



## Walrus teeth as biomonitors of trace elements in Arctic marine ecosystems

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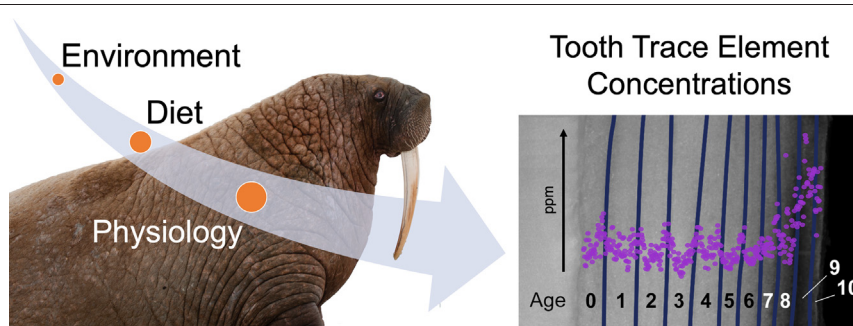
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### HIGHLIGHTS

- Trace elements in Pacific walrus teeth varied with age, sex, and calendar year.
- Physiology was important in shaping patterns of trace element accumulation.
- Confounding factors limit potential of walrus teeth as trace element biomonitors.
- Future research is needed to improve tooth trace element data interpretability.

### GRAPHICAL ABSTRACT



### ARTICLE INFO

#### Article history:

Received 10 December 2020

Received in revised form 24 January 2021

Accepted 25 January 2021

Available online 2 February 2021

Editor: Julian Blasco

#### Keywords:

Contaminants

Marine mammals

LA-ICP-MS

*Odobenus rosmarus*

Vanadium

### ABSTRACT

Effective biomonitoring requires an understanding of the factors driving concentrations of the substances or compounds of interest in the tissues of studied organisms. Biomonitoring of trace elements, and heavy metals in particular, has been the focus of much research; however, the complex roles many trace elements play in animal and plant tissues can make it difficult to disentangle environmental signals from physiology. This study examined the concentrations of 15 trace elements in the teeth of 122 Pacific walrus (*Odobenus rosmarus divergens*) to investigate the potential for walrus teeth as biomonitors of trace elements in Arctic ecosystems. Elemental concentrations were measured across cementum growth layer groups (GLGs), thereby reconstructing a lifetime history of element concentrations for each walrus. The locations of GLGs were used to divide trace element time series into individual years, allowing each GLG to be associated with an animal age and a calendar year. The elements studied exhibited a great deal of complexity, reflecting the numerous factors responsible for generating tooth trace element concentrations. Generalized linear mixed models were used to investigate the importance of age and sex in explaining observed variation in trace element concentrations. Some elements exhibited clear physiological signals (particularly zinc, strontium, barium, and lead), and all elements except arsenic varied by age and/or sex. Pearson's correlations revealed that elements were more strongly correlated among calendar years than among individual walruses, and correlations of trace elements within individual walruses were generally inconsistent or weak. Plots of average elemental concentrations through time from 1945 to 2014 further supported the correlation analyses, with many elements exhibiting similar patterns across the ~70-year period. Together, these results indicate the importance of physiology in modulating tooth trace element concentrations in walrus tooth cementum, but suggest that many trace elements reflect a record of environmental exposure and dietary intake/uptake.

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## 1. Introduction

The use of organisms to assess the abundance and distribution of substances or compounds of interest in the environment, referred to as biomonitoring, is a common approach for tracking the impacts of anthropogenic activities on natural systems (Rainbow, 1995; Wang, 2016; Wren, 1986). Plants, animals, and fungi may retain or concentrate substances that are ephemeral, spatially heterogeneous, or otherwise difficult to measure directly in the environment, thereby increasing the ease of sampling (Harguinteguy et al., 2013; Morrison et al., 2017; Yin et al., 2008). Further, biomonitoring can provide information about the bioavailability of individual substances or compounds, and may give insight into how they move within ecosystems and food webs (Kraak et al., 1991; Wang, 2016; Yin et al., 2008).

Many biomonitoring efforts focus on concentrations of potentially toxic trace elements, including heavy metals. Concentrations of these elements may reflect anthropogenic contamination; however, they also exist naturally in the environment in measurable concentrations and some may be essential to plant and animal physiology in trace quantities (Mertz, 1981). In the ocean, concentrations of these elements are driven by numerous interacting factors, including release from underlying geology and sediments, weathering and terrestrial input, surface deposition of windblown particles, seawater chemistry, and sea ice melt, as well as by aggregation by marine organisms, and contamination via anthropogenic processes (Beattie et al., 2014; Bryan, 1984; Duce et al., 1991; Kim et al., 2015; Martin et al., 1976; Mendez et al., 2010; Tovar-Sánchez et al., 2010). Understanding the natural variability in baseline or background elemental concentrations is critical to detecting increases in these trace elements associated with anthropogenic activities. Further, the bioavailability and toxicity of some potentially toxic elements depends on the form in which they are found, thus absolute concentrations in water or sediment may not be a strong indicator of the impact of contamination on biological systems (Borgmann, 2000; Wang, 2016).

Tissues exhibiting incremental growth, such as coral skeletons and tree rings, can act as archives of trace element concentrations (Outridge et al., 1995). Mammalian teeth are one such tissue, and are particularly useful for reconstructing records of trace element concentrations, because tooth growth layers are metabolically inert after deposition, thus each layer represents a discrete point in time (Klevezal, 1996). Marine mammal teeth are considered to be valuable biomonitors of ocean ecosystems (e.g., Aubail et al., 2010; De María et al., 2021; Ozersky et al., 2017). These animals often move across broad geographic ranges and tend to occupy relatively high trophic positions, thus elemental concentrations in marine mammal teeth may provide information about concentrations of trace elements within regional food webs, as well as individual point sources. That said, little research has been conducted to quantitatively link concentrations of trace elements in teeth to those in other tissues, or to examine factors that could possibly confound the use of marine mammal teeth as biomonitors, such as the roles of physiology and age-related patterns of accumulation in shaping tooth trace element concentrations.

Pacific walrus (*Odobenus rosmarus divergens*) are key members of sub-Arctic and Arctic ecosystems in the Bering and Chukchi seas, and are important to the culture and subsistence of Russian and Alaska Native communities. Monitoring trace element concentrations, particularly potentially toxic elements, such as heavy metals, is thus of critical importance. Though their diet consists primarily of bivalves, walrus are generalist foragers and consume a wide variety of taxa including other pinnipeds (Fay, 1982; Seymour et al., 2014; Sheffield and Grebmeier, 2009). Pacific walrus exhibit a sex-segregated migration (Fay, 1982). Males and females spend the high sea ice season (late winter and spring) together in the Bering Sea. Females and juveniles typically follow the sea ice edge north in to the Chukchi Sea during summer, whereas most males move to the coastal regions of the Bering Sea, including Bristol Bay, Alaska, and Chukotka, Russia. Examining trace

elements in Pacific walrus teeth may provide important insight into elemental concentrations in benthic food webs in the Bering and Chukchi seas. Further, dissimilarities in element accumulation by males and females may provide some insight into differences between these two basins. Walrus teeth are regularly collected as part of Alaska Native subsistence harvests and are widely available in museum collections. This availability, as well as their large size and broad cementum layer, makes walrus teeth ideal candidates for trace element analyses. As such, a relatively large body of work has been dedicated to examining walrus tooth trace elements (e.g., Evans et al., 1995; Jay et al., 2008; Outridge and Stewart, 1999), and they are considered to be good candidates for reconstructing long-term records of environmental element concentrations (Evans et al., 1995). Further, indications of stock structure from whole tooth elemental concentrations support the idea that element concentrations in teeth reflect differences in geographic location or diet composition (Jay et al., 2008). That said, much remains to be learned about the factors affecting trace element concentrations in marine mammal teeth, including diet, physiology, and baseline environmental concentrations, before they can be effectively used as biomonitors.

The primary objective of this research was to assess the suitability of walrus teeth as tools for monitoring trace elements in the marine environment through time. To accomplish this, concentrations of 15 trace elements were measured in the growth layers of walrus teeth collected between 1880 and 2016. Patterns of element accumulation were then examined within the lives of individual walrus and by calendar year to 1) investigate the existence of any physiological signals that might impact biomonitoring efforts, 2) determine whether any elements exhibit age-related concentration increases or decreases, and 3) examine changes in average element concentrations through time at the population level.

## 2. Methods

### 2.1. Trace element analysis and data processing

Postcanine teeth from 122 Pacific walrus (Female:  $n = 93$ ; Male:  $n = 29$ ) were on loan from the University of Alaska Museum in Fairbanks, Alaska, and the National Museum of Natural History, in Washington DC. Specimens were collected between 1880 and 2016 (Table S1). The majority of these samples originated from Alaska Native subsistence harvests in the communities of Gambell and Savoonga on St. Lawrence Island, Alaska, though some of the earlier specimens were collected during scientific expeditions. Because specimens used in this study originated from museum collections and/or Alaska Native subsistence harvests, this research was Institutional Animal Care and Use Committee (IACUC) exempt. All specimens from contemporary subsistence harvests were transferred to UAF for analysis under a Letter of Authorization from the United States Fish and Wildlife Service (USFWS) to Dr. L. Horstmann.

A low speed, water-cooled saw equipped with a diamond blade was used to create a 1.5 mm-thick longitudinal cross-section of the center of the tooth. A 3000-grit diamond smoothing disc mounted on a rotary polishing wheel was then used to polish this cross-section. Samples were rinsed with ultra-pure water after polishing and allowed to air dry, then rinsed and air dried again immediately prior to analysis.

Trace element analyses were conducted at the Advanced Instrumentation Lab, University of Alaska Fairbanks (UAF), Fairbanks, Alaska. An Agilent 7500ce Inductively Coupled Plasma Mass Spectrometer (ICP-MS; fitted with a Cs lens stack to improve sensitivity), coupled with a New Wave UP213 laser, was used to measure concentrations of vanadium ( $^{51}\text{V}$ ), chromium ( $^{53}\text{Cr}$ ), manganese ( $^{55}\text{Mn}$ ), iron ( $^{57}\text{Fe}$ ), cobalt ( $^{59}\text{Co}$ ), nickel ( $^{60}\text{Ni}$ ), copper ( $^{63}\text{Cu}$ ), zinc ( $^{66}\text{Zn}$ ), arsenic ( $^{75}\text{As}$ ), strontium ( $^{88}\text{Sr}$ ), molybdenum ( $^{95}\text{Mo}$ ), silver ( $^{107}\text{Ag}$ ), cadmium ( $^{111}\text{Cd}$ ), barium ( $^{137}\text{Ba}$ ), and lead ( $^{208}\text{Pb}$ ) in walrus tooth cementum. Instrumental precision for the ICP-MS was  $\pm 5\%$ . The internal standard for these

analyses was calcium ( $^{43}\text{Ca}$ ), and the resulting calcium-normalized element concentrations are reported in parts per million (ppm). Measured elemental concentrations were compared with a United States Geological Survey microanalytical phosphate standard (MAPS-4), as well as a National Institute of Standards and Technology Standard Reference Material (NIST SRM 610). All laser transects were ablated using the following parameters: beam width = 25  $\mu\text{m}$ ; power = 55%; pulse frequency = 10 Hz; transect speed = 5  $\mu\text{m/s}$ . Dwell times ranged from 0.002–0.15 s (Table S2). Locations of ablation transects were selected to maximize distance from the root, where cementum growth layer groups converge and become distorted, while also avoiding areas of tooth wear near the crown, where not all cementum layers are present. Transects were ablated starting at the interface between the dentin and the cementum (first year of life), and ending at the outer edge of the tooth (final year of life). Thus, elemental time series generated during these analyses represented a lifetime record for each animal.

