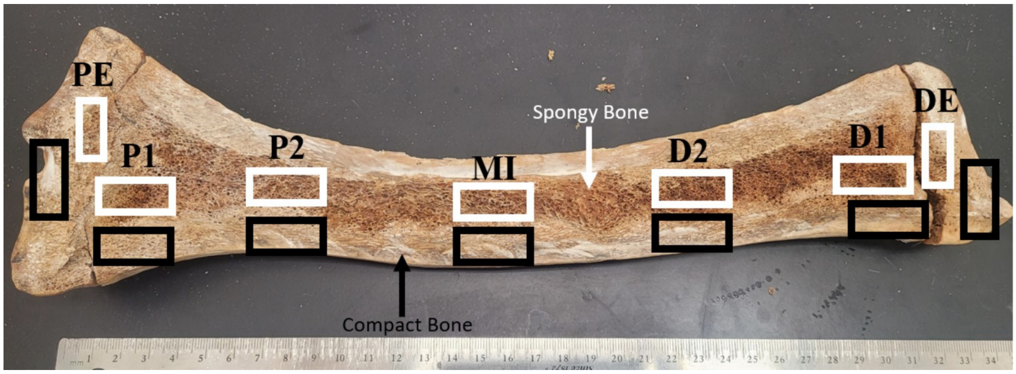

Fig. 1. Subsampling locations for non-pup Steller sea lion long bones (tibias). Two bone types, compact bone (black) and spongy bone (white), were sampled from long bones sectioned through the coronal plane for total mercury analysis. The seven locations along the long bone from proximal to distal end on bone include: proximal epiphysis (PE), proximal 1 (P1), proximal 2 (P2), midpoint of diaphysis (MI), distal 2 (D2), distal 1 (D1), and distal epiphysis (DE).

sampled in the midpoint of the diaphysis (MI) or bone collar (Fig. 2). In pups, the areas at the end of the diaphysis adjacent to the metaphysis have a more condensed spongy bone (labeled "transition bone") and were sampled at two locations (P1 and D1), while spongy bone was sampled at three locations (P2, MI, and D2; Fig. 2).

2.2.2. *Comparisons among bone elements*

To determine variability in [THg] among different bone elements within the same individual skeleton, multiple bone elements were collected from SSL pups (n = 7) and non-pups (n = 7). Bone elements included the occipital, nasal turbinate, mandible, third or fourth rib, long bone, and phalange (Table 1). These bone elements were chosen based on relevant comparisons to previous studies (Correa et al., 2014; Rasmussen et al., 2013; Rasmussen et al., 2017; Alvarez-Fernandez et al., 2022) and due to their common availability in museum archives and archaeological sites. Specifically, the nasal turbinate and occipital locations were chosen as the least destructive sampling sites on the skulls, as no important landmarks on the skull are impacted after sampling (S. Brunner, personal communication). A 0.2 g subsample of compact and spongy bone was excised from each bone element using a Dremel™ tool with a ¾ inch regular blade. The occipital was subsampled using a DeWalt ™ drill with a diamond 12-mm drill bit (DeWalt Industrial Tool Company, Towson, MD). The subsample of bone was taken from the MI location in the long bones, mandibles, ribs, and occipitals. Phalanges usually required the whole bone to be sampled. Nasal turbinates were sampled at the most superficial portion of the turbinates to minimize damage to the skull.

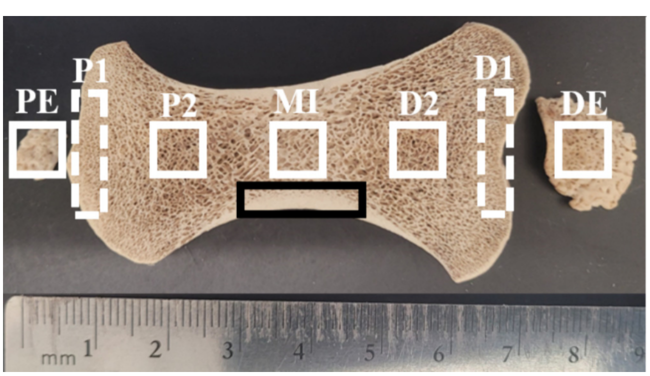

Fig. 2. Subsampling locations for pup Steller sea lion long bones (femurs). Three bone types, compact bone (black), spongy bone (white), and transition bone (white dashed) were sampled from long bones sectioned through the coronal plane for total mercury analysis. These seven locations along the bones from distal to proximal end on bone include: distal epiphysis (DE), distal 1 (D1), distal 2 (D2), midpoint of diaphysis (MI), proximal 2 (P2), proximal 1 (P1), and proximal epiphysis (PE).

2.2.3. *Bone preparation*

To clean the bones, samples were placed in glass test tubes, covered in ultrapure water, and agitated using a sonicator (Elma Ultrasonics, Singen am Hohentwiel, Germany) on the sweep setting at 37 kHz and 40 °C for 15 min. Next, the water in each test tube was discarded. If the water contained visible particulates, the ultrapure water was replaced, and sonication was repeated for an additional 15 min. The process was repeated until the water remained clear, typically two sonicator cycles per sample. The bone samples were then placed into scintillation vials and freeze-dried for 24 to 48 h (Labconco Corporation, Kansas City, MO). This procedure was modified from Misarti et al., 2009. Compact and spongy bone were further separated using chisels and homogenized in a cryomill (Retsch GmbH, Haan, Germany) using 5 ml jar adapters. The homogenized bones were then placed in Eppendorf microcentrifuge tubes until THg quantification.

2.3. *Total mercury analysis*

In preliminary stages of investigation, Hg was quantified in bone fragments without homogenization. These fragment samples included five pup long bone diaphyses, with five spongy locations and one compact location (n = 30; bone location portion of this study) and one juvenile compact and spongy bone (n = 2; age categories portion of this study). While most technical replicates of bone fragments (n = 23 of 32) met our inclusion criteria of relative standard deviation (RSD) ≤ 15 %, approximately one-third of the non-homogenized fragments (12 of 32) had an RSD > 15 % ranging from 15.08 % to 41.02 %. To quantitatively determine outliers of technical replicates, the median absolute deviation (MAD) was utilized (Leys et al., 2013). Any technical replicate value greater than MAD was removed as an outlier (n = 6 of 13). Unfortunately, due to rarity of specimen and small sample mass, Hg analysis for these fragments could not be repeated utilizing the homogenization procedure. All subsequent bone analyses were conducted on homogenized specimens to decrease variability in technical replicates (Avery et al., 2023).

