Title: Whiskers provide time-series of toxic and essential trace elements, Se:Hg molar ratios, and stable isotope values of an apex Antarctic predator, the leopard seal

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ABSTRACT

In an era of rapid environmental change and increasing human presence, researchers need efficient tools for tracking contaminants to monitor the health of Antarctic flora and fauna. Here, we examined the utility of leopard seal whiskers as a biomonitoring tool that reconstructs time-series of significant ecological and physiological biomarkers. Leopard seals (*Hydrurga leptonyx*) are a sentinel species in the Western Antarctic Peninsula due to their apex predator status and top-down effects on several Antarctic species. However, there are few data on their contaminant loads. We analyzed leopard seal whiskers (n =18 individuals, n = 981 segments) collected during 2018 – 2019 field seasons to acquire longitudinal profiles of non-essential (Hg, Pb, and Cd) and essential (Se, Cu, and Zn) trace elements, stable isotope ($\delta^{15}N$ and $\delta^{13}C$) values and to assess Hg risk with Se:Hg molar ratios. Whiskers provided between 46 and 286 cumulative days of growth with a mean ~125 days per whisker (n = 18). Adult whiskers showed variability in non-essential trace elements over time that could partly be explained by changes in diet. Whisker Hg levels were insufficient (< 20 ppm) to consider most seals being at “high" risk for Hg toxicity. Nevertheless, maximum Hg concentrations observed in this study were greater than that of leopard seal hair measured two decades ago. However, variation in the Se:Hg molar ratios over time suggest that Se may detoxify Hg burden in leopard seals. Overall, we provide evidence that the analysis of leopard seal whiskers allows for the reconstruction of time-series ecological and physiological data and can be valuable for opportunistically monitoring the health of the leopard seal population and their Antarctic ecosystem during climate change.

KEYWORDS: Mercury, Antarctica, Trace Elements, Leopard Seal, Stable Isotopes, Whisker

1.1 INTRODUCTION

Trace elements have concentrations ≤ 1000 ppm that originate from natural and anthropogenic activities, are transported to areas based on atmospheric and geochemical processes, and are made bioavailable to organisms through uptake at base trophic levels (Bradl, 2005; De Moreno et al., 1997; Lambert et al., 1990). While exposure to trace elements can occur through various pathways, diet is the main route of exposure for mammals (Kershaw and Hall, 2019; Tchounwou et al., 2012). Trace elements include those that are non-essential to physiological processes and may disrupt normal biochemical processes, such as, heavy metals mercury (Hg), lead (Pb), and cadmium (Cd) (Tchounwou et al., 2012). In contrast, essential trace elements play key roles in biological processes (Tchounwou et al., 2012) and include selenium (Se), zinc (Zn), and copper (Cu). Due to their important roles in either disruption (non-essential) or vital function (essential) of health, trace elements are an important research topic in wildlife assessments.

Pinnipeds (Pinnipedia; seals, sea lions, and walruses) are considered sentinel species in marine ecosystems and therefore can be used to assess trace element exposure and population/ecosystem health (Bossart, 2011; Clark et al., 2021; Lehnert et al., 2017; Rea et al., 2020). Mercury, specifically in its methylated form of methylmercury (MeHg), bioaccumulates up through trophic levels and can cross the placental and brain-blood barriers to expose both developing fetuses and adults to high Hg concentrations (Das et al., 2002; De Moreno et al., 1997; Gray et al., 2008; Sun et al., 2020). Although not known to bioaccumulate up marine food webs (Cardwell et al., 2013; Dehn et al., 2006), high concentrations of Pb and Cd may disrupt neurological and cellular activities (Carpenter, 2001; Martelli et al., 2006). Pinnipeds have mechanisms to detoxify non-essential trace elements by binding them to specific essential trace elements (Ikemoto et al., 2004). For example, Se can form biologically inert Se-Hg granules as a way to mitigate negative Hg effects (Ikemoto et al., 2004; Nigro and Leonzio, 1996). The ideal tissues to assess acute exposure to trace elements are internal organs, liver, kidneys, and muscle, where trace elements are processed, metabolized, and/or stored (Das et al., 2002; Ikemoto et al., 2004b, 2004a; Szefer et al., 1994), however, collecting these tissues from live free-ranging pinnipeds is highly invasive and unethical, requiring collection of alternative tissues.

Whiskers, or vibrissae, are keratinous hard tissues that grow over discrete periods of time and have been used to assess various ecological and physiological biomarkers in pinnipeds (Hirons et al., 2001; Keogh et al., 2021, 2020; Rogers et al., 2016). Trace elements are stored and remain inert in keratinous tissues and have been measured in mammal hair, nails, claws, baleen, and whiskers (Ethier et al., 2013; Ferdinando, 2019; Legrand et al., 2004; Noël et al., 2016; Rodushkin et al., 2000; Shore et al., 2022). Assessing relative changes in trace element concentrations throughout whisker growth provides a time-series of non-essential and essential trace elements in pinnipeds (e.g., Noël et al., 2016). Addition of stable isotope (nitrogen [$\delta^{15}N$] and carbon [$\delta^{13}C$]) time-series can provide information on trophic level and foraging location (Ben-David and Flaherty, 2012), respectively, relative to trace element concentrations. For example, pairing trace elements and stable isotopes in teeth helped determine that Uruguayan pinnipeds feeding on benthic prey in a coastal environment resulted in higher exposure to Pb and Cu (De María et al., 2021). Thus, an integrative time-series of trace elements and stable isotopes measured in sentinel species may provide clarity on top predator and ecosystem health during environmental changes.

Leopard seals (*Hydrurga leptonyx*) are apex predators that reside year-round in circumpolar Antarctica (Rogers, 2018; Siniff and Stone, 1985). However, recently, leopard seals have been expanding their distribution as a possible response to climate change with increased sightings in urban areas, such as New Zealand (Hupman et al., 2020), which may bring them into greater contact with humans and anthropogenically sourced contaminants (e.g., trace elements). Generally, leopard seal foraging ecology varies depending on seasons, locations, prey availability, size, and sex (Hall-Aspland and Rogers, 2004; Krause et al., 2020; Walker et al., 1998). Leopard seals are generalists, foraging on Antarctic fur seals (*Arctocephalus gazella*, Boveng *et al.*, 1998), Adelie penguins (*Pygoscelis adeliae*), crabeater seals (*Lobodon carcinophagus*, Hall-Aspland and Rogers, 2004), Antarctic krill (*Euphausia superba*, herein krill, Botta et al., 2018; Casaux et al., 2009) and demersal fish (Hall-Aspland and Rogers, 2004; Krause et al., 2020, 2015). These life-history characteristics makes them a candidate for a sentinel species and could provide data on the health of the Antarctic ecosystem (Aguirre and Tabor, 2004).

Leopard seal habitat has dramatically changed over the past 65+ years. For example, in the Western Antarctic Peninsula (WAP), temperature and salinity of the coastal upper water columns has increased resulting in decreased sea ice extent and plankton diversity (Alexander Haumann et al., 2016; Lin et al., 2021). This warming has led to an increase in glacial melting leading to a subsequent flux of non-essential and essential trace elements into WAP waters (Kim et al., 2015). Early studies on trace elements in Antarctic wildlife revealed a "pristine" environment (De Moreno et al., 1997); however, since the start of the industrial revolution, Antarctica has been greatly affected by the transport of trace elements from various industrial activities (e.g., coal burning) (Bargagli, 2008; De Castro-Fernández et al., 2021). Trace elements have been measured in leopard seal tissues (Beck, 1955; Gray et al., 2008; Szefer et al., 1994, 1993) and provide a baseline of trace element data, however, changes in trace elements as a function of time has not been measured in individual leopard seals. Leopard seal whiskers contain up to 1-year of growth before being asynchronously shed during their molt (Rogers et al., 2016) and they have been used to assess temporal changes in $\delta^{15}N$ and $\delta^{13}C$ values (Botta et al., 2018; Hall-Aspland et al., 2005). Here, we expand the utility of leopard seal whiskers by using a novel approach of assessing trace element concentrations and corresponding stable isotope values in whisker segments to monitor and contextualize the levels of trace elements in the leopard seal population and Antarctic ecosystem.