Data extraction and processing was conducted in Igor Pro version 6.37 using the Iolite software package version 3.0. All statistical analyses were conducted using R version 4.0.2 (R Core Team, 2020) with RStudio version 1.3.959 (RStudio Team, 2015). Limits of detection were calculated separately for each analytical run using the standard method applied by Iolite (Longerich et al., 1996). A value of one half the limit of detection was used to replace data points that fell below the detection limits (U.S. Environmental Protection Agency, 2000). Data points more than 4 standard deviations from the mean were considered outliers and removed from analysis (Tukey, 1977). These data points were typically single, unrealistically high values, and were likely to represent instrumental errors, rather than actual changes in tooth trace element concentrations. Their removal is therefore unlikely to have impacted the results of this study.

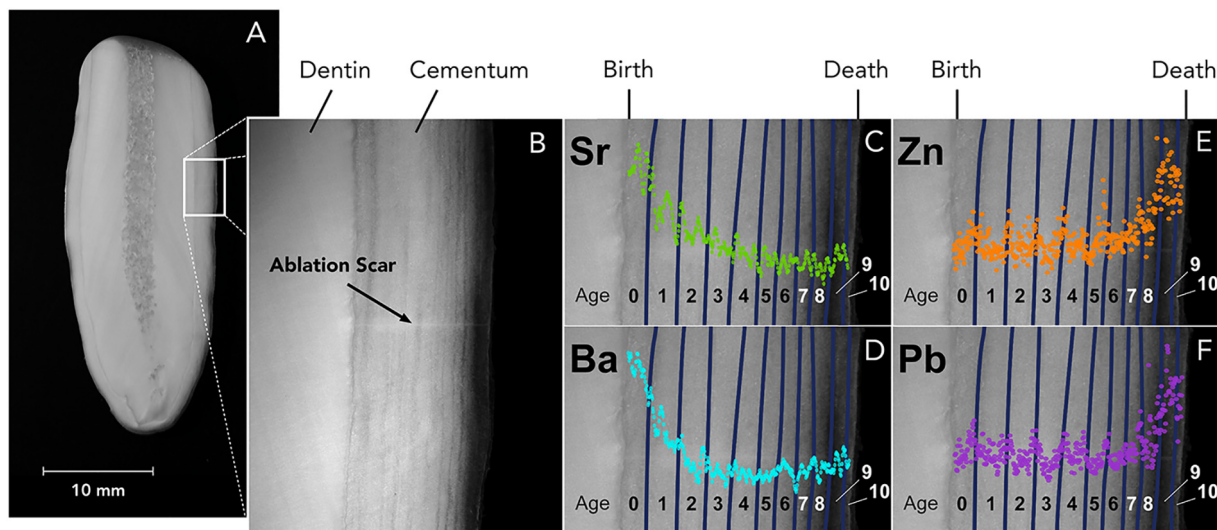
### 2.2. Growth layer group counts and designation of element concentrations to individual years

After trace element analysis, photographs of walrus teeth were taken using a Leica DFC295 camera coupled with a Leica M165 C optical microscope using reflected light. All growth layer groups (GLGs) in the tooth cementum were identified (Fay, 1982; Garlich-Miller et al., 1993) and marked collaboratively by the authors (C.T.C., L.H., and

N.M.), and their positions were revisited on at least two additional days to confirm their locations on the laser ablation transect (Fig. 1). Locations of the growth layers were used to assign measured elemental concentrations to individual years of life, with L1/D1 (the first light and dark layers) representing Age 0 (1st year of life), L2/D2 making up Age 1 (2nd year of life), and so on. All GLGs were counted to estimate the age of each animal at death, and this information was used in tandem with the year of death to associate GLGs with individual calendar years. Thus, an animal that was Age 5 when it was harvested in 1995 would have GLGs grown in 1990–1995. Only complete GLGs with a fully formed light and dark layer were used for analysis of trace element concentrations by animal age or calendar year.

### 2.3. Statistical methods

Trace element data were natural log-transformed prior to statistical analysis to ensure their distributions approximated normality. Generalized linear mixed models (GLMMs) were run using the R package ‘lme4’ (Bates et al., 2015) to test for relationships between concentrations of each trace element and individual walrus age, as well as test for differences between males and females. These analyses were restricted to ages 0–15, to ensure that  $\geq 15$  male and female walruses were represented at each age. Model selection was conducted using Akaike’s Information Criterion corrected for small sample sizes (AICc), where models with the lowest AICc score were considered to best explain the variability in the data (Burnham and Anderson, 2002). In instances where more than one model had a  $\Delta\text{AICc} < 2$ , the model with the fewest parameters was selected. Prior to running the GLMMs, random effects were selected using restricted maximum likelihood (“REML = TRUE” in the ‘lmer()’ function) and using AICc selection on the fully-parameterized models with varying random effects. Random effects tested included random intercepts for individual ID (“(1|id)”) and calendar year (“(1|year)”), and a combination of both of these intercepts, as well as correlated (“(age|id)”) and uncorrelated (“(age||id)”) random intercepts and slopes for individual ID by animal age, with and without a random intercept for calendar year. After choosing the random effects, model selection was conducted on five models with varying combinations of fixed effects for individual age and sex (Table S3). Both Sr and Ba exhibit large, non-linear changes in early life associated with nursing and weaning (Clark et al., 2020b), thus GLMMs were only conducted for



**Fig. 1.** Photograph of a longitudinal cross-section of the tooth of an adult female walrus (A), with an inset showing the locations of the dentin, cementum, and the laser ablation scar (B). Panels C–F show typical patterns of accumulation for Sr (C), Ba (D), Zn (E), and Pb (F), with the end of each cementum growth layer group marked with a dark blue line, and the animal’s age in each year denoted at the bottom. Figure adapted from Clark et al., 2020a, 2020b.

ages  $\geq 5$ , where the weaning signal is no longer present in the data. Individuals with five or more elemental concentrations classified as outliers (i.e., falling more than 4 standard deviations from the mean concentration of all individuals; Tukey, 1977) were excluded from the GLMM for that element. This resulted in the omission of one individual from the GLMMs for Cu and Pb. Model predictions and 95% confidence intervals were calculated using the 'bootpredictlme4' R package, which uses a bootstrapping approach (1000 iterations, in this case) to generate confidence intervals (Duursma, 2017).

Pearson's correlations were used to investigate relationships among trace elements within the lives of individual walrus, among walrus, and among calendar years. Correlation coefficients were calculated for the time series of 15 trace elements for each individual walrus, and the resulting correlation matrices were averaged for males and females to calculate mean within-individual correlations for each sex. There was high variability within the high-resolution elemental time series, possibly resulting from microscale variations in tooth structure or instrumental noise, which led to almost universally low correlations among elements within individual walrus. To better compare correlations among underlying trends in the data, the elemental time series were smoothed using a Savitzky-Golay filter from the R package *prospectr* (Stevens and Ramirez-Lopez, 2014) with a window of 15 data points. The Savitzky-Golay algorithm smooths the data by fitting a local polynomial regression of order  $p$  (3, in this case) to the data points in the window. Within-individual correlations were calculated using these smoothed time series. To examine correlations among walrus, mean (natural log-transformed) elemental concentrations were calculated for each individual, and correlations among these mean values were computed separately for males and females. Finally, to examine correlations among elemental concentrations by calendar year, mean (natural log-transformed) trace element concentrations were calculated for male and female walrus for each year. Correlations among calendar years were restricted to years in which elemental concentrations from at least three (and usually  $\geq 5$ ) individuals were available, which resulted in a time series from 1945 to 2014. Correlation coefficients were then calculated for each element across these years.

Changes in mean (untransformed) elemental concentrations from 1945 to 2014 were examined visually. As with the GLMMs, changes in Sr and Ba through time were calculated using only data from ages  $\geq 5$ . This resulted in slightly smaller sample sizes, but allowed for the inclusion of these two elements in this analysis. Pearson's correlations between male and female trace element concentrations were calculated for the period from 1945 to 2014 and interpreted alongside the visual examinations.

All data used in this study are archived with Dryad and can be accessed at doi:<https://doi.org/10.5061/dryad.q573n5thj> (Clark et al., 2021).

### 3. Results

All walrus teeth had detectable concentrations of each of the 15 trace elements examined in this study (Table 1). Median limits of detection for these elements ranged from 0.02 ppm for Co to 2.86 ppm for Fe. The median walrus age estimate generated from counts of cementum GLGs was 15 for both females (min = 4, max = 28) and males (min = 4, max = 26). The resulting time series of trace element data spanned the period from 1914 to 2015, not including a single individual from which data were available from 1866 to 1879.