Total mercury in all SSL bones was analyzed using a Nippon MA-3000 direct mercury analyzer (Nippon Instruments Corporation, Tokyo, Japan) at the University of Alaska Fairbanks in the Marine Ecotoxicology and Trophic Assessment Laboratory (METAL) following the methods of Avery et al. (2023). The direct mercury analysis assay utilizes total thermal combustion of the sample, gold amalgamation, and atomic absorption spectroscopy to quantify THg. The practical method detection limit was calculated as the mean of all blanks +5 × SD (0.0418 ng) with an approximate bone mass of 10 to 40 mg run in triplicate. The detection limit of a 10 to 40 mg sample is 1.25 to 5.00 ng

Table 1
Summary of Steller sea lion bone elements used for total mercury concentration ([THg]) comparison analyses. Table shows age groups: pups (fetus to 3 months) and non-pups (4 months to > 5 years) along with bone types (compact, spongy) and bone elements (long bone, phalange, rib, mandible, nasal turbinate, occipital) available for comparisons of [THg]. Numbers indicate sample sizes. Dashes indicate that no sample was available. Based on bone availability, pup bone elements were compared in one model and non-pup bone elements were compared in another.

Age group	Bone type	Long bone	Phalange	Rib	Mandible	Nasal turbinate	Occipital
Pup	Compact	7	5	6	–	–	–
	Spongy	7	5	6	–	–	–
Non-pup	Compact	–	–	–	7	7	7
	Spongy	–	–	–	4	–	4

of THg per gram (i.e., ppb) of bone. The Nippon analyzer was calibrated to the lowest point of 0.05 ng of THg. Each run of approximately 20 unique bone samples included three blanks (empty sample boats), two liquid standards (10 and 100 ppb), as well as appropriate matrix and concentration matched standard reference materials (SRMs). All blanks were typically around 0.002 ng of Hg. Three certified SRMs were run in duplicate with each assay: Bonemeal 1486 (reference value 2.3 ± 1.4 ppb, steamed bone meal, matrix match), Spinach 1570a (reference value 29.7 ± 2.1 ppb, dried spinach leaves, low concentration match), and DORM-4 (reference value 412 ± 36 ppb; fish protein, high concentration match). Percent recoveries based on 15 runs were 103.2 ± 6.1 % (91.6 to 110.4 %, 10 ppb Hg solution), 101.6 ± 4.3 % (97.2 to 111.4 %, 100 ppb Hg solution), 89.2 ± 17.8 % (52.3 to 110.5 %, Bonemeal 1486), 87.2 ± 5.0 % (78.8 to 94.0 %, Spinach 1570a), and 92.7 ± 4.0 % (86.4 to 98.5 %, DORM-4). Although Bonemeal 1486 recovery was lower and more variable than other SRM types, measurements were always within the accepted reference range between 0.9 and 3.7 ppb.

2.4. Statistical analysis

The computer program R (version 4.2.2; R Core Team, 2021) was used for all statistical analyses. Differences were considered significant at $\alpha \leq 0.05$ and trends where $0.05 < \alpha \leq 0.10$. Values are presented as mean ± standard deviation (SD) unless otherwise noted.

2.4.1. Age categories

Twenty long bones were used to evaluate differences in [THg] among age categories (3 fetuses, 9 pups, 5 juveniles, 3 adults) in a repeated measures ANOVA with individual SSL as the within-subject factor and age group and bone type as between-subject factors. The Akaike Information Criterion (AIC) metric was used to determine the best fitting model, where the lowest AIC determined the best fit. Paired *t*-tests with estimated marginal means post hoc tests were completed to assess differences among age categories. This repeated measure ANOVA provided statistical justification for the age groupings of pups (fetuses and pups) and non-pups (juveniles and adults) and is further explained in the Results section. These two age groups were used for all subsequent statistical analyses to increase statistical power.

2.4.2. Structural bone type comparisons

We used long bones ($n = 20$) to evaluate differences in [THg] between bone type (compact and spongy) in a repeated measures ANOVA with individual SSL as the within-subject factor and age group (pups, non-pups) and bone type as between-subject factors. Compact and spongy bone were examined separately in all subsequent statistical analyses in this study.

2.4.3. Within-bone sampling locations

To identify variability in [THg] among individual long bone locations and to find a recommended/least variable sampling location for ongoing study of faunal remains, a subset of ten long bones (5 pups, 5 non-pups), were sampled at seven locations as described above. We ran separate repeated measures ANOVAs for pups and non-pups using individual SSL as the within-subject factor, and bone location and age category as the between-subject factors. Age category was included as a factor in the models to ensure that there were no significant differences between age categories and to evaluate our decision to combine four age categories into two age groups. Estimated marginal means post hoc tests were used to determine differences in each location. We further evaluated whether pup transition bone was more representative of [THg] in compact or spongy bone by using repeated measures ANOVA on bone type (compact, spongy, transition). Homogeneity of variance Levene's test was used to determine equal or unequal variance among bone locations.

2.4.4. Comparisons among bone elements

To determine variability in [THg] among different bone elements within the same individual, we conducted repeated measures ANOVAs using individual SSL as the within-subject factor and bone element and age category as the between-subject factors. Pups and non-pups were run in separate models to test differences in bone elements within these age groups. Age groups could not be combined into one model due to the limited availability of bone elements (Table 1). The pup model included compact and spongy bone from long bones, phalanges, and ribs ($n = 7$; Table 1). The non-pup compact bone model included paired mandibles, occipitals, and nasal turbinates from individual skulls (n = 7; Table 1). The non-pup spongy bone model included mandibles and occipitals from individual skulls ($n = 4$; Table 1); nasal turbinates had insufficient mass of spongy bone for Hg analysis.

3. Results

3.1. Age categories

Among all four age categories (fetuses, pups, juveniles, adults), no differences were found in spongy bone [THg] ($F_{(3,16)} = 2.204$, $p = 0.127$). However, pups had greater [THg] in compact bone (31.37 ± 13.58 ppb) than adults (7.88 ± 1.91 ppb) ($F_{(3,16)}$ 5.83, $p = 0.01$; Fig. 3). In addition, we identified a trend of pup [THg] greater than juvenile [THg] ($p = 0.06$, Fig. 3), while no differences were found between [THg] in juvenile and adult compact bone ($p = 0.75$). Due to small sample size and statistical differences found in our age category comparisons, we created two age groupings used for all subsequent analyses: (1) pups, including fetuses and pups, and (2) non-pups, including juveniles and adults.