The objectives of this study were to characterize trace elements (Hg, Pb, Cd, Se, Cu, and Zn) and stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) in leopard seal whiskers to; 1) provide additional baseline data on leopard seal trace elements and foraging ecology in relation to sex and biometrics; 2) assess the suitability of whiskers as a biomonitoring tissue of trace element changes over time with stable isotope values ($\delta^{15}N$ and $\delta^{13}C$) while accounting for sex and biometrics; and 3) assess Hg risk in leopard seals and determine if Se is utilized to buffer Hg loads by analyzing Se:Hg molar ratios.

2.1 METHODS

2.1.1 Whisker collection

Leopard seal whiskers were collected between April – May in 2018 and 2019 during field work conducted at the U.S. Antarctic Marine Living Resources (AMLR) Program research station on Cape Shirreff, Livingstone Island, Antarctic Peninsula (National Marine Fisheries Service permit #19439 and Antarctic Conservation Act permit #2018-016) (Fig. 1). Leopard seals were chemically immobilized using a butorphanol-midazolam protocol administered with a jab stick following Pussini and Goebel, (2015). While sedated, morphometric data were collected (e.g., mass, kg; length, cm; girth, cm). The longest whisker was plucked with the root intact from the muzzle of each seal and stored in a Whirl-Pak® (Madison, WI, USA) at ambient temperature.

The final study collection consisted of 18 whiskers from 15 females (n = 1 juvenile and n = 14 adults) and 3 males (n = 3 adults). Following shipping to Baylor University, whiskers were stored at –80 °C until analysis. Whisker length and mass were measured using digital calipers (± 0.01 mm, Neiko 01407A) and a Mettler Toledo microbalance.

2.1.2 Scaled body mass index (SBM index)

Scaled body mass (SBM) index was calculated for each sampled leopard seal using the equation (Peig and Green, 2009):

$$\widehat{Mi} = Mi\left(\frac{Lo}{Li}\right)^{bsma}$$

Where *Mi* was the mass (kg) of the seal, *Li* was the seal's standard length (cm), *Lo* was the mean standard length of the different age classes (juvenile n = 1 female) or adult (n = 17 [n = 3 males and n = 14 females]), and b_{sma} was the scaling exponent from plotting natural log transformed mass by standard length for all seals and the $\widehat{Mi}$ was the predicted mass of the individual seal when standardized to *Lo*. This SBM index has been shown to scale more accurately with growth and mass compared to other condition indices used in wildlife studies, including pinnipeds (DeRango et al., 2019; Peig and Green, 2009).

2.1.3 LA-ICP-MS analysis of trace elements along whiskers

Whiskers were prepared for laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) analysis by removing visible surface contaminants (e.g., external root sheath fragments) by wiping with a Kimwipe moistened with a 2:1 chloroform methanol solution (Keogh et al., 2021; Rea et al., 2015), and drying for ≥ 24 hours in a ventilation hood. Cleaned whiskers were shipped in sealed Whirl-Pak® bags to The University of Texas at Austin, and then stored in a desiccator until LA-ICP-MS analysis.

Continuous elemental (Hg, Pb, Cd, Se, Cu, Zn) base-to-tip whisker concentrations were measured by LA-ICP-MS, using an ESI NWR193 excimer laser ablation system (193 nm, 4 ns pulse width) coupled to an Agilent 7500ce ICP-MS. Whiskers, ranging from 48.3 – 99.4 mm in length, were mounted on double-sided stick tape. The most stable mounts were achieved by allowing whiskers to best retain their natural curvatures in 2D; whiskers mounted in a straight line were found to move over time. Coordination of whisker transects involved establishing long segmented lines with 1-2 nodes placed per mm, and each node adjusted in x-y to follow the central growth axis. The z axis was adjusted to maintain laser focus along the surface of the traverse as whiskers greatly taper from base-to-tip. The LA-ICP-MS system was optimized for sensitivity across the atomic mass unit (AMU) mass range and low oxide production (ThO/Th: 0.28 ± 0.01) by tuning on a standard (NIST 612). Final parameters where whisker ablations were obtained from trial transects on representative areas of trial whisker samples (via iterative tests of energy density, repetition rate, and gas flow) to obtain robust and consistent elemental signals free from spectral skew). Following pre-ablation (100 µm spot, 100 µm/s scan rate, 2.7 J/cm^2 energy density [fluence]) to remove shallow superficial contaminants, a single base-to-tip transect was performed along the center of each whisker, using a 90 µm diameter spot, 100 µm/s scan rate, 2.44 ± 0.07 J/cm^2 energy density, 10 Hz repetition rate, and carrier gas flows (L/min) of 0.85 for Ar and 0.85 for He. The quadrupole time-resolved method measured eight masses with integration times of 10 ms (^{34}S, ^{63}Cu, ^{64}Zn, ^{83}Zr), 205 ms (^{202}Hg), and 100 ms (^{82}Se, ^{114}Cd, ^{208}Pb). Measured intensities were converted to elemental concentrations (ppm) using iolite software (Paton et al., 2011), with ^{34}S as the internal standard and a S index value of 5 wt % for whisker unknowns (Legrand et al., 2004; Noël et al., 2016; Rodushkin and Axelsson, 2003; Stadlbauer et al., 2005). Signals were converted to base-to-tip distance (µm) along the whisker based on the scan rate and duty cycle. Any data points that fell below detectable concentrations were assigned ½ the concentration of the limit of detection calculated for the analytical run of each whisker (Clark et al., 2021; Gilbert, 1987, Table S1). Outliers were defined as concentrations measured along the whisker greater than 4 standard deviations above the mean and removed from statistical analysis (Clark et al., 2021; Tukey, 1977).

Previous researchers have used commercially available human hair as an appropriate reference standard (wt % S content, keratin matrix) for mammal hair and whisker LA-ICP-MS studies (e.g., Noël et al., 2016, 2014). However, these standards have only sub-ppm concentrations for several of the metals assessed (Se, Hg) during this study. Thus, we made and validated reliable standards using methods described in the *Supplementary Material.*

2.1.4 Stable isotope analysis and timestamps

After whiskers were analyzed for trace elements using LA-ICP-MS analysis they were returned to Baylor University for sampling of bulk carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope analysis (Fig. 2). Lipids were removed by cleaning each whisker with a 1:1 ethanol: methanol solution, following previous leopard seal whisker stable isotope ratios studies (Botta et al., 2018; Rogers et al., 2016). After allowing whiskers to dry in a ventilation hood ($\leq$ 24 hr.), whiskers were sectioned into 0.50 ± 0.01 mm lengths (using digital calipers and a hand chisel) to enable fine-scale comparison with the LA-ICP-MS trace element time-series; segment lengths were somewhat longer near the frayed tip of whiskers to obtain the minimum required mass (~0.3 mg) for stable isotope analysis.