Results of the GLMMs (Table S3) and visual examinations of mean element concentrations plotted by animal age (from age 0 to age 15) revealed distinct differences in elemental accumulation by male and female walrus in this study (Fig. 2). Females had consistently higher concentrations of Zn, Sr (Ages 5+), Mo, Ag, Cd, Ba (Ages 5+), and Pb. In contrast, male walrus had higher concentrations of Cr, Fe, Co, Ni, and Cu. There were no sex differences in V or As concentrations, and Mn was more complex, with young females exhibiting lower

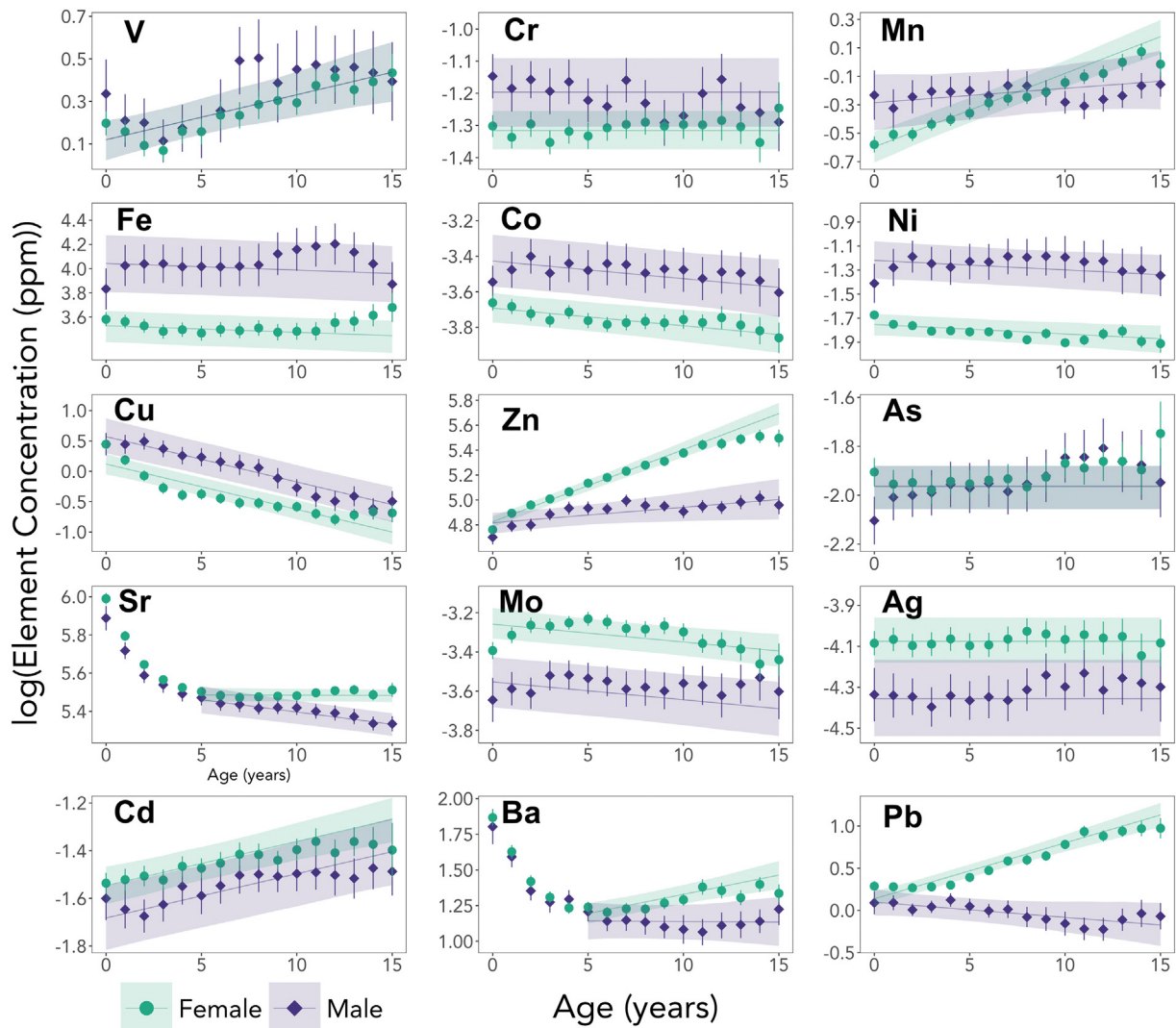
**Table 1**

Means and standard deviations (1 SD) of calcium-normalized trace element concentrations (ppm) in female ( $n = 93$ ) and male ( $n = 29$ ) Pacific walrus (*Odobenus rosmarus divergens*) tooth cementum, as well as median limits of detection (ppm) for each element examined in this study.

Females ( $n = 93$ )	<sup>51</sup> V	<sup>53</sup> Cr	<sup>55</sup> Mn	<sup>57</sup> Fe	<sup>59</sup> Co	<sup>60</sup> Ni	<sup>63</sup> Cu	<sup>66</sup> Zn
Mean (ppm)	1.53	0.46	1.11	42.51	0.04	0.29	1.83	181.14
SD	0.83	0.15	1.48	23.45	0.01	0.07	3.19	39.92
	<sup>75</sup> As	<sup>88</sup> Sr	<sup>95</sup> Mo	<sup>107</sup> Ag	<sup>111</sup> Cd	<sup>137</sup> Ba	<sup>208</sup> Pb	
Mean (ppm)	0.27	292.12	0.06	0.03	0.43	5.28	2.07	
SD	0.19	61.80	0.02	0.02	0.15	1.44	0.97	
Males ( $n = 29$ )	<sup>51</sup> V	<sup>53</sup> Cr	<sup>55</sup> Mn	<sup>57</sup> Fe	<sup>59</sup> Co	<sup>60</sup> Ni	<sup>63</sup> Cu	<sup>66</sup> Zn
Mean (ppm)	2.08	0.48	1.10	81.66	0.05	0.49	1.80	144.64
SD	1.38	0.11	0.69	62.01	0.03	0.30	1.16	31.07
	<sup>75</sup> As	<sup>88</sup> Sr	<sup>95</sup> Mo	<sup>107</sup> Ag	<sup>111</sup> Cd	<sup>137</sup> Ba	<sup>208</sup> Pb	
Mean (ppm)	0.23	263.02	0.05	0.02	0.35	4.65	1.23	
SD	0.11	48.37	0.02	0.01	0.12	1.42	0.44	
Median limit of detection (ppm)	<sup>51</sup> V	<sup>53</sup> Cr	<sup>55</sup> Mn	<sup>57</sup> Fe	<sup>59</sup> Co	<sup>60</sup> Ni	<sup>63</sup> Cu	<sup>66</sup> Zn
	0.03	0.23	0.12	2.88	0.02	0.10	0.10	0.52
	<sup>75</sup> As	<sup>88</sup> Sr	<sup>95</sup> Mo	<sup>107</sup> Ag	<sup>111</sup> Cd	<sup>137</sup> Ba	<sup>208</sup> Pb	
	0.14	0.18	0.03	0.02	0.13	0.35	0.05	

concentrations than males, and older females having higher concentrations. Elemental concentrations in the cementum of female walrus increased with age for V, Mn, Zn, Cd, Ba (Ages 5+), and Pb, whereas males exhibited increases in V, Mn, and Zn. In females, concentrations of Fe, Co, Ni, Cu, Sr (Ages 5+, minor decrease), and Mo decreased with age. In males, Fe, Co, Ni, Cu, Sr (Ages 5+), Mo, and Pb concentrations declined as animals aged. Only Cr and Ag did not change with animal age for male or female walrus. The expected physiological signals in Zn, Sr, Ba, and Pb (Clark et al., 2020a, 2020b) were clearly visible in plots of mean elemental concentrations by age, with females experiencing strong increases in Zn and Pb over their lives, and both sexes exhibiting steep declines in Sr and Ba concentrations within the first five years of life (Fig. 2).

Trace element correlations differed within and among individuals, as well as among calendar years (Fig. 3, Tables S4–S6). Even after smoothing, within-individual correlations averaged by sex were generally weak, with the strongest correlations between Zn and Pb (females:  $r = 0.59$ ; males:  $r = 0.50$ ) and Sr and Ba (females:  $r = 0.31$ ; males:  $r = 0.30$ ). All other correlations within individuals had an absolute value of  $\leq 0.26$  for females and  $\leq 0.21$  for males. The mean magnitude (absolute value) of the within-individual correlations was 0.05 for both sexes. Correlations among individuals were generally more pronounced, with females exhibiting the strongest correlations between Mo and Ag ( $r = 0.76$ ) and Co and Ag ( $r = 0.64$ ). All other correlations among female walrus had an absolute value  $\leq 0.59$ , and the overall mean magnitude of the correlation coefficients was 0.22. The strongest correlations for males were between Fe and As ( $r = 0.80$ ), Fe and Ni ( $r = 0.78$ ), Fe and Co ( $r = 0.76$ ), Co and Ni ( $r = 0.74$ ), and Mn and As ( $r = -0.73$ ). The absolute value of all other correlations among males was  $\leq 0.69$ , and the overall mean magnitude was 0.31. Trace element concentrations were most strongly correlated among calendar years. Females exhibited the strongest correlations between Fe and As ( $r = 0.90$ ), Fe and Ag ( $r = 0.80$ ), As and Ag ( $r = 0.78$ ), Ni and Zn ( $r = -0.74$ ), and Co and Ag ( $r = 0.72$ ). All other correlations among years for females had an absolute value  $\leq 0.68$ , and the mean magnitude of all correlation coefficients was 0.35. Among-year correlations were similarly high for males, with the strongest between Fe and Ni ( $r = 0.86$ ), Co and Ni ( $r = 0.83$ ), and Ni and As ( $r = 0.80$ ). All other correlations



**Fig. 2.** Mean ( $\pm 1$  standard error, SE) of natural log-transformed elemental concentrations for Pacific walrus, plotted by animal age. Males are represented by purple diamonds, and females by green circles. Lines represent predictions from generalized linear mixed models (GLMMs), and shaded ribbons represent the 95% confidence intervals of the modeled output. Due to non-linear early-life changes in Sr and Ba, only data from ages  $\geq 5$  were modeled using GLMMs. Elements where the model output for males and females overlaps completely (V, As) are those for which the GLMMs did not indicate sex differences.

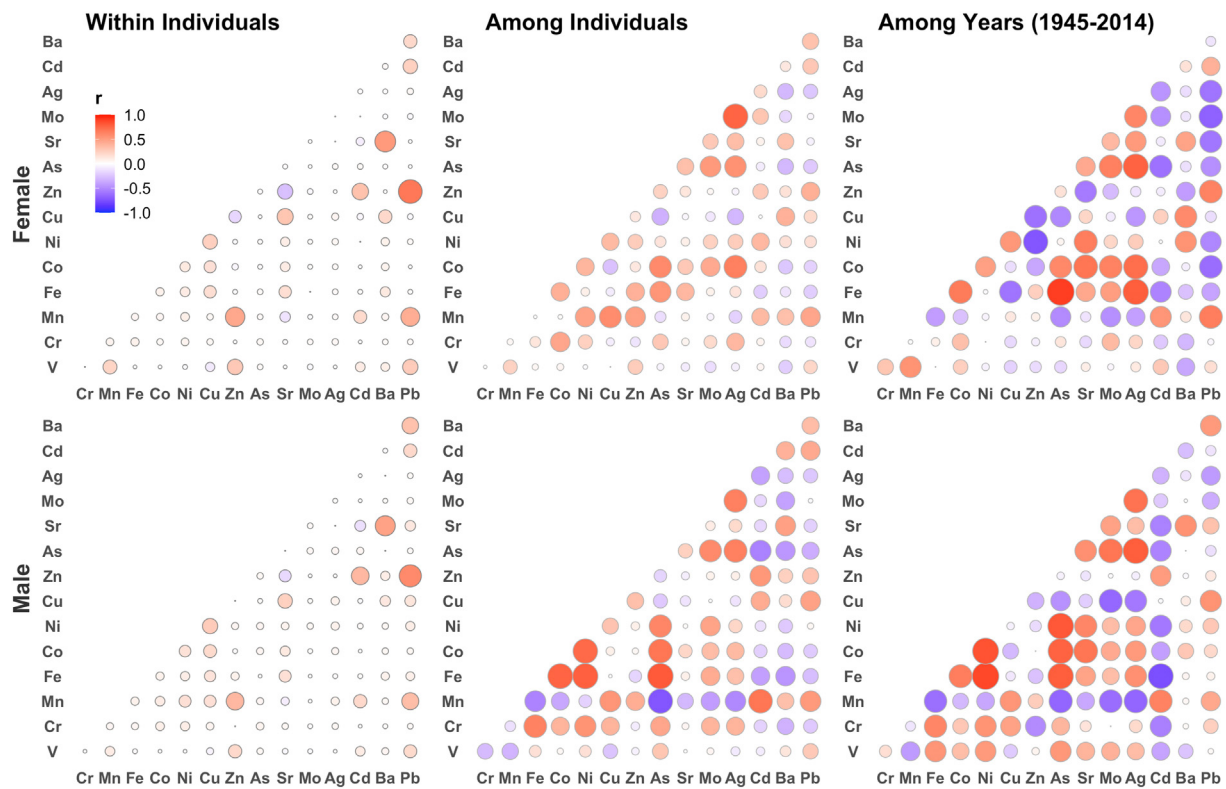
among years for males had an absolute value  $\leq 0.79$ , and the overall mean magnitude was 0.37.