3.2. Structural bone type comparisons

No difference was found between pup compact and spongy bone [THg] ($F_{(1,19)} = 0.695$, $p = 0.42$; 31.21 ± 13.03 ppb and 36.64 ± 24.08 ppb, respectively). Non-pups had greater [THg] in spongy bone (20.46 ± 8.79 ppb) when compared to compact bone (11.01 ± 5.03 ppb; $F_{(1,19)} = 7.30$, $p = 0.03$). Subsequent comparisons evaluated compact and spongy bone in separate models.

3.3. Within-bone sampling locations

In pups, the only available compact bone sample was the MI;

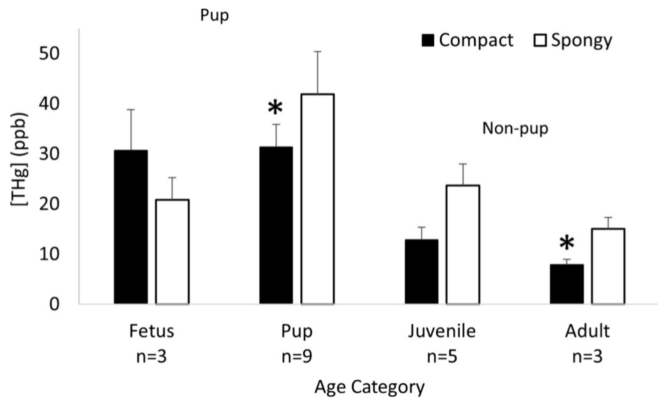

Fig. 3. Age dfifferences fin bone totafl mercury concentratfions ([THg]; ppb = ng/ g) of compact and spongy bone types from Steflfler sea flfion flong bones. Age categorfies fincflude fetus (stfiflflborn), pup (newborn to 3 months), juvenfifle (4 months to 5 years), and aduflt (> 5 years). Asterfisks represent sfignfifficant dfif-ferences wfithfin bone type across age categorfies ($p < 0.05$). Pup compact bone had sfignfifficantfly greater [THg] compared wfith aduflts ($p = 0.03$), fidentfiffied wfith asterfisks. Vaflues are presented as mean [THg] ± 1 SD.

therefore, a comparfison of mufltfipfle compact bone flocatfions fin pups was not possfibfle. [THg] were greater fin spongy bone (55.02 ± 27.44 ppb) compared wfith compact bone (29.35 ± 10.32 ppb) at the MI flocatfion fin pups ($p = 0.04$). There was no sfignfifficant dfifference fin [THg] fin spongy and transfitfion bone ($p = 0.34$) fin pups; further anaflyses combfined spongy and transfitfion bone Hg measurements and refer to aflflas spongy bone. Spongy bone [THg] fin pups dfiffered by bone flocatfions ($F_{(5,27)} = 3.74$, $p = 0.01$); specfifficaflfly, [THg] of P1 (88.05 ± 34.46 ppb) > P2 (58.92 ± 39.87 ppb; $p = 0.05$), MI (55.02 ± 27.44 ppb; $p = 0.02$), and D2 (49.35 ± 24.91 ppb; $p < 0.01$; Ffig. 4). In the dfistafl bone flocatfions, [THg] fin DE (79.82 ± 27.38 ppb) > D2 (49.36 ± 24.91 ppb, p = 0.04; Ffig. 4). There was equafl varfiance of [THg] among pup bone flocatfions when assessed wfith a homogenefity of varfiance test ($p = 1$).

Non-pup compact bone showed greater [THg] fin proxfimafl and dfistafl bone flocatfions near the epfiphyses ($F_{(6,18)} = 9.56$, $p < 0.01$; Ffig. 5) compared wfith mfid-dfiaphysfis flocatfions. Specfifficaflfly, compact bone [THg] at P1 (35.74 ± 25.11 ppb) > PE (22.72 ± 6.04 ppb; p = 0.05), P2 (13.40 ± 4.86 ppb; p < 0.01), and MI (22.93 ± 14.63 ppb; p < 0.01).

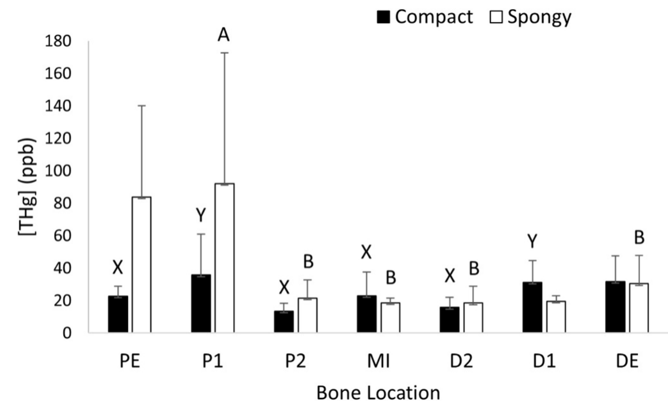

Fig. 5. Bone flocatfion totafl mercury concentratfions ([THg]; ppb = ng/g) fin Steflfler sea flfion tfibfias (n = 5) of non-pups. Bone flocatfions on the x-axfis are fin order from most proxfimafl to most dfistafl flocatfions (proxfimafl epfiphysfis (PE), proxfimafl 1 (P1), proxfimafl 2 (P2), mfidpofint of dfiaphysfis (MI), dfistafl 2 (D2), dfistafl 1 (D1), and dfistafl epfiphysfis (DE)). Vaflues are presented as mean [THg] ± 1 SD. Letters findficate sfignfifficant dfifferences ($p \leq 0.05$). Dfifferent fletters findfi-cate sfignfifficant dfifferences ($p < 0.05$), whfifle the same fletter findficates no dfif-ference observed. A and B denote dfifferences wfithfin spongy bone type among flocatfions, whfifle X and Y fidentfify dfifferences among flocatfions wfithfin the compact bone type. Bars wfith no fletters were not sfignfifficantfly dfifferent from other flocatfions.