Carbon and nitrogen stable isotope analysis was performed in the Baylor University Stable Isotope Facility, using an Elemental Analyzer (EA) Costech 4010 Elemental Combustion System (ECS) paired with a Conflow IV interphase (Thermo Scientific) and Thermo Delta V Advantage continuous flow Isotope Ratio Mass Spectrometer (EA-IRMS). Prior to combustion and isotopic analysis, whisker segments were placed into pre-weighed tin capsules (Costech 5 x 9 mm), tin capsules reweighed with a Mettler Toledo XP26 digital scale (± 0.001 mg). Whisker nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotope values are reported as the ratio of the heavy to light isotope relative to international standards; atmospheric nitrogen and Vienna Peedee Belemnite (VPDB), respectively, using the following equation:

$$\delta X = [(R_{sample}/R_{standard})-1] *1000,$$

where X is the targeted isotope (nitrogen or carbon) ratio expressed in delta notation (δ) with units per mil (‰), R_{sample} is the isotopic ratio of heavy to light isotopes ($^{15/14}N$ or $^{13/12}C$) of the sample, and $R_{standard}$ is the isotopic ratio of heavy to light isotopes measured in the standard. A two-point calibration curve for calculating nitrogen $\delta^{15}N$ and $\delta^{13}C$ values of samples was established using USGS-40 and USGS-41A international standards. The accuracy and precision of isotopic measurements was calculated based on the long-term mean and standard deviation of 105 replicates of an internal lab standard (Acetanilide, reported $\delta^{13}C = -29.53 \pm 0.01$ ‰, $\delta^{15}N = 1.18 \pm 0.02$ ‰) measured during each analytical run (n = 3 replicates/run). The replicate grand averages obtained are within ($\delta^{15}N = 1.28 \pm 0.17$ ‰) or very close to ($\delta^{13}C = -29.45 \pm 0.05$ ‰) analytical uncertainty of reported values.

Acceptable atomic C:N ratios of whisker segments ranged from approximately 3.0 – 3.8 based on previous leopard seal whisker isotope studies (Botta et al., 2018; Rogers et al., 2016). Nearly all whisker segments had acceptable atomic C:N ratios (3.47 ± 0.13, 3.03 – 4.00, mean ±

standard deviation (SD), min – max, respectively (Fig. S1); one segment with anomalously low atomic C:N ratio < 2.8, was excluded from statistical analysis.

Whisker segments were assigned approximate timestamps relative to date of collection based on leopard seal whisker growth characteristics and the application of a discrete Von Bertalanffy growth model (Hall-Aspland et al., 2005; Rogers et al., 2016; von Bertalanffy, 1938). The discrete Von Bertalanffy equation can be written as:

$$\delta L/\delta T = K(L_a - L_p - 1),$$

where $\delta L/\delta T$ is growth rate for sections $_{p-1}$ to $_p$. This can be rearranged to $\delta L = (L_p - {}_{Lp-1})$ and $\delta T = (T_p - T_{p-1})$ allowing calculation of time intervals for whisker section(s) $_{p-1}$ to $_p$ as done in Hall-Aspland et al., (2005) and Rogers et al., (2016):

$$(T_p - T_{p-1}) = (L_p - L_{p-1}) / [K(L_a - L_{p-1})],$$

where L_p is the total length of the whisker, L_{p-1} is the remaining length of the whisker after sampling section $_p$, K is the growth coefficient, and L_a is the asymptotic length of leopard seal whisker. We used the recommended K value of 0.013 and L_a of 101.2 mm developed by Rogers et al., (2016) for leopard seal whiskers and applied this equation to each section to acquire approximate segment growth in days. Cumulative δT values over respective segment intervals were then subtracted from the collection date of each whisker to develop an approximate timestamp for individual segments.

2.1.5 Aligning trace element and stable isotope data

The accurate alignment of trace element and stable isotope data per whisker was standardized to the segment lengths submitted for stable isotope analysis. For example, the first section of a whisker (i.e., section "1") with a length of 0.50 mm submitted for isotope analysis meant a number "1" was assigned to all trace element data obtained during the 0.0 - 0.50 mm of whisker sampled during LA-ICP-MS (Fig. 2). This was repeated for subsequent sections until trace element data were assigned a section number that directly corresponded with stable isotope data. Mean and standard deviation values were calculated for trace element data based on its respective section number to accurately be paired with stable isotope data (Fig. 2). This approach worked for all but three whiskers (n = 3), where cumulative segment lengths for stable isotope analysis were greater than the trace element whisker length data. This most likely was due to error when measuring and sampling individual segments for stable isotope analysis. To correct this error in the three whiskers, the total sampling error was calculated (i.e., total whisker length prior to sampling minus cumulative segment lengths post sampling) for these three whiskers and the total error was divided by the total number of whisker segments. The resulting values ranged from 0.06 – 0.08 mm and were subtracted from section lengths for all segments. The process of integrating trace element with stable isotope data was rerun with the corrected segment lengths and resulted in all trace element data being assigned to a corresponding stable isotope segment.

2.1.6 Statistical analysis

Type II ANOVAs (F-tests for linear models) were used to determine significant differences in mean log10 transformed whole whisker trace elements, $\delta^{15}N$, and $\delta^{13}C$ values between sexes, standard length, and SBM index in respective linear models (Garcia-Cegarra et al., 2021). Due to only having one juvenile, age class was not assessed among these whole whisker trace element and stable isotope data. Whole whisker analyte concentrations calculated means across all whisker segments per individual, whereas segment analyte concentrations are averaged only across segment length (Fig. 2).

Pearson correlations of transformed trace elements were performed within and among individuals of pooled sexes (n = 15 females, n = 3 males) using segmented and whole whisker trace element data, respectively. Among individual correlations were calculated using average whole whisker trace element concentrations (i.e., correlations among mean trace element concentrations for each individual seal) and can provide general insight into trace element associations within leopard seal whiskers (e.g., individuals with high concentrations of element "A" tend to have low concentrations of element "B"). In contrast, within individual correlation coefficients were calculated using a smoothed time series of trace element concentrations across the whisker of each individual. The resulting Pearson correlation coefficients were then Fisher-Z transformed, averaged across all individuals, and transformed back to a mean Pearson correlation coefficient. This approach reveals correlations among trace elements through time (i.e., along the whisker) that are consistent across seals, thus are likely to represent processes or phenomena that affect most or all leopard seals in this study, which may include things like physiological, temporal, and spatial intrinsic influences on trace element intake or uptake (e.g., Clark et al., 2021).

The R package SIBER was used to calculate intraindividual standard ellipse area (SEA) corrected for small sample sizes (SEAc) across whisker segment $\delta^{13}C$ and $\delta^{15}N$ values for each seal (Jackson et al., 2011; Scholz et al., 2020). The SEAc calculates the variability among $\delta^{13}C$ and $\delta^{15}N$ values across whisker segments to provide insight into range of trophic level and foraging locations (i.e., trophic niche) of an individual seal. We used SEAc values to understand how trophic niche width related to whole whisker trace element concentrations. We also calculated a "population" SEAc using whole whisker $\delta^{15}N$ and $\delta^{13}C$ values from all whiskers (n = 18) to compare with a previous leopard seal whisker study (Botta et al., 2018). Bivariate linear models of log10 transformed trace element data and ANOVAs (F-tests for linear models) were used to determine relationships of trace elements with intraindividual SEAc values. Linear models and Type II ANOVAs (F-tests for linear models) were used to assess intraindividual SEAc with sex, standard length, and SBM index.