Visual examinations of average trace element concentrations through time revealed distinct changes for both males and females across the period from 1945 to 2014 (Fig. 4). In general, elements that were strongly correlated among years in the above analyses exhibited visually similar patterns. Females, for example, exhibited a peak in Fe, As, and Ag values around 1990, which declined into the early 2000s. A similar pattern can be observed for Sr, though this plot was generated only with data from ages 5+, thus it is not directly comparable to the correlation analyses. Elements with strong among-year correlations in males (e.g., Fe, Co, Ni, As) tended to start with low values that increased sharply around 1975 and, with the exception of Co, sustained high values for the remainder of the record. In general, the magnitudes of the changes exhibited by males were larger than those of female walrus, though this may be a result of the lower sample size of male walrus allowing individuals to have a greater impact on the mean. Of the 15 elements examined, changes in male and female concentrations through time were positively correlated for 10 elements, and negatively correlated for the remaining five. The elements with the strongest positive correlations were Fe ( $r = 0.60$ ) and Sr (Age 5+;  $r = 0.59$ ), whereas

Cu ( $r = -0.41$ ) and Ni ( $r = -0.37$ ) exhibited the strongest negative correlations.

#### 4. Discussion

Analysis of trace elements in walrus teeth revealed much complexity, with nearly all elements exhibiting some combination of sex differences, age-related changes, and fluctuations in average concentrations through time. This complexity reflects the multitude of interacting factors responsible for determining elemental concentrations in walrus teeth, ranging from regional differences in geology to poorly understood aspects of mammalian trace element physiology. The primary obstacle standing in the way of the effective use of walrus teeth as trace element biomonitors is a poor understanding of how elemental concentrations in teeth relate to those in the diet and in other parts of the body. Several studies have suggested that marine mammal teeth may be valuable tools for biomonitoring (e.g., Aubail et al., 2010; De María et al., 2021; Ozersky et al., 2017); however, only two have compared elemental concentrations in teeth to those in soft tissues. Both focused on evaluating the use of beluga whale (*Delphinapterus leucas*) teeth as biomonitors, one for mercury (Hg), and the other for selenium (Se). In belugas,

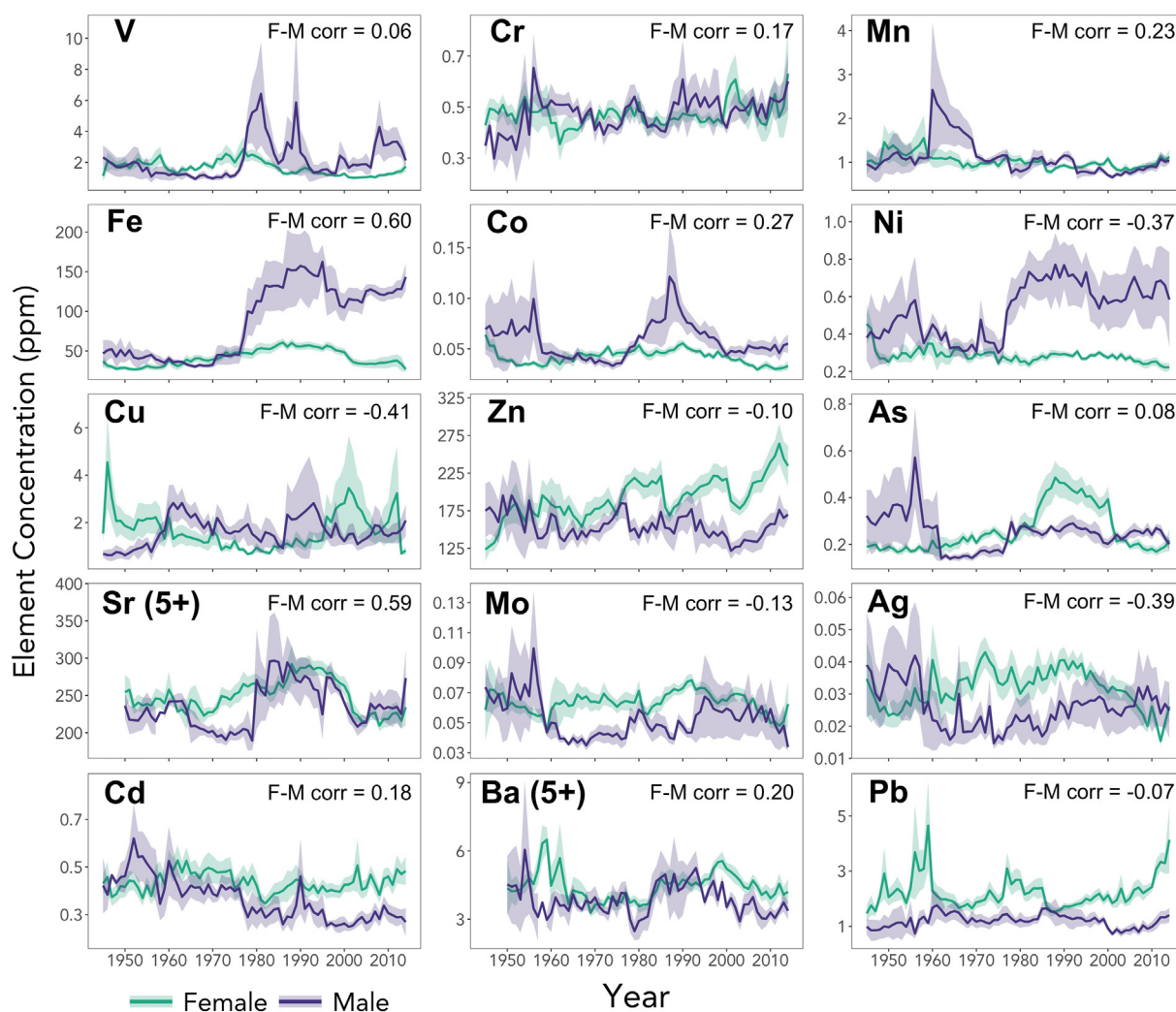


**Fig. 3.** Pearson's correlation plots for trace elements in female (top) and male (bottom) walrus teeth. Bubble size is scaled to the absolute value of the correlation coefficient, and the color inside each bubble represents the strength of the correlation (dark blue =  $-1.0$ ; dark red =  $1.0$ ). Mean correlations within individual walruses (correlation coefficients calculated on lightly smoothed data, then averaged across all individuals) are in the left column. The middle column contains correlations among individual walruses (trace element concentrations averaged for each walrus, and correlations calculated among all individuals). Correlations among calendar years (average concentrations calculated across all individuals for each year, and correlations calculated among all years) from 1945 to 2014 are in the column on the right. This date range was selected to ensure that no individual year contained data from fewer than three walruses, and the majority of years included at least five individuals.

tooth Hg concentrations were positively correlated with those in the kidney, liver, muscle, and skin (Outridge et al., 2000), whereas Se in the teeth was positively correlated with liver and muscle concentrations, but only weakly related to those in the kidney and skin (Kinghorn et al., 2008). In terrestrial mammals, including humans, concentrations of Cu (Blanuša et al., 1990, but see also Fosse and Justesen, 1978), Zn (Attramadal and Jonsen, 1976; Blanuša et al., 1990), Cd (Wesenberg et al., 1979, 1981), and Pb (Blanuša et al., 1990; Wesenberg et al., 1979) in teeth are linked to dietary intake or environmental exposure. This suggests that teeth may be a good indicator of trace element intake, uptake, and/or exposure in mammals. Though the Pacific walrus population is panmictic, with little internal stock structure (Beatty et al., 2020), cementum trace element concentrations revealed differences among individuals from two geographically isolated breeding aggregations (Jay et al., 2008). This may be further evidence that trace elements in teeth reflect underlying variability in environmental element concentrations, or perhaps differences in the diets of animals in the two breeding groups.

Some inference about the relationship between tooth trace elements and concentrations in other tissues can perhaps be gained by comparing age-related patterns of element accumulation in walrus teeth to those reported for marine mammal soft tissues. Positive correlations between marine mammal age and tissue concentrations are widely reported for V (Agusa et al., 2008, 2011a; Anan et al., 2002; Mackey et al., 1996; Saeki et al., 1999) and Cd (Agusa et al., 2011a, 2011b; Anan et al., 2002; Dehn et al., 2005, 2006; Goldblatt and Anthony, 1983; Hansen et al., 1990; Honda et al., 1983; Honda and Tatsukawa, 1983; Krone et al., 1999; Mackey et al., 1996; Miles et al., 1992), both of which increased linearly with age in the cementum of walruses in the present

study. Liver Cu concentrations are negatively related to body length and/or age in some Arctic cetaceans (Dehn et al., 2006; Woshner et al., 2001), and a similar age-related decline also appears in the walrus teeth (Fig. 2). The relationship between teeth and soft tissues is less clear for other elements. Pacific walrus soft tissues exhibit age-related increases in Zn and As, but not Pb (Warburton and Seagars, 1993), though two studies of other marine mammal species found a negative correlation between age and liver Zn concentrations (striped dolphins, *Stenella coeruleoalba*: Agusa et al., 2008; harbor seals, *Phoca vitulina*: Akmajian et al., 2014). This may indicate that the increase in Zn concentrations associated with sexual maturity is exclusive to walruses, or could reflect relatively greater levels of Zn in the benthic invertebrates that make up the majority of walrus diet (Sheffield and Grebmeier, 2009) as compared to the primarily fish diets of the dolphins and seals in the other two studies. Similarly, positive correlations between age and soft tissue Pb concentrations exist for some marine mammals (e.g., Agusa et al., 2008; Miles et al., 1992), though these relationships are less commonly reported and are typically weaker than those between age and V or Cd. Only female walruses in the present study exhibited increasing Pb concentrations with age, likely due to physiological factors related to attaining sexual maturity (Clark et al., 2020a), whereas male cementum Pb concentrations typically declined slightly across an animal's life. Examining males and females together could possibly have dampened the age-related signals of both sexes in previous studies. Alternatively, other species may exhibit different patterns, or tooth Pb concentrations might not be representative of those of soft tissues. Together, the evidence indicates that the concentrations of at least some elements in walrus teeth are closely related to those in other tissues, thus, presumably, to dietary intake and environmental



**Fig. 4.** Mean yearly trace element concentrations for female (green) and male (purple) walrus. The bold line represents the mean and the shaded ribbon is  $\pm 1$  standard error. Correlations between female and male means are included in the top right. Due to early-life physiological signals associated with weaning, only ages  $\geq 5$  were used when plotting strontium (Sr) and barium (Ba).

concentrations; however, the infrequent and often conflicting reports of age-related trace element changes in soft tissues of many species suggest that these links may be species- or taxon-specific.