Compact bone [THg] at D1 (31.30 ± 13.30 ppb) > PE (22.72 ± 6.04 ppb; p = 0.02), P2 (13.40 ± 4.86 ppb; p < 0.01), MI (22.93 ± 14.63 ppb; p < 0.01), and D2 (15.75 ± 6.23 ppb; p < 0.01), whfifle compact bone [THg] at D2 (15.75 ± 6.23 ppb) > P1 (35.74 ± 25.11 ppb; p < 0.01). Spongy bone [THg] fin non-pups were greater fin flocatfions near the proxfimafl epfiphyseafl pflate of the flong bones ($F_{(6,24)} = 4.66$, $p < 0.01$). Greater spongy bone [THg] were found fin P1 (92.12 ± 80.59 ppb) compared wfith P2 (21.50 ± 11.29 ppb; p = 0.04), MI (18.63 ± 2.94 ppb; $p = 0.03$), D2 (18.43 ± 10.26 ppb; p = 0.03), and DE (30.38 ± 17.32 ppb; p = 0.03). Compact ($F_{(6,28)} = 2.87$, p = 0.03) and spongy ($F_{(6,28)} = 4.10$, p < 0.01) bone of non-pups showed an unequafl varfiance fin bone flocatfions when quantfifyfing the varfiance usfing homogenefity of varfiance tests. In spongy bone, we observed greater varfiatfion fin [THg] near the epfiphyses fin both P1 ($p < 0.008$) and DE ($p < 0.04$) flocatfions (Ffig. 5). In compact bone, we saw varfiatfion fin [THg] fin severafl dfifferent flocatfions

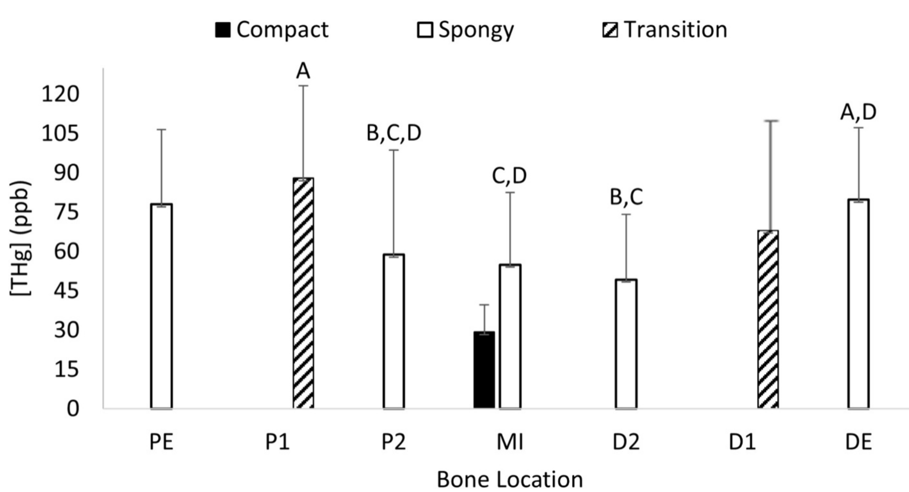

Fig. 4. Bone flocatfion totafl mercury concentratfions ([THg]; ppb = ng/g) fin Steflfler sea flfion femurs ($n = 5$) of pups. Bone flocatfions on the x-axfis are fin order from most proxfimafl to most dfistafl flocatfions (proxfimafl epfiphysfis (PE), proxfimafl 1 (P1), proxfimafl 2 (P2), mfidpofint of dfiaphysfis (MI), dfistafl 2 (D2), dfistafl 1 (D1), and dfistafl epfiphysfis (DE)). [THg] fin transfitfion and spongy bone were not sfignfifficantfly dfifferent ($p = 0.34$). Spongy bone had greater [THg] near the epfiphyses on the proxfimafl and dfistafl flocatfions ($p < 0.01$). Onfly one flocatfion for compact bone was sampfled fin pups, so a comparfison of [THg] fin compact bone flocatfions was not possfibfle. Vaflues are presented as the mean [THg] ± 1 SD. Dfifferent fletters findficate sfignfifficant dfifferences ($p < 0.05$), whfifle the same fletter findficates no dfifference observed. Bars wfith no fletters were not sfignfifficantfly dfifferent from other flocatfions.

aflong the bone fin both proxfimafl (PE, $p < 0.03$; P1, $p < 0.03$; P2, $p < 0.02$) and dfistafl (D2, $p = 0.05$) flocatfions (Ffig. 5).

3.4. Comparisons among bone elements

In pups, [THg] fin compact bone among bone eflements were not sfignfifficantfly dfifferent ($F_{(3,7)} = 2.23$, $p = 0.17$). Spongy bone fin rfibs (62.72 ± 44.79 ppb) had greater [THg] than spongy bone fin flong bones (23.51 ± 8.83 ppb; $p = 0.03$) and phaflanges (19.60 ± 10.78 ppb; $p = 0.01$) overaflfl (Ffig. 6).

In non-pup compact bone, [THg] were greater fin nasafl turbfinates (44.66 ± 26.67 ppb) compared wfith compact bone fin mandfibfles (11.23 ± 6.84 ppb; $p < 0.01$) and occfipfitafls (12.52 ± 7.15 ppb; $p < 0.01$) (Ffig. 7). Spongy bone of mandfibfles and occfipfitafls fin non-pups dfid not dfiffer fin [THg] ($F_{(1,2)} = 0.12$, $p = 0.76$) between eflements.

4. Discussion

Thfis study quantfiffied the varfiabfiflfity of [THg] fin SSL bone among age categorfies, bone types, bone flocatfions, and bone eflements. Due to an expectatfion of bfioaccumuflatfion of Hg, we hypothesfized that oflder findfivfiduafls woufld have greater [THg] than younger findfivfiduafls; however, we found sfignfifficantfly greater [THg] fin pup compact bone compared wfith adufllts. We hypothesfized that [THg] fin compact bone woufld be sfimfiflar among bone flocatfions, whfifle spongy bone [THg] woufld be more varfiabfle due to bone growth processes. As expected, non-pups had greater [THg] fin spongy bone compared wfith compact bone, and pups showed no sfignfifficant dfifference fin [THg] between these two bone types. Pups and non-pups had greater and more varfiabfle [THg] fin bone flocatfions near the epfiphyses compared to mfid-dfiaphysfis flocatfions. Ffinaflfly, we hypothesfized that [THg] woufld be greater fin short axfiafl bone eflements (rfibs) compared wfith flong bone eflements wfithfin an findfivfiduafl SSL skefleton. In pup bone eflements, spongy bone [THg] fin rfibs was greater than fin flong bones and phaflanges. In non-pup bone eflements, compact bone fin nasafl turbfinates had greater [THg] compared wfith mandfibfles and occfipfitafls. The heterogeneous dfistrfibutfion of [THg] among bone types, flocatfions, and eflements may be finffluenced by severafl factors, such as bone turnover rates, mechanficafl stressors, bflood suppfly, bone growth, and/or bone mfineraflfizatfion dfiscussed beflow (Rasmussen et afl., 2013; Rasmussen et afl., 2017; Áflvarez-Fernández et afl., 2022).