Linear mixed models (LMMs) were constructed to determine relationships among trace elements with changes in $\delta^{15}N$ and $\delta^{13}C$ over time while incorporating sex and biometrics (standard length and SBM index) as covariates. All trace element data were log10 transformed to approximate normal distribution to meet LMM assumptions. Trace element data from adult leopard seal whiskers were modeled (n = 17 adults [n = 3 males and n = 14 females]). Full models were constructed using the R Studio software (RStudio Team, 2020) and the package lme4 (Bates et al., 2015) based on our objectives to assess temporal relationships among trace elements with changes in diet ($\delta^{15}N$ and $\delta^{13}C$ values), that also incorporated sex and biometric data (Zuur and Ieno, 2016). A numbered "Week" of the year (1-52) was assigned to individual segments relative to the earliest segment timestamp and only retained "Weeks" that included a minimum of three unique seals (total n = 17 seals, n = 834 segments, and n = 28 consecutive weeks). The full model took the form of: log10(trace element) ~ Standard Length (numeric)+ SBM index (numeric)+Sex (factor, "Male" or "Female") + Week (numeric)+Carbon (numeric, $\delta^{13}C$ of segment) + Nitrogen (numeric, $\delta^{15}N$ of segment) + Carbon*Week + Nitrogen*Week + (1|FieldSeason) (random intercept, controlling for whisker collection year) +(1|Seal.ID) (random intercept, controlling for differences in average concentrations among individuals). Biologically relevant permutations with the fixed effects of the full model were constructed to compare with the full model (Table S2). The selected model was determined based on lowest AICc and highest AIC weight (Burnham et al., 2011; Table S3). We assessed the full and selected models for each trace element by plotting residuals with fitted values, residuals with all covariates, and assessed the distribution of residuals (Zuur and Ieno, 2016).

Selected LMMs for each trace element had fitted and 95% confidence intervals constructed using the "bootpredictlme4" package in R using n = 500 iterations to estimate the fit of selected models with the trace element data (Clark et al., 2021; Duursma, 2021). If the interaction terms were retained in the selected model, we predicted trace element concentrations over time keeping $\delta^{15}N$ and $\delta^{13}C$ at biologically relevant “lower”, “median”, and “upper” values, while keeping the other isotope and/or main effects at their median values, if applicable. For $\delta^{15}N$, the lower value was 10.15 ‰ (12.5 % range of our $\delta^{15}N$ values, between 0 and 1st quartile), median value was 10.83 ‰, and upper value was 12.47 ‰ (87.5 % range of data, between 3rd and 4th quartiles of our $\delta^{15}N$ values). For $\delta^{13}C$, the lower value was -22.74 (12.5 % range of data, between 0 and 1st quartile of our $\delta^{13}C$ values), median value = -21.98, and upper value was = -21.41 ‰ (87.5 % range of data, between 3rd and 4th quartiles of our $\delta^{13}C$ values). We then visually assessed the fit of model predictions with the leopard seal whisker trace element data from the four seals that fell within those stable isotope categories (i.e., “lower”, “median”, and “upper” $\delta^{13}C$ and $\delta^{15}N$ values) (Clark et al., 2021; Zuur and Ieno, 2016).

Mercury is a relatively well-studied toxin in pinnipeds with published Hg toxicological thresholds from hair concentrations (McHuron et al., 2019; O’Hara and Hart, 2018; Rea et al., 2020), which also correlate with whisker Hg concentrations (Noël et al., 2016). Previous studies suggest different toxicity thresholds for hair Hg concentrations that are associated with deleterious effects to wildlife and humans including; 5.4 ppm [µg/g dw] in brain tissue of polar bears (*Ursus maritimus*) which correlated with a reduction in genomic DNA methylation and NMDA receptors, 10 ppm in human infants was correlated with delayed development, and ~30 ppm in mink (*Neogale vison*) hair that had resulted in acute Hg toxicity and death in some individuals (Van Hoomissen et al., 2015; Yates et al., 2005). Since hair and whisker Hg thresholds are unknown for leopard seals, we followed Rea et al., (2020) and used Hg toxicological thresholds of < 10 ppm to assign whole whisker and individual segment concentrations as "Low" risk, 10 to 20 ppm as "Moderate", and "High" risk of Hg toxicity if Hg concentrations were > 20 ppm (O'Hara and Hart, 2018; Rea et al., 2020). Additionally, molar Se:Hg ratios were calculated for each segment using the formula: (Se ppm /78.96)/(Hg ppm/200.59) following McCormack et al., (2021). An ANOVA with Tukey's Post Hoc honestly significant difference (HSD) was used to determine overall differences in mean Se:Hg molar ratios among risk groups ("low", "moderate", and "high"). We then visually assessed how Se:Hg molar ratios patterns changed over time with respect to Hg risk classification to analyze the potential importance of Se to leopard seals as a Hg detoxicant (Rea et al., 2020). An alpha level of 0.05 was used for threshold of significance for ANOVAs (F-tests for linear models). All data presented in figures are median ± interquartile range (IQR) due to high variability among data.

3.1 RESULTS

Timestamps of whiskers represented a mean ~125 days per whisker (n = 18), representing ~4.5 months of paired trace element and stable isotope ($\delta^{15}N$ and $\delta^{13}C$) data for each individual leopard seal. The time represented by whiskers ranged from approximately 1.5 months (46 days) to 10 months (286 days) of growth, which falls within previous estimates (max ~1 year) of whisker growth in another leopard seal study (Rogers et al., 2016). These timestamps were based on whisker length; mean whisker length was 74.3 ± 14.1 mm (mean ± standard deviation, SD) with a range from 48.3 – 99.4 mm; this equated to a mean mass of 21.7 ± 7.3 mg and ranged from 12.3 – 33.8 mg.

3.1.1 Whole whisker trace element, stable isotope, and trace element correlation data

All trace elements had detectable concentrations above their limits of detection (LOD) along the lengths of all whiskers (Table S1). All trace elements (Hg, Pb, Cd, Se, Cu, and Zn) had similar whole whisker mean concentrations among sexes and exhibited no significant relationships with standard length or SBM index (Table 1, ANOVAs, $p > 0.05$ all trace elements).

Whole whisker $\delta^{15}N$, $\delta^{13}C$, and trophic niche width (SEAc) were compared between sexes and assessed with biometric data. Mean $\delta^{15}N$ values did not significantly differ between sexes (female $\delta^{15}N = 11.38 \pm 0.99$ ‰, male $\delta^{15}N = 9.89 \pm 1.32$ ‰). Further, $\delta^{15}N$ values did not exhibit significant relationships with standard length or SBM index (ANOVAs, $p > 0.05$ for all). Similarly, mean $\delta^{13}C$ values were not significantly different between sexes (female $\delta^{13}C = -22.02 \pm 0.71$ ‰, male $\delta^{13}C = -22.08 \pm -0.62$ ‰) with no significant relationships with standard length and SBM index (ANOVAs; sex: $p > 0.05$ for all). Trophic niche width (SEAc) did not differ between sexes or exhibit significant relationships with standard length and SBM index (ANOVAs, $p > 0.05$ for all, Fig. S2). The population level SEAc trophic niche width value derived from whole whisker $\delta^{15}N$ and $\delta^{13}C$ variability was 2.45 (Fig. S3). Summary data of $\delta^{15}N$, $\delta^{13}C$, and SEAc values from whole whiskers are presented in Table 1.