When considering walrus teeth as potential biomonitors of environmental element concentrations, the role of physiology in determining tooth trace element concentrations cannot be ignored. Patterns of accumulation of Zn and Pb in walrus teeth have been linked to attainment of sexual maturity (Clark et al., 2020a). Similarly, early-life shifts in Sr and Ba in walrus teeth may be associated with nursing and weaning (Clark et al., 2020b). These physiological signals are apparent in the age-related changes exhibited by each of these elements (Fig. 2). Both Zn and Pb increase steeply with age in females, but not males. Declines in Sr and Ba in the first five years of life are pronounced enough to render their relationship with age non-linear, thus precluding the inclusion of these years in the GLMMs. The correlation between Zn and Pb is by far the strongest observed within individual walrus, followed by that of Sr and Ba (Fig. 3). The role of physiological factors in shaping the observed concentrations of other trace elements is unknown. Elements that are important for physiological function might be expected to change in relation to age or life stage, as observed with Zn, Sr, Ba, and Pb, or their concentrations in the body might be closely regulated and maintained within an optimal window. For example, Fe is necessary for oxygen transport and storage, and is critically important for diving

marine mammals (Kooyman, 1989). The GLMMs predict a slight decrease in Fe with age; however, the confidence intervals are wide, and the mean values appear to remain relatively constant across life for both males and females (Fig. 3). In contrast, the relatively strong increase in Mn concentrations with age observed only in female walrus is suggestive of physiological differences between the sexes. This essential element plays an important role in enzyme function (Schramm, 2012), and Mn deficiency inhibits reproductive function in mammals (Boyer et al., 1984; Plumlee et al., 1956; Rojas et al., 1965). Little information is available about the role of Mn in the bodies of marine mammals, but the data presented here indicate either a sex-related difference in Mn physiology, or differential intake of this element by female and male Pacific walrus.

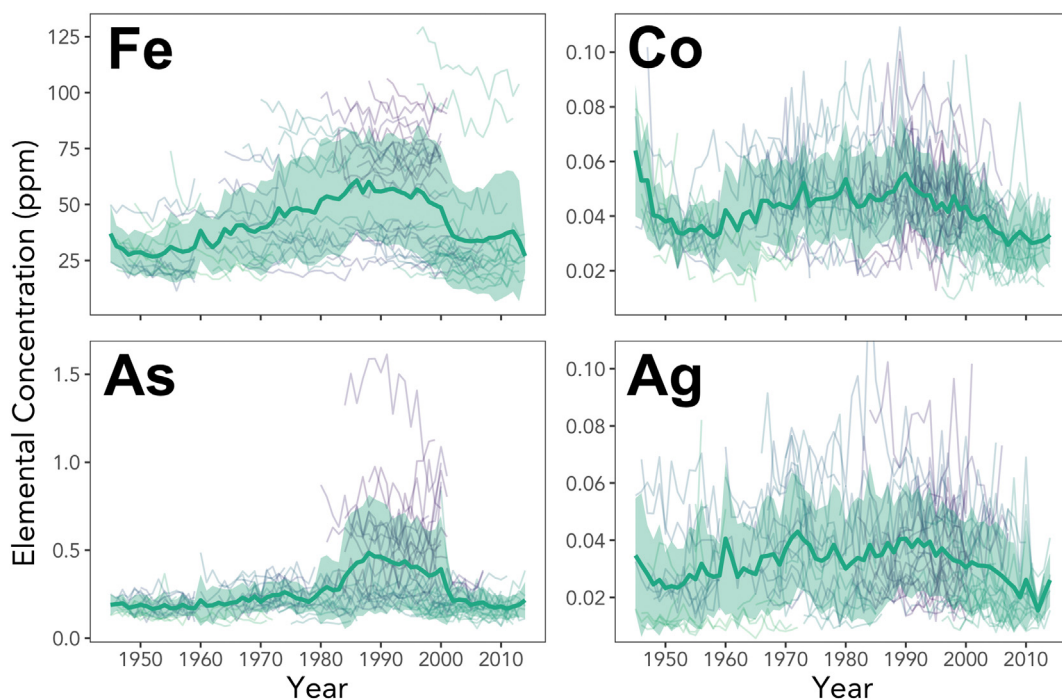
Despite the numerous factors potentially masking such signals, walrus tooth elemental concentrations exhibited much variability through time that did not appear to be driven by age-related accumulations or declines. A random intercept for calendar year was included in the random effects structure of the GLMMs for eight elements, and a model including “year” appeared among the top models ( $\Delta AIC_c < 2$ ) for four of the remaining seven elements (Table S3). The top model (i.e., the model without the random intercept for “year”) was selected in those instances, but the presence of calendar year among the top models indicates that this term was important for explaining variability in the

majority of the elements examined. Further, correlation analysis revealed that the strongest relationships among walrus tooth trace element concentrations were those among calendar years, rather than within- or among-individual walruses (Fig. 3). The fact that the changes in elemental concentrations through time were consistent across a suite of elements, and did not simply reflect underlying patterns of trace element accumulation typical to walruses, supports the idea that they represented actual fluctuations in exposure, intake, and/or uptake. Finally, visual examination of the mean trace element concentrations for male and female walruses across the period from 1945 to 2014 revealed marked changes through time for many elements (Fig. 4). These plots were generated from the raw data, and the age-related patterns of accumulation may thus influence interpretation, as can be most clearly seen in the female Zn and Pb data. The average concentrations of these elements rise steeply towards the end of the record, reflecting age-related increases and a lack of younger animals in the last years of the dataset, rather than a change in average Zn concentrations at the population level. Despite this, distinct changes through time are apparent for many elements that cannot be explained by age-related increases or declines. For female walruses, the elements most strongly correlated among calendar years (Fe, As, Ag) tended to increase from ~1950 to ~1990, then decline for the remainder of the record, though the large magnitude of the changes exhibited by males (likely as a result of lower sample size allowing individuals to have a greater effect on the mean) sometimes made the changes in average female elemental concentrations difficult to see. Average Ba concentrations for animals age 5+ exhibited a similar pattern to Fe, As, and Ag, but the correlation with other elements is unknown, because the first 5 years of life were included when correlations were generated. In contrast to the females, the elements most strongly correlated with calendar year for males (Fe, Co, Ni, As) were generally characterized by a steep increase in concentrations at ~1975 (though this increase was relatively small for As). These high values were sustained for the remainder of the record for Fe and Ni, the two most strongly correlated elements among calendar years for males. Their sex-segregated migration means that male and

female walruses spend much of the year apart, foraging in distinctly different habitats. Thus, the sex-related differences in elemental accumulation through time further support the idea that the record of trace element concentrations in walrus teeth changes in proportion to dietary intake, and is likely reflective of environmental concentrations.

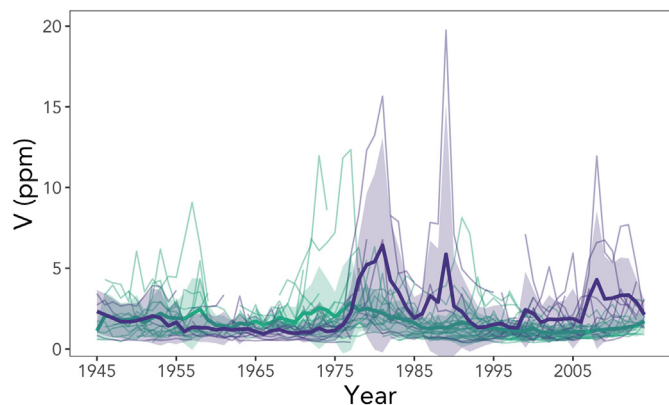
When interpreting changes in yearly trace element concentrations, attention should be given to whether shifts in mean values are caused by a change in the population as a whole, or by individuals with especially high (or low) elemental concentrations. For example, in female walruses, concentrations of Fe, Co, As, and Ag were strongly positively correlated among years; however, when the distributions of the underlying data used to calculate the means are examined, stark differences can be seen (Fig. 5). For both Fe and As, changes in average concentrations are driven almost exclusively by the presence (or absence) of individuals with high elemental concentrations. In contrast, changes in Co and Ag appear to primarily result from shifts in average concentrations across the majority of animals sampled. Thus, though these elements are all strongly correlated through time, the contrasting distributions shaping the average values of these elements likely represent important differences in the forces responsible for driving these patterns. The relatively low magnitude, population-level changes exhibited by Co and Ag are suggestive of shifts in baseline environmental concentrations or walrus diet, occurring gradually and affecting all sampled individuals in a similar way. In contrast, the patterns present in the Fe and As data appear more likely the result of exposure of some individuals to high concentrations of those elements, or by distinct differences in physiology, diet, or foraging location that caused those individuals to exhibit sustained high concentrations throughout their lives.

Determining the cause of observed changes in walrus tooth trace elements remains challenging, even if a strong link between elemental exposure/intake and tooth concentrations can be made. The time series of V concentrations in walrus teeth is a good example (Fig. 6). Mean V concentrations were relatively stable for females, but periodically increased by an order of magnitude in the teeth of males. Male V concentrations exhibited a major peak ~1980, another ~1990, and a third,



**Fig. 5.** Concentrations of the four trace elements that were most highly correlated among calendar years in female walrus teeth from 1945 to 2014. The bold green line represents the annual mean concentration and the shaded ribbon represents the  $\pm 1$  standard deviation. Element concentrations of individual walruses are represented by thin lines.