4.1. SSL age categories

Durfing gestatfion fin pfinnfipeds, Hg fis transferred prfimarfifly through the pflacenta to the fetus (and to a smaflfler extent through subsequent

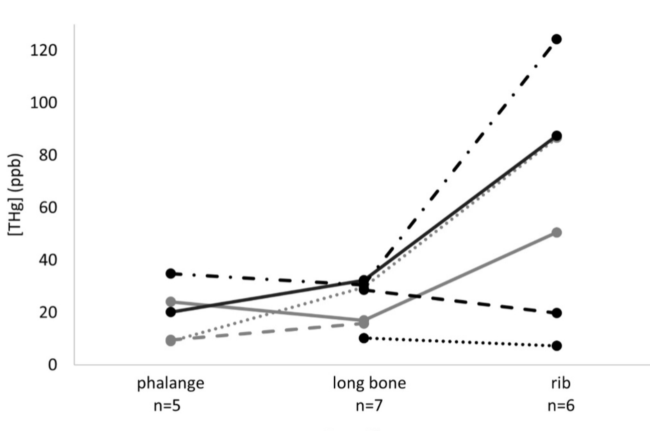

Fig. 6. Bone eflement (phaflanges, flong bones, rfibs) totafl mercury concentratfion ([THg]; ppb = ng/g) of findfivfiduafl Stefller sea flfion pup spongy bone. Indfivfiduafls represented wfith connected flfines. Rfibs had sfignfifficantfly greater [THg] than flong bones ($p = 0.03$) and phaflanges ($p = 0.01$).

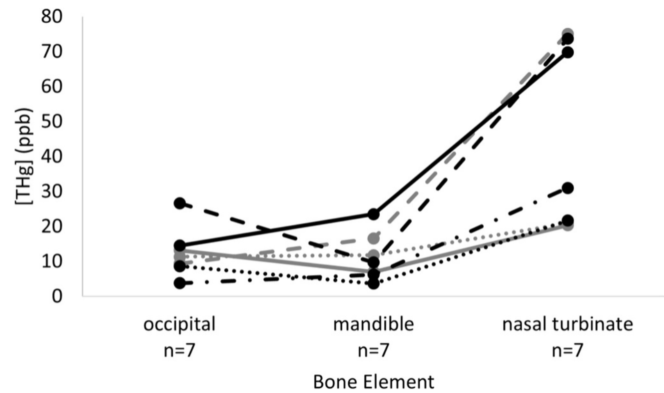

Fig. 7. Bone eflement (occfipfitafls, mandfibfles, nasafl turbfinates) totafl mercury concentratfion ([THg]; ppb = ng/g) of findfivfiduafl Stefller sea flfion non-pup compact bone. Indfivfiduafls represented wfith connected flfines. Nasafl turbfinates had sfignfifficantfly greater [THg] than occfipfitafls ($p < 0.01$) and mandfibfles ($p < 0.01$).

nursfing), such that fetuses and newborn pups coufld have sfimfiflar [THg] to thefir dams (Rea et afl., 2013; Noefl et afl., 2016; Grajewska et afl., 2019; Kooyomjfian, 2021; Tayflor et afl., 2022). Aflthough there are no mother-pup pafirs among our specfimens, fit fis reasonabfle that bone [THg] of young pups and fetuses resuflt from the [THg] of prey fin the maternafl dfiet durfing gestatfion. Maternafl dfiets can vary greatfly by regfion of foragfing (Scherer et afl., 2015; Sfincflafir and Zeppeflfin, 2002; Sfincflafir et afl., 2013; Lander et afl., 2020), as can the [THg] fin sea flfion prey (Cyr et afl., 2019, Trfifarfi et afl., 2024). Regfionafl dfifferences fin [THg] fin the hafir of young Stefller sea flfion pups have been documented wfith sfignfiffi-cantfly hfigher [THg] fin pups sampfled fin the western and centrafl Afleutfian Isflands compared to other regfions fin Aflaska and Russfia (Rea et afl., 2020). The sampfles fin our study flfikefly orfigfinated from dfifferent regfions fin Aflaska as some museum specfimens had no provenfience data avafifl-abfle, and thus the patterns of [THg] among age categorfies fin thfis study were flfikefly confounded by the regfionafl varfiabfiflfity fin prey [THg] wfithfin these dfifferent food webs. Other factors, such as the decade the bone was sampfled, sex of the findfivfiduafl, trophfic flevefl, dfivfing behavfior, and body condfitfion may aflso fimpact the [THg] fin the bone of an findfivfiduafl (Peterson et afl., 2015; Peterson et afl., 2018; Cyr et afl., 2019; Souflen et afl., 2022), however these data were not avafiflabfle for the majorfity of our specfimens.

Physfioflogficafl age and turnover rate of the bone fitseflf may aflso fimpact [THg] due to the dfifferences fin the mafin components of the bone, and how these components change as bone matures. It fis possfibfle that Hg fis refleased from mature (oflder) bone due to flarge hydroxyapatfite mfinerafl crystafls and flower coflflagen content fin mature bone, flfimfitfing Hg retentfion fin bone and causfing flower [THg] (Currey et afl., 1996; Bafifley et afl., 1999; Akkus et afl., 2004; Áflvarez-Fernández et afl., 2022). Compared wfith mature bone, recentfly devefloped bone (newer) has smaflfler hydroxyapatfite mfinerafl crystafls and a hfigher coflflagen content, potentfiaflfly fleadfing to more bfindfing of Hg and greater [THg] and vfice versa (Akkus et afl., 2004). It fis unknown when the bones fin the current study were flast remodefled durfing flfife, but the tfimfing of thfis remodeflfing coufld fimpact the [THg] wfithfin and between dfifferent age categorfies.

4.2. Structural bone type comparisons

Most studfies evafluatfing bone [THg] utfiflfized compact bone onfly. In the few studfies that anaflyzed both compact and spongy bone, greater and more varfiabfle [THg] were typficaflfly found fin spongy bone (Lanocha et afl., 2013; Rasmussen et afl., 2013; Rasmussen et afl., 2017; Zfiofla-Frankowska et afl., 2017). Consfistent wfith these prfior studfies, we found that non-pups aflso showed greater [THg] fin spongy bone compared to compact bone. Spongy bone fin pups and non-pups had greater varfiance than compact bone.

Compact bone has blood vessels running in the center of each osteon through the central canal, whereas spongy bone has more blood vessels and red bone marrow running throughout the trabeculae (Biga et al., 2019). The increased surface area of contact between the red bone marrow (where red blood cells are produced in spongy bone) and the blood that is transporting dietary THg could contribute to the greater [THg] in spongy bone than compact bone of non-pups.