Trace elements were not significantly related to trophic niche width (ANOVA, $p > 0.05$ for all trace elements, Fig. S4). However, visual assessment of Hg with trophic niche width demonstrates that seals with a narrow trophic niche width had generally higher $\delta^{15}N$, enriched

δ^{13}C, and high Hg concentrations (Fig. S4, Fig. S5). Seals showed positive and negative correlations among and within individuals' trace element data, which can be found in Fig. 3 and Table S4.

3.1.2 Trace element changes over time with nitrogen and carbon isotopes, sex, and biometrics

3.1.2.1 Non – essential trace elements (Hg, Pb, and Cd)

Linear mixed models were used to assess the relationships of trace elements, including the non-essential Hg, Pb, and Cd, in whiskers with changes over time with δ^{15}N values, δ^{13}C values, sex, and biometrics. Mercury, Pb, and Cd concentrations were dependent on the following fixed effects; SBM index (Hg only), Week, δ^{15}N, δ^{13}C, and the interaction effect of Week*δ^{15}N and Week*δ^{13}C (Table S3).

General trends in monthly median Hg were relatively constant from November to January before increasing during February and March then decreasing in April and increasing in May (Table 2, Fig. S6). Seals with a greater SBM index had higher Hg concentrations (0.00081 log10 ppm/kg, Table S3). Mercury LMM results showed differences in accumulation over time based on different δ^{15}N and δ^{13}C values.

Generally, Hg concentrations in seal whiskers with lower δ^{15}N (~10.15 ‰) values decrease throughout the year at approximately -0.0023 log10 ppm/week equating to a decrease of approximately 1.46 ppm (Fig. 4). For seals with median δ^{15}N values (~10.83 ‰), models predicted Hg would accumulate more slowly (0.00036 log10 ppm/week) equating to a 0.23 ppm increase compared to the seals with higher δ^{15}N (Fig. 4). Seals that maintained high δ^{15}N (~12.47 ‰) values throughout the year accumulated Hg at the greatest rate of 0.0044 log10 ppm/week with a total increase of 2.84 ppm (Fig. 4). Mercury concentrations in seal whiskers with lower

$\delta^{13}C$ values (~ -22.74 ‰) increased over time at approximately 0.0032 log10 ppm/week with approximately a 2.00 ppm increase in Hg concentrations (Fig. 4). Seal whiskers with median $\delta^{13}C$ values (~ -21.98 ‰) accumulated Hg over time, however, at a slower rate (0.00035 log10 ppm/week) and resulted in a slight increase of ~0.22 ppm of Hg over time compared to seals with lower $\delta^{13}C$ values (Fig. 4). Seals with the highest $\delta^{13}C$ values (assuming -21.41 ‰) exhibited a decline in Hg concentrations over time at approximately -0.0030 log10 ppm/week and had an overall decrease of 1.90 ppm over the study period (Fig. 4). Overall, model predictions of Hg over time with changes in $\delta^{15}N$ and $\delta^{13}C$ captured general, not fine-scale (possibly non-linear), trends in observed data (Fig. 4, Table S3).

Whisker Pb and Cd concentrations decreased over time, given low, median, or high $\delta^{15}N$ values ([low] ~10.15 ‰, -0.054 log10 ppm/week; Pb, -0.082 log10 ppm/week; Cd, [median] ~10.83 ‰, -0.055 log10 ppm/week; Pb, -0.081 log10 ppm/week; Cd, [high] ~12.47 ‰, -0.059 log10 ppm/week; Pb, -0.078 log10 ppm/week) (Fig. 4). Lead and Cd concentrations were highest near the end of January (Fig. S6) and decreased until April for all seals, regardless of their $\delta^{15}N$ values (Fig. 4). Seal whiskers with low (~-22.74 ‰), median (~-21.98 ‰), or high (~ -21.41 ‰) $\delta^{13}C$ values exhibited high Pb and Cd concentrations in January, which slowly decreased over time, with Pb and Cd concentrations in whiskers of high $\delta^{13}C$ values declining at a faster rate (-0.063 log10 ppm/week; Pb, -0.089 log10 ppm/week; Cd) than in whiskers with median (-0.055 log10 ppm/week; Pb, -0.081 log10 ppm/week; Cd) and low (-0.045 log10 ppm/week; Pb, -0.070 log10 ppm/week; Cd) $\delta^{13}C$ values (Fig. 4). Model predictions fit observed data when sample sizes reached above n = 6 seals per week (January – April) but fit poorly when sample sizes dropped below n = 6 seals per week (November – December, Fig. 4). General trends in median Pb and Cd exhibited high variability throughout the study period (Table 2, Fig. S6).

3.1.2.2 Essential trace elements (Se, Cu, and Zn)

Selenium concentrations were dependent on sex and the interaction terms Week*δ^{15}N and Week*δ^{13}C (Table S3). The selected model predicted that whiskers from male leopard seals contained greater Se compared to those of females throughout the study period (Fig. 5). In general, model predictions did not capture the variability in male leopard seal Se levels over time but fit the data from female seals from January to April (Fig. 5). Females with higher δ^{15}N (~ 12.47 ‰, 0.033 log10 ppm/week; female Se) accumulated Se the slowest compared to females with other δ^{13}C and δ^{15}N values (~ -22.74 ‰ and 10.83 ‰, 0.038 log10 ppm/week; lower δ^{13}C values and median δ^{15}N values, respectively, -21.98 ‰, 0.038 log10 ppm/week; median δ^{13}C values, -21.41 ‰, 0.037 log10 ppm/week; higher δ^{13}C values, ~10.15 ‰, 0.039 log10 ppm/week; lower δ^{15}N values) (Fig. 5). Nevertheless, females maintaining higher δ^{15}N values from mid-February through mid-March had higher median Se concentrations compared to seals with median and lower δ^{15}N values (Fig. 5). For males, model fit was poor for both δ^{15}N and δ^{13}C values (Fig. 5). Overall trends in Se over time showed relatively low and stable Se from November through March before increasing during April (Table 2, Fig. S6).

Copper concentrations were dependent on the interaction of time (Week) with δ^{15}N and δ^{13}C values (Table S3). Copper, like all other trace elements, displayed high variability throughout time (Fig. S6, Fig. S7). Overall, regardless of the δ^{15}N or δ^{13}C values seals exhibited, models predicted declines in Cu concentrations over time; however, rates of decline differed based on δ^{15}N and δ^{13}C values. Differences in rates of decline over time were similar with varying δ^{13}C values (~-22.74 ‰ [lower], ~-21.98 ‰ [median], ~-21.41 ‰ [higher] values, -0.0093 log10 ppm/week; lower δ^{13}C values, -0.012 log10 ppm/week; median δ^{13}C values, -0.013

log10 ppm/week; higher $\delta^{13}C$ values) compared to different $\delta^{15}N$ values (~10.15 ‰ [lower], ~10.83 ‰ [median], ~12.47 ‰ [higher] values, -0.010 log10 ppm/week; lower $\delta^{15}N$ values, -0.012 log10 ppm/week; median $\delta^{15}N$ values, -0.016 log10 ppm/week; higher $\delta^{15}N$ values, Fig. S7). These model predictions only fit observed trends from January – April (Fig. S7).