**Fig. 6.** Vanadium (V) concentrations (in ppm) for female (green) and male (purple) walrus for the years from 1945 to 2014. Bold lines illustrate annual means and shaded ribbons represent  $\pm 1$  standard deviation. Thin lines indicate V concentrations of individual walrus.

smaller peak starting in ~2005. Examining the individual-level data reveals brief episodes of extremely high elemental concentrations occurred in walrus of both sexes and typically spanned several years before declining (Fig. 6). These short-term spikes in V concentrations suggest acute exposure to or intake of this element, and the fact that elevated levels of V were typically only observed for ~1–3 years followed by a sharp decline indicates that they were not simply the result of the age-related changes in V.

Increased V concentrations (as well as Cr, Mn, Fe, Ni, Cu, Zn, As, Cd, Ba, and Pb) in marine sediments and benthic organisms have been linked with offshore petroleum production (Boothe and Presley, 1987; Breuer et al., 2004, 2008; Lacerda et al., 2013; Marcaccio et al., 2003), and V is considered a tracer of crude oil in marine sediments (Khalaf et al., 1982; Trefry et al., 1985). The Alaskan Arctic has been the focus of widespread exploration for oil and gas reserves since the 1940s and, after the discovery of oil near Prudhoe Bay in 1968, these activities were greatly intensified, reaching a maximum in the 1980s (Naidu et al., 2012). Given the correspondence between the timeline of oil and gas-related activities and the spikes in V concentrations in walrus teeth, it is tempting to attribute the observed spikes in V in walrus teeth to petroleum-related activities. Alaska North Slope crude oil contains high concentrations of V, as do drilling muds and cuttings on the Alaskan outer continental shelf (Coleman, 1978; Naidu et al., 2012). The increased V concentrations in the 1970s – 1980s could possibly have resulted from inadvertent input of crude oil and drilling byproducts into benthic systems, or from natural crude oil seeps. Analysis of sediment cores from the Beaufort Sea, however, indicate that offshore oil and gas exploration has likely not been a significant source of V input to Arctic marine food webs (Naidu et al., 2012; Venkatesan et al., 2013). No significant changes occurred in the composition or concentration of hydrocarbons in Beaufort Sea sediments from 1976 to 1997 (Venkatesan et al., 2013). Naidu et al. (2012) detected decreasing V concentrations in marine sediments in Beaufort Lagoon from 1977 to 2003. Near Prudhoe Bay, sediment V concentrations increased from 1977 to 1985 and remained elevated through at least 1997. The trends observed in Beaufort Lagoon and Prudhoe Bay may have been associated with the closure of a Distant Early Warning Line (military observation) station and combustion of petroleum products at a gas flaring facility in Deadhorse, AK, respectively (Naidu et al., 2012). These localized sources may have been enough to significantly increase V concentrations in coastal sediments; however, it remains unclear whether such local sources would have released enough V to cause widespread increases in concentrations of this element in walrus teeth, particularly because walrus rarely visit the Beaufort Sea and almost never occur as far east as Prudhoe Bay (Beatty et al., 2016; Fay, 1982). Other localized

sources closer to the walrus range could play a role in determining walrus tooth V concentrations, and non-local sources may also contribute to increased V concentrations in the Arctic. Atmospheric deposition of trace metals in this region increased during the 20th century (Boutron and Delmas, 1980), and air pollution events referred to as the “Arctic Haze” are generated primarily by lower-latitude pollution sources containing relatively large amounts of V (Rahn and McCaffrey, 1980). Thus, variability in the intensity or composition of this airborne pollution, or perhaps changes in input of V into the oceans associated with sea ice melt (Tovar-Sánchez et al., 2010), could have been responsible for changes in V concentrations in the walrus in this study.

Alternatively, the observed changes in V may have nothing to do with anthropogenic activities, instead resulting from changes in walrus diet or foraging location. Some species of invertebrates, including ascidians (Michibata and Sakurai, 1990) and polychaete worms (Fattorini and Regoli, 2012) accumulate large amounts of V in their bodies. Polychaetes are an important prey item for walrus and, though consumed less frequently than polychaetes, ascidians (specifically *Halocynthia aurantium*, the sessile tunicates referred to as “sea peaches”) are relatively common in walrus diet (Sheffield and Grebmeier, 2009). Episodes of increased consumption of these organisms could cause spikes in V concentrations like those observed in walrus teeth. This might result from changes in availability of these prey items (e.g., as a result of periodic recruitment events or environmental shifts), or due to walrus moving to a new foraging location where these species were abundant. Little information is available about whether the ascidian and polychaete species found in the Pacific walrus habitat are among those that accumulate V. One study of traditional human diet items and environmental contaminants in Chukotka, Russia, found that the levels of V in ascidians were relatively low compared to those found in other regions, but still high enough to potentially explain the spikes in V observed in walrus teeth (Dudarev et al., 2019). The highest V concentrations were found in sea peaches. No information is available in the literature for Arctic polychaetes; however, some Antarctic invertebrates tend to concentrate higher levels of V than temperate species (Fattorini and Regoli, 2012).

The difficulty of assigning causality to observed changes in trace element concentrations in walrus teeth means that any attempts to use walrus teeth as biomonitors of trace elements should be carried out with caution and with awareness of the potentially confounding factors. The results of this study demonstrate that walrus teeth contain an enormous amount of information about animal physiology, age-related trends in the accumulation of trace elements, and exposure, intake, and/or uptake of elements from the environment via diet. The wide geographic range and generalist diet of walrus means that the trace element concentrations in their tissues represent the incorporation of a multitude of signals. Elements with relatively constant concentrations across the ~70 year record presented here could possibly be considered to represent baseline values, with little influence from anthropogenic sources. Alternatively, these elements might be physiologically regulated to remain within a narrow range of concentrations. In contrast, elements that changed substantially between 1945 and 2014 may reflect anthropogenic impacts on the environment, as well as the rapid environmental change occurring in the Arctic, which itself could potentially influence changes in physiology.

Further research that quantitatively links elemental concentrations in teeth to those in diet and generates a more complete understanding of the role of physiology in shaping tooth element concentrations will improve the interpretability of this type of dataset. As such, we advocate for several key areas for future research. First, we recommend a broader exploration of physiological patterns of trace element accumulation in marine mammal teeth, including testing whether the phenomena observed in walrus (i.e., Zn and Pb as indicators of female maturity; Sr and Ba as indicators of weaning) are applicable to other species and taxa. Second, controlled feeding experiments and quantification of the relationship between tooth trace element concentrations and those in

other tissues will be critical to developing methodologies for using tooth trace elements to monitor environmental trace element concentrations. Given the difficulty of conducting controlled feeding experiments on marine mammals, particularly when tooth sampling is required, studies comparing concentrations of trace elements in stomach contents, soft tissues, and the outermost (most recently grown) portion of the tooth cementum in free-ranging, subsistence harvested animals may provide important information in the shorter term. Studies focusing on species with more restricted geographic ranges and fewer potential prey items might lend themselves particularly well to this type of research, and would likely be more effective biomonitors than widely ranging, generalist predators like Pacific walruses. Third, pairing trace element data with other types of analyses, including stable isotopes, hormone concentrations, and growth layer widths might provide insight into the roles of physiology and diet shifts in changing tooth trace element concentrations. Finally, the development or adaptation of new or improved statistical techniques for analyzing the high-resolution time series produced by trace element analysis on the LA-ICP-MS may facilitate interpretation of future research results.

### CRedit authorship contribution statement

**Casey T. Clark:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization, Funding acquisition. **Lara Horstmann:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Nicole Misarti:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition.

### Declaration of competing interest

The authors declare no competing interests.

### Acknowledgements

This work was supported by the National Science Foundation Arctic SEES Program [Grant Number 1263848], with supplementary funds from the Bureau of Ocean Energy Management; Coastal Marine Institute; North Pacific Research Board; Cooperative Institute for Alaska Research; National Institutes of Health Biomedical Learning and Student Training Program [Award Numbers UL1GM118991, TL4GM118992, or RL5GM118990]; and NOAA Cooperative Agreements NA15OAR4320063 and NA20OAR4320271. We thank the Alaska Native subsistence hunters of St. Lawrence Island, Eskimo Walrus Commission, USFWS, North Slope Borough Department of Wildlife Management, Alaska Department of Fish and Game, University of Alaska Museum, National Museum of Natural History (Smithsonian Institution), K. Spaleta, K. Gagne, and the rest of the Advanced Instrumentation Laboratory at the University of Alaska Fairbanks, B. Konar, P. Lemons, and K. Severin for providing input on this research, and P. Charapata, C. Heninger, N. Taylor, E. Van Dam, L. Wendling for laboratory support.

### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2021.145500>.