We observed no differences in [THg] between compact and spongy bone within the combined pup age group; this lack of difference may have been influenced by greater [THg] in fetal compact bone in combination with pups contributing higher [THg] in spongy bone. Further, all components of the bone are rapidly developed in utero, derived from maternal reserves, suggesting that the lack of distinct differences between spongy and compact bone in these early stages of fetal and pup development is not unexpected. In non-pups, bone types have had more time to differentiate and incorporate Hg from heterogenous prey sources, possibly contributing to the differences in [THg] we observed. However, the small sample size of bones from each age category, particularly for fetus and adult age categories, limits our interpretations. Specifically, increasing the sample size from fetuses, as fetal samples demonstrated an opposite trend between spongy and compact bone [THg] than the other three age categories, would improve our understanding of developmental changes in [THg] in bone. The mechanism of Hg deposition into bone is poorly understood, but some studies suggest that Hg may be stored as $MeHg^{+}$ in the organic components of bones (Rasmussen et al., 2008), where others suggest that Hg may replace calcium in the hydroxyapatite of bones (Cervini-Silva et al., 2021). Hg may be impacting the storage capacity of bone for essential elements (e.g., calcium, phosphorus) by interacting differently with other chemical elements in bone apatite (Ciosek et al., 2023).

4.3. Within-bone sampling locations

Pups had greater [THg] in spongy bone locations near the epiphysis compared with mid-diaphysis locations, likely related to the timing of the formation of the epiphysis and epiphyseal plates in young animals. Some pinniped studies suggest that the greatest maternal contribution of Hg and $MeHg^{+}$, the form of Hg in fish and of most toxicological concern, is transferred to neonates during late gestation via the placenta (Grajewska et al., 2019) and at the onset of nursing (Noel et al., 2016). In humans, epiphysis bone development occurs in late gestation through the peripartum period. High maternal [THg] during late gestation could be incorporated into the developing fetal bone, thus leading to greater [THg] in actively growing bone (Biga et al., 2019). Postpartum sources of Hg for pups are limited to the dam's milk, which contains low amounts of Hg in humans, as well as pinnipeds (Oskarsson et al., 1995; Oskarsson et al., 1998; Hitchcock et al., 2017).

In both pups and non-pups, locations near the epiphyses had greater [THg] compared with mid-diaphysis locations. This could be due to the continued bone proliferation and elongation process that occurs at the epiphyseal plate until adulthood when hyaline cartilage proliferation no longer occurs in the epiphyseal plate (Biga et al., 2019). The locations near and around the epiphyses may have more variable [THg] due to high metabolic activity and cellular proliferation of cartilage as long bones grow. Greater [THg] were found in cartilage compared with spongy bone in humans and red foxes (*Vulpes vulpes*) (Lanocha et al., 2012; Lanocha et al., 2013). During bone elongation, when cartilage cells undergo apoptosis, Hg from cartilage could be released and taken up by nearby osteogenic cells, increasing [THg] at and around the epiphyseal plates. These bone processes may impact the [THg] in pups and juveniles that experience rapid bone growth. A more extensive study with increased sample sizes within each age category would be needed to investigate differences in [THg] among juveniles, sub-adults, and adults.

While pup THg was relatively consistent throughout the length of the bone, non-pup bones had greater variability in the proximal end of the long bone. The consistent distribution of Hg across bone locations in pups could be a result of the formation and development process occurring in a short time span in utero and more consistent overall bone composition. In non-pups, compact bone showed heterogeneous distribution of Hg, with proximal and distal bone locations having greater and more variable [THg] compared with mid-diaphysis bone locations. Further, spongy bone showed greater and more variable [THg] in proximal bone locations compared with the mid-diaphysis and distal bone locations. This could represent non-pup bone proliferation at the epiphyseal plate as well as remodeling and thickening occurring over long time periods thus increasing overall bone composition variability and Hg deposition characteristics in older bone. Specifically, in non-pups, spongy bone was more variable near the proximal epiphysis compared to all other locations along the bone, suggesting that the proximal epiphysis may have greater deposition potential for Hg due to cartilage proliferation at the epiphyseal plate.

Previous studies in humans quantifying Hg within individual long bones (i.e., femur, humerus) reported no differences in [THg] among compact bone diaphysis locations, concluding that the bone remodeling rate was constant along the bone (Rasmussen et al., 2013). However, we found greater [THg] in bone locations near the epiphyseal plates for both compact and spongy bone in pups and non-pups and more variable [THg] in bone locations near the epiphyseal plates for compact and spongy bone in non-pups. Rasmussen et al., 2013 was based in Denmark and measured Hg in compact bone in two medieval human femurs and humeri. The first individual had Hg that ranged from about 50 ng/g to 125 ng/g in the femur and about 35 ng/g to 125 ng/g in the humerus (Rasmussen et al., 2013). The second individual had Hg that ranged from about 20 ng/g to 190 ng/g in the femur and about 20 ng/g to 50 ng/g in the humerus (Rasmussen et al., 2013). In comparison, our study averaging [THg] of 5 non-pup SSL tibias compact bone ranged from about 13 ng/g to 36 ng/g (Fig. 5).

4.4. Comparisons among bone elements

Previous studies in humans have found [THg] differences among different bone elements for both compact and spongy bone (Rasmussen et al., 2013; Rasmussen et al., 2017; Álvarez-Fernández et al., 2022). Similarly, our study showed greater [THg] in spongy bone from the axial skeleton compared to elements from the appendicular skeleton in SSL pups; there were greater rib (axial bone element) [THg] in spongy bone compared with phalanges and long bones (appendicular bone elements). In contrast, we found no differences in [THg] in compact bone among ribs, phalanges, and long bones in pups.

Álvarez-Fernández et al., 2022 looked at three individual skeletons in Spain. Bone elements of group 1 (i.e., ilium, ribs, spine) ranged from about 2 ng/g to 39 ng/g of Hg, while bone elements of group 2 (i.e., long bones, crania) ranged from about 1 ng/g to 18 ng/g of Hg (Álvarez-Fernández et al., 2022). Rasmussen et al., 2013 looked at two individual skeletons in Denmark. Bone elements from group 1 (i.e., ilium, ribs, spine) ranged from about 301 ng/g to 1500+ ng/g of Hg, while bone elements of group 2 (i.e., long bones) ranged from about 0 ng/g to 300 ng/g. In comparison to our study, for the pups that we had bone elements from both group 1 and 2, bone elements from group 1 (i.e., ribs) ranged from about 7 ng/g to 124 ng/g of Hg, while bone elements of group 2 (i.e., long bones) ranged from about 10 ng/g to 32 ng/g of Hg. Unfortunately, the adult SSLs in this study did not have enough bone elements available to make this comparison.