Zinc concentrations were dependent on the interactions of time of year (Week) with $\delta^{13}C$ and $\delta^{15}N$ values (Table S3). Seals with median $\delta^{13}C$ (~-21.98 ‰) and $\delta^{15}N$ (~10.83 ‰) values accumulated Zn comparably over time (0.000010 log10 ppm/week; median $\delta^{13}C$, 0.0000020 log10 ppm/week; median $\delta^{15}N$, Fig. S8). However, seals that maintained higher $\delta^{13}C$ (~21.09 ‰, -0.0020 log10 ppm/week) or lower $\delta^{15}N$ values (~10.15 ‰, -0.0020 log10 ppm/week) exhibited decreases in Zn over time (Fig. S8), while seals with higher $\delta^{15}N$ (~12.47 ‰, 0.0050 log10 ppm/week) and lower $\delta^{13}C$ (~-22.74 ‰, 0.0027 log10 ppm/week) values accumulated Zn at the fastest rates (Fig. S8). Zinc concentrations oscillated throughout the study period, with peaks in December and February through April (Table 2, Fig. S6).

3.1.3 Hg risk and Se:Hg molar ratios over time

Based on whole whisker Hg concentrations, leopard seals in this study were classified as having a "low" (Hg < 10 ppm; n = 11, 6.73 ± 1.61 ppm, mean ± SD), "moderate" (Hg 10 - 20 ppm; n = 5, 13.40 ± 2.47 ppm) and "high" (Hg > 20 ppm; n = 2) Hg toxicity risks. The two high risk seals were both adult females with whole whisker Hg concentrations of 21.05 and 22.98 ppm. When assessing whole whisker Se:Hg molar ratios with Hg risk, mean Se:Hg molar ratios were similar among "low" (2.22 ± 0.75 (SD)), "moderate" (2.50 ± 0.95), and "high" (1.66 ± 0.23) Hg risk thresholds (ANOVA, $p = 0.06$). However, our whisker transect/segment time-series allowed us to assess temporal changes in molar Se:Hg in relation to Hg risk classifications.

Mercury concentrations show leopard seals were classified as either “low” or “moderate” Hg toxicity risk from August through December and maintained a Se:Hg molar ratios > 1.0 (Table 2, Fig. 6). However, after December, Hg in whisker segments from these two individuals increased above 20 ppm and were classified as “high” risk. The first Hg segments to drop below Se:Hg molar ratios of 1.0 were from seals that were classified as “moderate” and “low” risk based on their whole whisker Hg concentrations (Fig. 6). From February through March, seals categorized as “low” Hg risk maintained Se:Hg molar ratios > 1.0, while “moderate” risk seals had whisker segments above and below Se:Hg molar ratios of 1.0, and “high” risk seals maintained Se:Hg molar ratios < 1.0. Although data are limited in May (Table 2), Se:Hg molar ratios for all three risk groups rose well above 1.0 (Fig. 6). Se: Hg molar ratios exhibited a possible exponential decrease with increasing Hg concentrations, where “low” Hg risk seal whisker segments maintained Se:Hg molar ratios > 1.0 and “high” Hg risk whisker segments with the greatest Hg concentrations (> 20 ppm) were mostly below Se:Hg molar ratios < 1.0 (Fig. S9).

4.1 DISCUSSION

Leopard seal whiskers recorded paired time-series trace element and stable isotope data. Some non-essential (Hg) and essential (Se and Zn) trace element concentrations increased at different rates over time depending on variations in trophic level and foraging location (paired $\delta^{15}N$ and $\delta^{13}C$ values, respectively), whereas other non-essential (Pb and Cd) and essential (Cu) trace element concentrations decreased over time at varying rates based on trophic group patterns (i.e., $\delta^{15}N$ values). Leopard seal whiskers can therefore be used to monitor trace element concentrations and accrual of trace elements is partly explained by changes in general trophic level and foraging location. Leopard seals were generally classified as "low" or "moderate" risk for Hg toxicity risk based on whole whisker Hg. Yet, fine-scale changes in Se:Hg molar ratios over time in whiskers relative to Hg toxicity risk and paired Hg concentrations supports the hypothesis Se is a detoxifier of Hg in leopard seals.

4.1.1 Relationships of diet with sex and biometrics

Mean trophic level ($\delta^{15}N$ values), foraging location ($\delta^{13}C$ values), and trophic niche width (SEAc values) data from whiskers did not reveal any significant relationships with sex or biometric data, similar to previous whisker assessments in leopard seals (Botta et al., 2018). Whole whisker mean $\delta^{15}N$ values ranged from 10.07 – 13.14 ‰ for females and 8.37 – 10.70 ‰ for males; however, similar mean $\delta^{15}N$ values suggest females and males foraged at similar trophic levels (Table 1). Consistent with this interpretation are similar mean whole whisker $\delta^{13}C$ values between sexes, suggesting that both sexes foraged in similar areas (Table 1). However, other studies have shown females and males exhibit diverse summer foraging strategies (Krause et al., 2020, 2015). The warming of the WAP has possibly altered $\delta^{13}C$ isoscapes based on changes in primary producer composition (Kerr et al., 2018; Seyboth et al., 2018) possibly converging $\delta^{13}C$ values of different foraging areas of different sexes resulting in similar whole whisker $\delta^{13}C$ values. The paired $\delta^{13}C$ (and $\delta^{15}N$) in whisker segments may better represent individual foraging strategies of males and females compared to assessing their whole whisker values. Additionally, similarities of $\delta^{15}N$ and $\delta^{13}C$ values between sexes should be cautiously interpreted due to our disparate sample sizes between males (n = 3) and females (n = 14).

While leopard seal trophic niche width has been assessed at population and subgroup levels using whiskers (Botta et al., 2018), this is the first study assessing intraindividual trophic niche width in leopard seals. Leopard seals exhibit wide ranges of intraindividual trophic niche widths (SEAc, Table 1, Fig. S2). The median intraindividual trophic niche width of females (SEAc = 1.02) was not within 95% confidence intervals of leopard seal trophic niche width at a population (SEAc = 2.51 – 3.13) nor high or low trophic level groupings of leopard seals (1.87 - 2.44 and 1.38 - 2.02, SEAc values respectively (Botta et al., 2018)); however two of the three male intraindividual trophic niche widths (SEAc = 1.64 and 2.74) did fall within the low trophic and population level groupings, respectively. The greater SEAc ranges reported in Botta et al., (2018) are most likely due to the increase in trophic niche width at the population compared to an individual level (Scholz et al., 2020). The population SEAc value (SEAc = 2.45), fell just below the population level trophic niche width range (SEAc = 2.51 – 3.13) reported by Botta et al., (2018). This suggests leopard seals exhibited comparable population level trophic niche widths in 2018 and 2019 (this study) similar to WAP leopard seals during 2011, 2014, and 2016 (Botta et al., 2018). Overall, female and male leopard seals in this study exhibited a wide range of intraindividual trophic niche widths (Table 1), indicating leopard seals are capable of dynamic foraging strategies which may be beneficial with reduced sea ice habitat and changing prey abundance (Botta et al., 2018; Krause et al., 2020; Meade et al., 2015).

4.1.2 Trace element relationships with trophic niche width

Leopard seal trophic niche width was not related to whole whisker trace element concentrations (Fig. S4). Few studies are available as a comparison, but similar results have been found for different cetacean species with Hg measured in skin (Pinzone et al., 2019). We sampled three seals (n = 3) whose SEAc values reflected a greater change in $\delta^{13}C$ and/or $\delta^{15}N$ values during whisker growth, resulting in higher SEAc values compared to other seals (e.g., Fig.