### References

- Agusa, T., Nomura, K., Kunito, T., Anan, Y., Iwata, H., Miyazaki, N., Tatsukawa, R., Tanabe, S., 2008. Interelement relationships and age-related variation of trace element concentrations in liver of striped dolphins (*Stenella coeruleoalba*) from Japanese coastal waters. *Mar. Pollut. Bull.* 57, 807–815. <https://doi.org/10.1016/j.marpolbul.2008.01.039>.
- Agusa, T., Yasugi, S.Y., Iida, A., Ikemoto, T., Anan, Y., Kuiken, T., Osterhaus, A.D.M.E., Tanabe, S., Iwata, H., 2011a. Accumulation features of trace elements in mass-stranded harbor seals (*Phoca vitulina*) in the North Sea coast in 2002: the body distribution and association with growth and nutrition status. *Mar. Pollut. Bull.* 62, 963–975. <https://doi.org/10.1016/j.marpolbul.2011.02.047>.
- Agusa, T., Nomura, K., Kunito, T., Anan, Y., Iwata, H., Tanabe, S., 2011b. Accumulation of trace elements in harp seals (*Phoca groenlandica*) from Pangnirtung in the Baffin Island, Canada. *Mar. Pollut. Bull.* 63, 489–499. <https://doi.org/10.1016/j.marpolbul.2011.02.022>.
- Akmajian, A., Calambokidis, J., Huggins, J.L., Lambourn, D., 2014. Age, region, and temporal patterns of trace elements measured in stranded harbor seals (*Phoca vitulina richardii*) from Washington inland waters. *Northwest. Nat.* 95, 83–91. <https://doi.org/10.1898/nwn13-26.1>.
- Anan, Y., Kunito, T., Ikemoto, T., Kubota, R., Watanabe, I., Tanabe, S., Miyazaki, N., Petrov, E.A., 2002. Elevated concentrations of trace elements in Caspian seals (*Phoca caspica*) found stranded during the mass mortality events in 2000. *Arch. Environ. Contam. Toxicol.* 42, 354–362. <https://doi.org/10.1007/s00244-001-0004-7>.
- Attramadal, A., Jonsen, J., 1976. The content of lead, cadmium, zinc and copper in deciduous and permanent human teeth. *Acta Odontol. Scand.* 34, 127–131. <https://doi.org/10.3109/00016357609002559>.
- Aubail, A., Dietz, R., Rigét, F., Simon-Bouhet, B., Caurant, F., 2010. An evaluation of teeth of ringed seals (*Phoca hispida*) from Greenland as a matrix to monitor spatial and temporal trends of mercury and stable isotopes. *Sci. Total Environ.* 408, 5137–5146. <https://doi.org/10.1016/j.scitotenv.2010.07.038>.
- Bates, D., Mächler, M., Bolker, B.M., Walker, S.C., 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67, 1–48. <https://doi.org/10.1126/science.1176170>.
- Beattie, S.A., Armstrong, D., Chaulk, A., Comte, J., Gosselin, M., Wang, F., 2014. Total and methylated mercury in arctic multiyear sea ice. *Environ. Sci. Technol.* 48, 5575–5582. <https://doi.org/10.1021/es5008033>.
- Beatty, W.S., Jay, C.V., Fischbach, A.S., Grebmeier, J.M., Taylor, R.L., Blanchard, A.L., Jewett, S.C., 2016. Space use of a dominant Arctic vertebrate: effects of prey, sea ice, and land on Pacific walrus resource selection. *Biol. Conserv.* 203, 25–32. <https://doi.org/10.1016/j.biocon.2016.08.035>.
- Beatty, W.S., Lemons, P.R., Sethi, S.A., Everett, J.P., Lewis, C.J., Lynn, R.J., Cook, G.M., Garlichmiller, J.L., Wenburg, J.K., 2020. Panmixia in a sea ice-associated marine mammal: evaluating genetic structure of the Pacific walrus (*Odobenus rosmarus* diversgens) at multiple spatial scales. *J. Mammal.* 101, 1–11. <https://doi.org/10.1093/jmammal/gyaa050>.
- Blanuša, M., Ivčić, N., Simeon, V., 1990. Lead, iron, copper, zinc and ash in deciduous teeth in relation to age and distance from a lead smelter. *Bull. Environ. Contam. Toxicol.* 45, 478–485. <https://doi.org/10.1007/BF01700618>.
- Boothe, P.N., Presley, B.J., 1987. The effects of exploratory petroleum drilling in the north-west gulf of Mexico on trace metal concentrations in near rig sediments and organisms. *Environ. Geol. Water Sci.* 9, 173–182. <https://doi.org/10.1007/BF02449949>.
- Borgmann, U., 2000. Methods for assessing the toxicological significance of metals in aquatic ecosystems: bio-accumulation-toxicity relationships, water concentrations and sediment spiking approaches. *Aquat. Ecosyst. Health Manag.* 3, 277–289.
- Boutron, C., Delmas, R., 1980. Historical record of global atmospheric pollution revealed in polar ice sheets. *Ambio* 9, 210–215. <https://doi.org/10.2307/4312583>.
- Boyer, P.D., Shaw, J.H., Phillips, P.H., 1984. Studies on manganese deficiency in the rat. *Current Topics in Cellular Regulation*. Elsevier, pp. 155–169.
- Breuer, E., Stevenson, A.G., Howe, J.A., Carroll, J., Shimmield, G.B., 2004. Drill cutting accumulations in the northern and Central North Sea: a review of environmental interactions and chemical fate. *Mar. Pollut. Bull.* 48, 12–25. <https://doi.org/10.1016/j.marpolbul.2003.08.009>.
- Breuer, E., Shimmield, G., Peppe, O., 2008. Assessment of metal concentrations found within a North Sea drill cuttings pile. *Mar. Pollut. Bull.* 56, 1310–1322. <https://doi.org/10.1016/j.marpolbul.2008.04.010>.
- Bryan, G.W., 1984. Pollution due to heavy metals and their compounds. *Mar. Ecol.* 5, 1289–1431.
- Burnham, K.P., Anderson, D.J., 2002. *Model Selection and Multimodel Inference*. Springer-Verlag, New York.
- Clark, C.T., Horstmann, L., Misarti, N., 2020a. Zinc concentrations in teeth of female walruses reflect the onset of reproductive maturity. *Conserv. Physiol.* 8, 1–13. <https://doi.org/10.1093/conphys/coaa029>.
- Clark, C.T., Horstmann, L., Misarti, N., 2020b. Evaluating tooth strontium and barium as indicators of weaning age in Pacific walruses. *Methods Ecol. Evol.* 11, 1626–1638. <https://doi.org/10.1111/2041-210X.13482>.
- Clark, C.T., Horstmann, L., Misarti, N., 2021. Data from: Walrus teeth as biomonitors of trace elements in Arctic marine ecosystems. Dryad, Dataset.
- Coleman, H.J., 1978. *Analysis of 800 Crude Oils from United States Oilfields*. Department of Energy, Office of Intergovernmental Affairs, Technical Information Center.
- Core Team, R., 2020. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
- De María, M., Szteren, D., García-Alonso, J., de Rezende, C.E., Araújo Gonçalves, R., Godoy, J.M., Barboza, F.R., 2021. Historic variation of trace elements in pinnipeds with spatially segregated trophic habits reveals differences in exposure to pollution. *Sci. Total Environ.* 750, 141296. <https://doi.org/10.1016/j.scitotenv.2020.141296>.
- Dehn, L.A., Sheffield, G.G., Follmann, E.H., Duffy, L.K., Thomas, D.L., Bratton, G.R., Taylor, R.J., O'Hara, T.M., 2005. Trace elements in tissues of phocid seals harvested in the Alaskan and Canadian Arctic: influence of age and feeding ecology. *Can. J. Zool.* 83, 726–746. <https://doi.org/10.1139/z05-053>.
- Dehn, L.A., Follmann, E.H., Rosa, C., Duffy, L.K., Thomas, D.L., Bratton, G.R., Taylor, R.J., O'Hara, T.M., 2006. Stable isotope and trace element status of subsistence-hunted bowhead and beluga whales in Alaska and gray whales in Chukotka. *Mar. Pollut. Bull.* 52, 301–319. <https://doi.org/10.1016/j.marpolbul.2005.09.001>.
- Duce, R.A., Liss, P.S., Merrill, J.T., Atlas, E.L., Buat-Menard, P., Hicks, B.B., Miller, J.M., Prospero, J.M., Arimoto, R., Church, T.M., Ellis, W., Galloway, J.N., Hansen, L., Jickells, T.D., Knap, A.H., Reinhardt, K.H., Schneider, B., Soudine, A., Tokos, J.J., Tsunogai, S., Wollast, R., Zhou, M., 1991. The atmospheric input of trace species to the world ocean. *Glob. Biogeochem. Cycles* 5, 193–259. <https://doi.org/10.1029/91GB01778>.