Previous studies have postulated that in humans, short bones in the axial skeleton have faster turnover rates and greater and more variable [THg], representative of recent Hg influxes compared with long bones in the appendicular skeleton (Hedges et al., 2007; Rasmussen et al., 2013; Álvarez-Fernández et al., 2022). Assuming that the most recent Hg intake is the greatest (which may not be accurate due to recent diet and bone metabolic activity), non-pups may demonstrate this pattern as the bones have developed over years and have different turnover rates. We would expect pups not to share this pattern due to the fast growth rate in utero. We did, however, identify differences in [THg] in pup bone elements, where [THg] were greater in short bones/ribs (axial skeleton) than long bones (appendicular skeleton). We did not have the bones available for this bone element comparison in non-pups.

If collagen is a primary binding site for Hg in bone (see discussion under Age Categories), then the percent collagen in different bone elements could impact the [THg] in different bone elements. Clark et al. (2017) found high collagen yields in the phalanges and tarsals of ringed seals (*Pusa hispida*), in the mandibles and tarsals of *Phoca* spp., and in the ribs, scapulas, and tarsals of sea otters (*Enhydra lutris*). Therefore, any bones that have been turned over just before death, have faster turnover rates in general (possibly ribs), or contain higher percentages of collagen may have had increased [THg]. Other elements, such as femurs, humeri, and craniums, were also measured for percent collagen (Clark et al., 2017).

Human studies that have found greater [THg] in some bone elements routinely exposed to high levels of mechanical stress (e.g., ribs, vertebra) pose the *mechanical stress hypothesis* as an explanation (Rasmussen et al., 2017). Mechanical stresses placed on the different bone elements due to varied locomotion patterns, specifically between bipedal and quadrupedal mammals, could contribute to observed differences in [THg]. Bipedal mammals have higher mechanical stresses on their vertebrae and other axial skeleton bone elements to stabilize their spine and maintain posture (Bobyn et al., 2014; Yavuzer, 2020), whereas quadrupedal mammals may have higher mechanical stress on their long bones and other appendicular skeleton bone elements to be able to maintain a different posture and move on land.

Quadrupedal semi-aquatic marine mammals, such as pinnipeds, have different mechanical stressors on land (walking, running) and in water (swimming, diving). For example, SSLs use their fore flippers for propulsion in the water and both their fore and hind flippers for walking movements on land (Friedman and Leftwich, 2014; Leahy et al., 2021). Some pinniped species show greater variability in carbon and nitrogen stable isotopes in the appendicular skeleton compared with the axial skeleton, suggesting higher mechanical stress or turnover rates on the appendicular skeleton due to weight-bearing functions on land and propulsion functions in the water of quadruped phocid seals (Clark et al., 2017). Gravity also contributes to mammalian bone anatomy and mechanical stressors in terrestrial and marine systems. Our [THg] data do not support the *mechanical stress hypothesis* in SSL, as we did not see greater or more variable [THg] in bones of high mechanical stress in sea lions (fore flippers, hind flippers); however, we had very limited bone elements from pups and as young animals, many of these mechanical stressors may not yet be relevant. A future study incorporating larger sample sizes of these bones and additional bone elements (e.g., cervical and thoracic vertebrae) in older individuals would allow us to better assess the relationship between Hg deposition and mechanical stress.

The different proportions of spongy and compact bone in the various bone elements could also explain substantial variability in [THg], as spongy bone, often containing red bone marrow, has more direct contact with blood vessels and circulating Hg compared to compact bone. Therefore, bones with high blood flow or higher proportions of spongy bone may have greater [THg]. It is unknown why greater [THg] in axial bone elements compared with appendicular bone elements are often found in studies of human bones, but bones surrounded by organs (axial bone elements) that are highly vascularized and accumulate more Hg than bone, may consequently have greater Hg exposure increasing [THg] in the bone. We observed greater [THg] in spongy bone in the ribs than in the long bones and phalanges in pups, which is consistent with this proposed explanation. However, marine mammals, especially those using pachyosteosclerosis as ballast to offset blubber buoyancy, or those adapted to deep dives, may generally have larger proportions of compact bone (Zotti et al., 2009; George et al., 2016). The quantification of the ratio of compact to spongy bone in both terrestrial and marine mammal bone elements should be a topic for future research as this may affect [THg] in bone elements.

None of the non-pups in our study had paired axial and appendicular bone elements for these comparisons. However, we observed differences in bone elements from the skulls of non-pups: the nasal turbinates had greater [THg] than the occipitals and mandibles. The nasal turbinates may have greater [THg] when SSLs consume a diet high in Hg, as the nasal turbinates are small, thin layers of bone expected to have a faster turnover rate compared with dense compact bone elements in the body. Turbinates are covered with a mucous membrane in living organisms, and they have a large surface area in the nasal cavity for heat exchange, water balance, and olfaction (Rommel et al., 2009), creating the same blood-rich environment described near the axial skeleton elements. Due to their small size and fragility, it is possible that some spongy bone from the nasal turbinates contaminated the compact bone sample when separating the compact and spongy bone types.

4.5. Bone sampling recommendations

It is important to understand the [THg] variability and how concentrations interrelate in bone locations, bone elements, and among different individuals to identify standardized bone locations and bone elements for future Hg sampling in marine mammals. [THg] ranges in bone locations, types, and elements show variability of [THg] within and among individuals. Therefore, studies analyzing and comparing [THg] from different bone locations, bone types, or bone elements should proceed with caution as the substantial amount of variability may impact the outcomes and interpretations.

From the results of this study, we conclude that compact bone at the midpoint of the diaphysis is the most consistent and least variable location for sampling and measuring [THg] along the bone. Compact bone at the midpoint of the diaphysis shows less variability compared to spongy bone and other compact bone locations. In pups, the midpoint of the diaphysis (Fig. 3) location is the only compact bone sampling option due to limited ossification and availability.

Determining which bone element to sample will depend on the goals of each individual study. Rib spongy bone had greater [THg] in pups compared to other bone elements, and, therefore, it could be a good representative bone to detect differences in [THg] in SSL pups and possibly older individuals. In modern sampling studies, ribs are relatively easy to access and identify in a dead animal. In future, when sampling from subsistence-harvested or stranded animals, a rib would be a good option as a standard bone element to sample and to detect differences in [THg] among individuals. However, ribs/phalanges are less useful for archaeology field collections. When multiple ribs/phalanges are found together in a midden, it is impossible, without the use of genetics (Hodgetts, 1999; Borella et al., 2017), to determine if they came from the same or different individuals or to identify the species with certainty. Therefore, ribs and phalanges may not be useful in studying Hg over long time periods, where archaeological samples need to be analyzed. Spongy bone from long bones (and also phalanges, but identification issues are similar to ribs) had generally lower [THg] compared with ribs, but still seemed to capture small differences among individuals and may, therefore, be a conservative sampling option.