S10 and Fig. S11), which potentially made them their own "prey/location switching" subgroup. When the "prey switching" seals were excluded, the remaining seals that displayed a more specialized foraging strategy (i.e., low SEAc) had higher Hg concentrations as well as higher $\delta^{15}N$ and $\delta^{13}C$ values (Fig. S5). These specialized seals (SEAc values ~ 0.5), mostly adult females, appeared to have fed almost exclusively at higher trophic levels, which might explain the higher Hg concentrations compared to more generalist (i.e., high SEAc) seals with lower $\delta^{15}N$ values (Fig. S5, Aubail et al., 2011; Das et al., 2002). Although no other trace elements displayed a clear relationship with SEAc, prey choice rather than trophic niche width appears to be a more important factor for accumulating trace elements as demonstrated by Hg (Pinzone et al., 2019). Mercury did not exhibit strong positive correlations with other trace elements which could also explain why no other trace elements exhibited a similar relationship with SEAc (Fig. 3, Fig. S5).

4.1.3 Model results - trace element changes over time with nitrogen and carbon stable isotope ratios, sex, and biometrics

A main objective of this study was to assess suitability of leopard seal whiskers as a tool for biomonitoring of trace elements over time. Model predictions for trace elements did not closely fit the data, most likely due to small sample sizes; however, these predictions revealed general trends in trace element accumulation of adult, mostly female, leopard seals (Fig. 4). Sample sizes were greatest during January – April ($n \geq 6$ seals), thus, we discuss results during these months. Results from November – December and May ($n \geq 3$ and ≤ 6 seals) are included for reference.

4.1.3.1 Non-essential trace elements (Hg, Pb, and Cd)

Leopard seals in better body condition (i.e., high SBM index) had higher Hg compared to seals in poorer body condition (i.e., low SBM index) (Table S3). The SBM index is a measure of an animal's energy reserves (Peig and Green, 2009). The lipid-rich blubber tissue is where pinnipeds store the majority of their surplus resources, (Noren and Mangel, 2004), however, previous blubber Hg studies in phocids indicate it is not a storage site for Hg (~3.5 % total Hg burden in grey seals (*Halichoerus grypus*) (Habran et al., 2013, 2012). Instead, muscle, liver, and hair are the major Hg reservoirs for phocids (Correa et al., 2014; Ikemoto et al., 2004b). In elephant seals (*Mirounga angustirostris*), females in good body condition after foraging trips exhibited greater muscle mass and Hg concentrations compared to fasting periods (i.e., low body condition) (Peterson et al., 2018). Our results suggest adult leopard seals in better body condition may have greater muscle stores and Hg concentrations compared to leopard seals in lower body conditions. Scaled body mass index explained a relatively small amount of variability of Hg in leopard seal whiskers compared to changes in their $\delta^{15}N$ and $\delta^{13}C$ values (Table S3).

Leopard seals with higher $\delta^{15}N$ values accumulated greater Hg concentrations in their whiskers than did seals with lower $\delta^{15}N$ values (Fig. 4). Considering Hg exhibits biomagnification with increasing trophic levels (Bargagli et al., 1998; Das et al., 2002; Sun et al., 2020), it was expected that Hg would accumulate over time if seals maintained high $\delta^{15}N$ values (~ 12.47 ‰, Fig. 4). Although leopard seals have demonstrated dynamic "prey switching" behavior between lower and higher trophic levels throughout a year (Botta et al., 2018; Hall-Aspland et al., 2005; Rogers et al., 2016), we provide additional evidence (Botta et al., 2018; Krause et al., 2015) that individuals may reduce their trophic niche width and feed mainly at high trophic levels over time relative to whisker growth (mean = 152 days total), resulting in higher

Hg concentrations (Fig. S4 and Fig. S11). During January – February of the austral summer, adult females finish mating activity and their annual molt, which requires energy dense prey, such as the Antarctic fur seals (*Arctocephalus gazella*) and penguins (Krause et al., 2020; Rogers, 2018). Feeding at higher trophic levels could explain the higher whisker $\delta^{15}N$ values and Hg concentrations during this time period (Fig. 4). Despite the higher $\delta^{15}N$ values of these seals, leopard seals are known to supplement their diet during the summer with lower trophic prey, mainly krill, with additional supplements of demersal fish (Hall-Aspland and Rogers, 2004; Krause et al., 2020, 2016, 2015), which could help explain the variability in Hg relative to $\delta^{15}N$ on an estimated weekly basis (Fig. 4).

Mercury concentrations during March – April generally decreased across assumed low (10.15 ‰), median (10.83 ‰), and higher (12.47 ‰) $\delta^{15}N$ values (Fig. 4), despite whisker stable isotope data implying seals were generally foraging at higher trophic levels (Fig. S6). This decline in Hg concentrations may correspond with physiological and ecological processes occurring during this period. The fall season comes after lactation in December (i.e., finished weaning pups) and molting in February (Rogers, 2018), for which adult females in Cape Shireff require large amounts of available energy dense prey (e.g., Antarctic fur seal pups) to meet these energetic activities (Krause et al., 2020). Intake of higher trophic level prey may decline during the following months (March – April), which could explain the declines in Hg concentrations across varying trophic levels (Fig. 4). The gestation period for pregnant female leopard seals begins in March – April (Atkinson, 1997; Boyd, 1991). The decreases in whisker Hg during this time could be due to transplacental transfer to developing fetuses (Castellini et al., 2012; Noël et al., 2016). Blood Hg or female reproductive status were not available, so further studies are needed to confirm these interpretations.

Variability in whisker $\delta^{13}C$ values may further explain variation in Hg during January – April (Table S3). The LMM model predicting Hg concentrations based on varying $\delta^{13}C$ values fit poorly for seals with lower $\delta^{13}C$ values compared to seals with higher and median $\delta^{13}C$ values (Fig. 4). Recent research along coastal areas in the Western Antarctic Peninsula and South Shetland Islands, indicates that trace elements are evenly distributed by local biological processes at the base of the food chain (De Castro-Fernández et al., 2021). This makes it difficult to predict trace element accumulation in whiskers based on general foraging location from $\delta^{13}C$ values, but availability of paired tracking data could help contextualize $\delta^{13}C$ and trace element relationships (Peterson et al., 2015; Walters et al., 2020). Leopard seals are generally shallow divers in coastal habitats, but expand their range as sea ice increases (Krause et al., 2015; Meade et al., 2015). The lower median $\delta^{13}C$ in leopard seal whisker values suggests offshore foraging compared to seals with higher whisker median $\delta^{13}C$ values that may be feeding closer to shore or near sea ice (Jia et al., 2016; Mincks et al., 2008).

Lead and Cd were positively correlated within individual whiskers and exhibited similar general accumulation patterns over time relative to whisker growth (Fig. 3 and Fig. 4). Positive correlations among Pb and Cd are documented in muscle of Antarctic seals, in leopard seals, and in growth layers in teeth of male and female Pacific walruses (*Odobenus rosmarus divergens*) (Clark et al., 2021; Szefer et al., 1994). Median Pb and Cd concentrations were highest during January then declined continuously until mid-March regardless of $\delta^{15}N$ and $\delta^{13}C$ values (Fig. 4). Pb and Cd are not consistently biomagnified up trophic levels in aquatic ecosystems (Cardwell et al., 2013; Dehn et al., 2006), thus, these decreases over time may not be associated with changes in trophic level even though the interaction of $\delta^{15}N$ and time was retained in the top Pb and Cd models (Table S3). Instead, seals may be switching foraging locations or encountering changing food web components during this time resulting in changing exposure to Pb and Cd (Alekseev and Abakumov, 2020; De Castro-Fernández et al., 2021; De María et al., 2021). Lead and Cd increased during mid-March – April, regardless of their diet (Fig. 4).