- Dudarev, A.A., Chupakhin, V.S., Vlasov, S.V., Yamin-Pasternak, S., 2019. Traditional diet and environmental contaminants in coastal Chukotka III: metals. *Int. J. Environ. Res. Public Health* 16, 1–17. <https://doi.org/10.3390/ijerph16050699>.
- Duurmsma, R., 2017. bootpredictlme4: predict method for lme4 with bootstrap, R package version 0.1.
- Evans, R.D., Richner, P., Outridge, P.M., 1995. Micro-spatial variations of heavy metals in the teeth of walrus as determined by laser ablation ICP-MS: the potential for reconstructing a history of metal exposure. *Arch. Environ. Contam. Toxicol.* 28, 55–60. <https://doi.org/10.1007/BF00213969>.
- Fattorini, D., Regoli, F., 2012. Hyper-accumulation of vanadium in polychaetes. In: Michibata, H. (Ed.), *Vanadium: Biochemical and Molecular Biological Approaches*. Springer, Dordrecht, pp. 73–92. <https://doi.org/10.1007/978-94-007-0913-3>.
- Fay, F.H., 1982. Ecology and biology of the Pacific walrus, *Odobenus rosmarus divergens* Illiger. *North Am. Fauna* 1–279.
- Fosse, G., Justesen, N.P.B., 1978. Zinc and copper in bone and teeth of mice. *Int. J. Environ. Stud.* 12, 111–120. <https://doi.org/10.1080/00207237808709770>.
- Garlich-Miller, J.L., Stewart, R.E.A., Stewart, B.E., Hiltz, E.A., 1993. Comparison of mandibular with cemental growth-layer counts for ageing Atlantic walrus (*Odobenus rosmarus rosmarus*). *Can. J. Zool.* 71, 163–167.
- Goldblatt, C.J., Anthony, R.G., 1983. Heavy metals in northern fur seals (*Callorhinus ursinus*) from the Pribilof Islands, Alaska. *J. Environ. Qual.* 12, 478–482.
- Hansen, C.T., Nielsen, C.O., Dietz, R., Hansen, M.M., 1990. Zinc, cadmium, mercury and selenium in minke whales, belugas and narwhals from West Greenland. *Polar Biol.* 10, 529–539. <https://doi.org/10.1007/BF00233702>.
- Harguinteguy, C.A., Schreiber, R., Pignata, M.L., 2013. *Myriophyllum aquaticum* as a bio-monitor of water heavy metal input related to agricultural activities in the Xanaes River (Córdoba, Argentina). *Ecol. Indic.* 27, 8–16. <https://doi.org/10.1016/j.ecolind.2012.11.018>.
- Honda, K., Tatsukawa, R., 1983. Distribution of cadmium and zinc in tissues and organs, and their age-related changes in striped dolphins, *Stenella coeruleoalba*. *Arch. Environ. Contam. Toxicol.* 12, 543–550. <https://doi.org/10.1007/BF01056550>.
- Honda, K., Tatsukawa, R., Itano, K., Miyazaki, N., Fujiyama, T., 1983. Heavy metal concentrations in muscle, liver and kidney tissue of striped dolphin, *Stenella coeruleoalba*, and their variations with body length, weight, age and sex. *Agric. Biol. Chem.* 47, 1219–1228. <https://doi.org/10.1080/00021369.1983.10863383>.
- Jay, C.V., Outridge, P.M., Garlich-Miller, J.L., 2008. Indication of two Pacific walrus stocks from whole tooth elemental analysis. *Polar Biol.* 31, 933–943. <https://doi.org/10.1007/s00300-008-0432-1>.
- Khalaf, F., Literathy, V., Anderlini, V., 1982. Vanadium as a tracer of oil pollution in the sediments of Kuwait. *Hydrobiologia* 91–92, 147–154. <https://doi.org/10.1007/BF00940104>.
- Kim, I., Kim, G., Choy, E.J., 2015. The significant inputs of trace elements and rare earth elements from melting glaciers in Antarctic coastal waters. *Polar Res.* 34. <https://doi.org/10.3402/polar.v34.24289>.
- Kinghorn, A., Humphries, M.M., Outridge, P., Chan, H.M., 2008. Teeth as biomonitors of selenium concentrations in tissues of beluga whales (*Delphinapterus leucas*). *Sci. Total Environ.* 402, 43–50. <https://doi.org/10.1016/j.scitotenv.2008.04.031>.
- Klevezal, G.A., 1996. *Recording Structures of Mammals. Determination of Age and Reconstruction of Life History*. AA Balkema, Rotterdam.
- Kooyman, G.L., 1989. *Diverse Divers: Physiology and Behavior*. Springer-Verlag, Berlin.
- Kraak, M.H.S., Scholten, M.C.T., Peeters, W.H.M., De Kock, W.C., 1991. Biomonitoring of heavy metals in the western European rivers Rhine and Meuse using the freshwater mussel *Dreissena polymorpha*. *Environ. Pollut.* 74, 101–114.
- Krone, C.A., Robisch, P.A., Tilbury, K.L., Stein, J.E., Mackey, E.A., Becker, P.R., O'Hara, T.M., Philo, L.M., 1999. Elements in liver tissues of bowhead whales (*Balaena mysticetus*). 15, 123–142.
- Lacerda, L.D., Campos, R.C., Santelli, R.E., 2013. Metals in water, sediments, and biota of an offshore oil exploration area in the Potiguar Basin, Northeastern Brazil. *Environ. Monit. Assess.* 185, 4427–4447. <https://doi.org/10.1007/s10661-012-2881-9>.
- Longerich, H.P., Jackson, S.E., Günther, D., 1996. Laser ablation inductively coupled plasma mass spectrometric transient signal data acquisition and analyte concentration calculation. *J. Anal. At. Spectrom.* 11, 899–904. <https://doi.org/10.1039/JA9961100899>.
- Mackey, E.A., Becker, P.R., Demiralp, R., Greenberg, R.R., Koster, B.J., Wise, S.A., 1996. Bioaccumulation of vanadium and other trace metals in livers of Alaskan cetaceans and pinnipeds. *Arch. Environ. Contam. Toxicol.* 30, 503–512. <https://doi.org/10.1007/BF00213402>.
- Marcaccio, M., Spagnoli, F., Frascari, F., 2003. Drilling mud as tracers of sedimentation and geochemical processes on continental shelves. *J. Coast. Res.* 19, 89–100.
- Martin, J.-M., Høgdahl, O., Philippot, J.C., 1976. Rare earth element supply to the ocean. *J. Geophys. Res.* 81, 3119–3124. <https://doi.org/10.1029/jc081i018p03119>.
- Mendez, J., Guieu, C., Adkins, J., 2010. Atmospheric input of manganese and iron to the ocean: seawater dissolution experiments with Saharan and North American dusts. *Mar. Chem.* 120, 34–43. <https://doi.org/10.1016/j.marchem.2008.08.006>.
- Mertz, W., 1981. The essential trace elements. *Science* (80-) 213, 1332–1338.
- Michibata, H., Sakurai, H., 1990. Vanadium in ascidians. *Vanadium in Biological Systems*. Springer, pp. 153–171.
- Miles, A.K., Calkins, D.G., Coon, N.C., 1992. Toxic elements and organochlorines in harbor seals (*Phoca vitulina richardsi*), Kodiak, Alaska, USA. *Bull. Environ. Contam. Toxicol.* 48, 727–732. <https://doi.org/10.1007/BF00195994>.
- Morrison, L., Bennion, M., McGrory, E., Hurley, W., Johnson, M.P., 2017. *Talitrus saltator* as a biomonitor: an assessment of trace element contamination on an urban coastline gradient. *Mar. Pollut. Bull.* 120, 232–238. <https://doi.org/10.1016/j.marpolbul.2017.05.019>.
- Naidu, A.S., Blanchard, A.L., Misra, D., Trefry, J.H., Dasher, D.H., Kelley, J.J., Venkatesan, M.L., 2012. Historical changes in trace metals and hydrocarbons in nearshore sediments, Alaskan Beaufort Sea, prior and subsequent to petroleum-related industrial development: part I. Trace metals. *Mar. Pollut. Bull.* 64, 2177–2189. <https://doi.org/10.1016/j.marpolbul.2013.10.012>.
- Outridge, P.M., Stewart, R.E.A., 1999. Stock discrimination of Atlantic walrus (*Odobenus rosmarus rosmarus*) in the eastern Canadian Arctic using lead isotope and element signatures in teeth. *Can. J. Fish. Aquat. Sci.* 56 (1), 105–112. <https://doi.org/10.1139/f98-155>.
- Outridge, P.M., Veinott, G., Evans, R.D., 1995. Laser ablation ICP-MS analysis of incremental biological structures: archives of trace-element accumulation. *Environ. Res.* 3, 160–170. <https://doi.org/10.1139/a95-007>.
- Outridge, P.M., Wagemann, R., McNeely, R., 2000. Teeth as biomonitors of soft tissue mercury concentrations in beluga, *Delphinapterus leucas*. *Environ. Toxicol. Chem.* 19, 1517–1522. [https://doi.org/10.1897/1551-5028\(2000\)019<1517:TABOST>2.3.CO;2](https://doi.org/10.1897/1551-5028(2000)019<1517:TABOST>2.3.CO;2).
- Ozersky, T., Pastukhov, M.V., Poste, A.E., Deng, X.Y., Moore, M.V., 2017. Long-term and ontogenetic patterns of heavy metal contamination in Lake Baikal seals (*Pusa sibirica*). *Environ. Sci. Technol.* 51, 10316–10325. <https://doi.org/10.1021/acs.est.7b00995>.
- Plumlee, M.P., Thrasher, D.M., Beeson, W.M., Andrews, F.N., Parker, H.E., 1956. The effects of a manganese deficiency upon the growth, development, and reproduction of swine. *J. Anim. Sci.* 15, 352–367.
- Rahn, K.A., McCaffrey, R.J., 1980. On the origin and transport of the winter arctic aerosol. *Ann. N. Y. Acad. Sci.* 338, 486–503. <https://doi.org/10.1111/j.1749-6632.1980.tb17142.x>.
- Rainbow, P.S., 1995. Biomonitoring of heavy metal availability in the marine environment. *Mar. Pollut. Bull.* 31, 183–192. [https://doi.org/10.1016/0025-326X\(95\)00116-5](https://doi.org/10.1016/0025-326X(95)00116-5).
- Rojas, M.A., Dyer, I.A., Cassatt, W.A., 1965. Manganese deficiency in the bovine. *J. Anim. Sci.* 24, 664–667.
- RStudio Team, 2015. *RStudio: Integrated Development Environment for R* (Boston, Massachusetts, USA).
- Saeki, K., Nakajima, M., Noda, K., Loughlin, T.R., Baba, N., Kiyota, M., Tatsukawa, R., Calkins, D.G., 1999. Vanadium accumulation in pinnipeds. *Arch. Environ. Contam. Toxicol.* 36, 81–86. <https://doi.org/10.1007/s002449900445>.
- Schramm, V.L., 2012. *Manganese in Metabolism and Enzyme Function*. Elsevier.
- Seymour, J., Horstmann-Dehn, L., Wooller, M.J., 2014. Proportion of higher trophic-level prey in the diet of Pacific walrus (*Odobenus rosmarus divergens*). *Polar Biol.* 37, 941–952. <https://doi.org/10.1007/s00300-014-1492-z>.
- Sheffield, G., Grebmeier, J.M., 2009. Pacific walrus (*Odobenus rosmarus divergens*): differential prey digestion and diet. *Mar. Mammal Sci.* 25, 761–777. <https://doi.org/10.1111/j.1748-7692.2009.00316.x>.
- Stevens, A., Ramirez-Lopez, L., 2014. An introduction to the prospectr package. R package vignette. <https://doi.org/10.1176/ajp.136.12.1628-a>.
- Tovar-Sánchez, A., Duarte, C.M., Alonso, J.C., Lacorte, S., Tauler, R., Galban-Malagón, C., 2010. Impacts of metals and nutrients released from melting multiyear Arctic sea ice. *J. Geophys. Res. C Ocean.* 115, 1–7. <https://doi.org/10.1029/2009JC005685>.
- Trefry, J.H., Trocine, R.P., Proni, J.R., 1985. *Drilling-Fluid Discharges into the Northwestern Gulf of Mexico, Wastes in the Ocean*. John Wiley & Sons, New York.
- Tukey, J.W., 1977. *Exploratory Data Analysis*. Addison-Wesley, Reading, Massachusetts, USA.
- U.S. Environmental Protection Agency, 2000. *Guidance for Data Quality Assessment - Practical Methods for Data Analysis*.
- Venkatesan, M.L., Naidu, A.S., Blanchard, A.L., Misra, D., Kelley, J.J., 2013. Historical changes in trace metals and hydrocarbons in nearshore sediments, Alaskan Beaufort Sea, prior and subsequent to petroleum-related industrial development: part II. Hydrocarbons. *Mar. Pollut. Bull.* 77, 147–164. <https://doi.org/10.1016/j.marpolbul.2013.10.012>.
- Wang, W.-X., 2016. Bioaccumulation and biomonitoring. *Mar. Ecotoxicol.* 99–119. <https://doi.org/10.1016/b978-0-12-803371-5.00004-7>.
- Warburton, J., Seagars, D.J., 1993. Heavy metal concentrations in liver and kidney tissues of Pacific walrus: continuation of a baseline study. *Tech. Rep. R7/MMM93-1* 23.
- Wesenberg, G.B.R., Fosse, G., Berg Justesen, N.P., Rasmussen, P., 1979. Lead and cadmium in teeth, bone and kidneys of rats with a standard Pb-cd supply. *Int. J. Environ. Stud.* 14, 223–230. <https://doi.org/10.1080/00207237908737397>.
- Wesenberg, G., Fosse, G., Rasmussen, P., Justesen, N.P.B., 1981. Cadmium content of indicator and target organs in rats after graded doses of cadmium. *Int. J. Environ. Stud.* 16, 147–155. <https://doi.org/10.1080/00207238108709863>.
- Woshner, V.M., O'Hara, T.M., Bratton, G.R., Suydam, R.S., Beasley, V.R., 2001. Concentrations and interactions of selected essential and non-essential elements in bowhead and beluga whales of arctic Alaska. *J. Wildl. Dis.* 37, 693–710. <https://doi.org/10.7589/0090-3558-37.4.693>.
- Wren, C.D., 1986. Mammals as biological monitors of environmental metal levels. *Environ. Monit. Assess.* 6, 127–144. <https://doi.org/10.1007/BF00395625>.
- Yin, X., Xia, L., Sun, L., Luo, H., Wang, Y., 2008. Animal excrement: a potential biomonitor of heavy metal contamination in the marine environment. *Sci. Total Environ.* 399, 179–185. <https://doi.org/10.1016/j.scitotenv.2008.03.005>.