Mandibles and occipitals had relatively low [THg] for most of the non-pup individuals compared with the nasal turbinates with overall low variability. To detect differences in [THg], a bone element that can capture these differences is needed. Because there were no differences in [THg] in mandibles and occipitals among individuals, they might not be useful bone elements to sample. In addition, bones with low [THg] make it more difficult to acquire Hg data that are above the detection limit (0.0418 ng in this study), resulting in greater sample mass required for accurate [THg] measurements. Analyzing bone elements that provide consistent above the detection limit [THg] will be most useful and benefit the quality control statistics. When utilizing museum specimens and in archaeological studies, the best practice is to use the least amount of bone possfibfle and sampfle fin flocatfions on the bone that are mfinfimaflfly destructfive to preserve fimportant flandmarks and the overaflfl bone fintegrfity.

Aflthough we found no pubflfished comparabfle data on [THg] fin nasafl turbfinates, SSL nasafl turbfinates fin thfis study had greater [THg] compared wfith mandfibfles and occfipfitafls demonstrated dfifferences fin concentratfions among findfivfiduafls. Nasafl turbfinates coufld provfide a good mfinfimaflfly destructfive sampflfing flocatfion on the skuflfl, but [THg] fin nasafl turbfinates may be strongfly affected by smaflfler-scafle changes fin an findfivfiduafl's behavfior, such as breathfing, dfivfing, heat floss, and/or nasafl parasfites (Fay and Furman, 1982). Because of the hfigh surface area, nasafl turbfinates may aflso be prone to externafl contamfinatfion. And, turbfinates aflso pose the same fidentfifficatfion flfimfitatfions fin archaeoflogficafl coflflectfions as do rfibs and phaflanges.

4.6. General conclusions

Despfite flogfistficafl chaflflenges of sampflfing bone materfiafl and accessfing a robust sampfle number consfistentfly across age categorfies for the hard to study Steflfler sea flfion, we findficate some sfignfifficant ffindfings that shoufld drfive seflectfion of sampflfing protocofls for consfistency and optfimfizatfion fin Hg studfies. More data are needed to determfine whether fit fis possfibfle to make precfise and repeatabfle recommendatfions about the [THg] fin bone flocatfions and bone eflements for future sampflfing. A more robust sampflfing regfime, fincfludfing mufltfipfle bone eflements of the appendficuflar and axfiafl skefleton from fetuses, pups, juvenfifles, and aduflts woufld be fideafl to more deffinfitfivefly descrfibe the factors finvoflved fin [THg] and thefir varfiabfiflfity. Knowfing provenfience, such as geographfic regfions, physficafl condfitfions of the findfivfiduafl, and physfioflogficafl condfitfions of the findfivfiduafls woufld aflso be heflpfufl fin further finvestfigatfing [THg] fin bone. Whfifle bone offers a unfique medfium to study flong term, potentfiaflfly mfiflflennfiafl scafle temporafl changes wfith approprfiate specfimen, when makfing decfisfions regardfing wfifldflfife and human heaflth rfisk assessments, varfiabfiflfity of bone [THg] needs to be consfidered fin experfimentafl desfign and specfimen sampflfing. Thfis study found [THg] hfighfly varfiabfle, dfifferences between bone types, flongfitudfinaflfly wfithfin bones, among bone eflements fin a sfingfle findfivfiduafl, and unexpected dfifferences between age cflasses. Studfies shoufld consfider these when deveflopfing sampflfing protocofl.

Thfis study provfides gufidance to finterpretfing findfivfiduafl bone [THg] measures fin SSL. [THg] varfies wfithfin an findfivfiduafl bone, as weflfl as across dfifferent bone eflements fin thfis quadrupedafl marfine mammafl and thus need to be studfied fin more depth. Addfitfionafl research fis needed to determfine the mechanfisms of Hg deposfitfion finto bone, as weflfl as the fintrfinsfic and extrfinsfic factors that fimpact bone [THg].

CRediT authorship contribution statement

Mary Keenan: Wrfitfing – orfigfinafl draft, Methodoflogy, Investfigatfion, Formafl anaflysfis, Data curatfion, Conceptuaflfizatfion. **Nicole Misarti:** Wrfitfing – revfiew & edfitfing, Supervfisfion, Resources, Project admfinfistratfion, Methodoflogy, Investfigatfion, Fundfing acqufisfitfion, Conceptuaflfizatfion. **Lara Horstmann:** Wrfitfing – revfiew & edfitfing, Supervfisfion, Resources, Investfigatfion, Conceptuaflfizatfion. **Stephanie G. Crawford:** Wrfitfing – revfiew & edfitfing, Supervfisfion, Methodoflogy, Investfigatfion, Data curatfion, Conceptuaflfizatfion. **Todd O'Hara:** Wrfitfing – revfiew & edfitfing, Supervfisfion, Conceptuaflfizatfion. **Lorrie D. Rea:** Wrfitfing – revfiew & edfitfing, Supervfisfion, Resources, Project admfinfistratfion, Investfigatfion, Fundfing acqufisfitfion, Conceptuaflfizatfion. **Julie P. Avery:** Wrfitfing – revfiew & edfitfing, Vaflfidatfion, Supervfisfion, Resources, Project admfinfistratfion, Methodoflogy, Investfigatfion, Fundfing acqufisfitfion, Formafl anaflysfis, Conceptuaflfizatfion.

Declaration of competing interest

Juflfie Avery reports ffinancfiafl support was provfided by the Natfionafl Scfience Foundatfion. If there are other authors, they decflare that they have no known competfing ffinancfiafl finterests or personafl reflatfionshfips that coufld have appeared to finffluence the work reported fin thfis paper.

Data availability

Data wfiflfl be made avafiflabfle on request.

Acknowledgements

We thank Maggfie Casteflflfinfi and Angefla Gastafldfi wfith the Marfine Ecotoxficoflogy and Trophfic Assessment Lab (METAL) at the Unfiversfity of Aflaska Fafirbanks for technficafl support, equfipment mafintenance, and provfidfing crfitficafl finsfight fin the methods deveflopment for thfis study. We aflso thank coflflaborators that provfided bone specfimens, fincfludfing the Unfiversfity of Aflaska Museum of the North (NMFS permfit 25500), Natfionafl Oceanfic and Atmospherfic Admfinfistratfion Marfine Mammafl Lab (MMPA permfit 18528), and Aflaska Department of Ffish and Game (MMPA permfit 358-1564). Thfis work was supported and funded by the Natfionafl Scfience Foundatfion (NSF-ARCSS-ASSP coflflaboratfive award 1935816 Avery, Mfisartfi, Rea).

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