Most leopard seals examined in this study were adult females (n = 14) and therefore physiological processes may influence excretion or accretion pathways of Pb and Cd in whiskers over time. Molting during January - February provides a pathway to excrete toxic non-essential trace elements while newly grown hair may accumulate trace elements (Gray et al., 2008; Ikemoto et al., 2004b), instead of whiskers. Females are likely pregnant during March – April (Rogers, 2018, 2009). As Pb and Cd are non-essential trace elements, they could be transferred through the placenta to the fetus, providing another excretion mechanism for Pb and Cd. This would decrease the amount of circulating levels of Pb and Cd, thus reducing their availability for incorporation into the whiskers (Habran et al., 2013, 2012; Noël et al., 2016; Rea et al., 2013). This is consistent with the marginally lower Pb and Cd in females compared to males (Table 1). In summary, changes in $\delta^{15}N$ and $\delta^{13}C$ as well as physiological mechanisms likely explain a Pb and Cd variability and decreases during January – March (Fig. 4).

4.1.3.2 Essential trace elements (Selenium)

There were differences between Se in male and female whiskers over time (Table S3, Fig. 5). During November – December, males retained greater Se concentrations compared to females, until January – April when Se concentrations dropped into a similar range as the female Se levels (Fig. 5). Selenium is mobilized and transferred to pups during lactation in seals (Habran et al., 2013, 2012) which is thought to occur during this time (Southwell et al., 2003) and differences in feeding intakes at different trophic levels occur between adult female and male leopard seals (Krause et al., 2020). These factors, alone or in combination, may explain the lower Se in females compared to males (Fig. 5). However, this is a cautious interpretation of this significant difference between sexes due to unequal sample sizes (n = 3 males and n = 14 females).

Diet changes may explain Se variability in whiskers (Fig. 5). In female leopard seal whiskers, Se concentrations remained relatively low and stable from January until mid-February, despite changing diet during the same time period (Fig. 5 and Fig. S6). However, average Se in key prey items of different trophic levels are higher in Antarctic fur seals (~13.1 – 26.6 ppm [hair]) (Yin et al., 2007) compared to krill (~2.93 ppm wet weight [whole body]) (Mirzoeva et al., 2022). This could indicate that something other than trophic level or foraging location controls Se accumulation in whiskers during this time. Because females increase their food intake during mid-February through April, higher Se in whiskers could follow from greater consumption of higher trophic level prey with elevated Se or other physiological processes that influence Se uptake during this time (Fig. 5, Krause et al., 2020; Majer et al., 2014). Selenium was the only studied trace element that exhibited stable concentrations during portions of whisker growth, independent of diet, and then increased at different rates depending on trophic level and foraging location (Fig. 5 and Table S3).

4.1.4 Leopard seal whiskers as a biomonitoring tool of Hg risk

Most leopard seals in this study were not exposed to neurotoxic levels of Hg. The whole whisker mean of < 20 ppm Hg for most seals (n = 16 of 18; 89%), suggests they were not exposed to or did not accumulate harmful levels of Hg for pinnipeds (O'Hara and Hart, 2018). Two adult females (n = 2), however, had whole whisker Hg means of 21.05 and 22.98 ppm, which are twice the maximum Hg concentration measured in leopard seal hair during 1999 – 2001 (max hair Hg concentration = 10.04 µg/g dry weight or ppm, Gray et al., 2008). This contrast in whisker Hg levels may indicate an increase in background levels over the past 17 years or regional differences in Hg, as the seals sampled in Gray et al., (2008) were from a different area of Antarctica (Prydz Bay) compared to Cape Shirreff (Fig. 1). Additionally, while Mercury measured in paired whiskers and hair are related (Noël et al., 2016), greater Hg in whiskers from this study compared to hair in Gray et al., (2008) may in part be due to using different methods for measuring Hg in different matrices. Although Antarctica was historically considered to be a "pristine" environment (De Moreno et al., 1997), heavy metal concentrations (e.g., Hg) have been increasing in organisms throughout trophic levels over the past two decades attributed to human emissions (e.g., coal burning) (Bargagli, 2008; Cossa et al., 2011; De Castro-Fernández et al., 2021).

We provide evidence that Se is a detoxifier of Hg in leopard seals. When assessing general patterns in Se:Hg molar ratios over time from whisker segments among seals we found leopard seals exhibited median Se:Hg molar ratios > 1.0 when assigned as "low" Hg risk, but those of "moderate" Hg risk had weeks where their molar ratios fell below 1.0 (Fig. 6). This could mean "low" Hg risk seals accumulated sufficient Se to neutralize potential Hg burden, whereas "moderate" risk seals undergo Se-deficit periods where uptake is limited, and they experience potentially harmful Hg levels (Rea et al., 2020). The two "high" risk seals maintained median Se:Hg molar ratios below 1.0, indicating they were deficient in Se and at risk of potential harmful effects of Hg (Correa et al., 2014) or utilize a different Hg detoxifying mechanism, such as other metallothionein proteins (Ikemoto et al., 2004a) (Fig. 6 and Fig. S9). All seals, irrespective of Hg risk classification including "high" risk seals, had Se:Hg molar ratios that increased during mid-April through May well above 1.0 (Fig. 6, Table 2), suggesting that this may be an important time for seals to accumulate Se to help deal with detoxifying non-essential trace elements (Ikemoto et al., 2004). While liver Se:Hg molar ratios provide the best assessment on availability of protective Se in a seal, our results suggest whisker Se:Hg molar ratios are a reasonable proxy for assessing general patterns in bioavailable Se (Correa et al., 2014).

4.1.5 Considerations and conclusions

Our results show that a combination of stable isotope and trace metal analysis along the length of whiskers can provide important information on the variation of trace elements relative to foraging location and diet. While we discuss changes in $\delta^{15}N$ values in relation to trace elements as mostly seals foraging at different trophic levels, baseline differences in $\delta^{15}N$ in different foraging locations could also contribute to variability in leopard seal $\delta^{15}N$ whisker values. Although these elemental components are incorporated during growth, maximum whisker growth durations are limited by seasonal molting cycles. Average growth durations in the present study were 152 days. This means that whisker records will generally be limited to monthly and not yearly timescales. Although physiological processes (e.g., lactating, molting, gestation) may be excretion or accumulation mechanisms for trace elements, addition of reproductive status information and paired blood data is critically important to strengthen these interpretations. Additionally, elements may accumulate differently over time, or with age, depending on trace element, species, and tissue (Clark et al., 2021; Ikemoto et al., 2004b). Our sample population was largely restricted to adult females, leaving a gap in knowledge of how trace elements accumulate over time in pups, juveniles, subadults of both sexes and adult males. Although toxicity thresholds for Hg used in this study were developed for other pinniped species

(O'Hara and Hart, 2018), assessment of Hg effects on leopard seals has not been conducted. Thus, Hg of 20 ppm or above may or may not impose deleterious effects on leopard seals.

Leopard seals live in a remote habitat where they play a crucial ecological role in their Antarctic ecosystem and whiskers may provide a means for continuously monitoring trace elements in their population and the Antarctic ecosystem. Further, leopard seals are being commonly sighted in more urban areas (e.g., New Zealand) (Hupman et al., 2020), resulting in possibly greater exposure to non-essential trace elements. Analysis of trace elements with stable isotope value data in whiskers provides a method to assess exposure to heavy metals over time in seals inhabiting either Antarctic or urban environments.

CONFLICT OF INTEREST STATEMENT

The authors declare there are no conflicts of interest.

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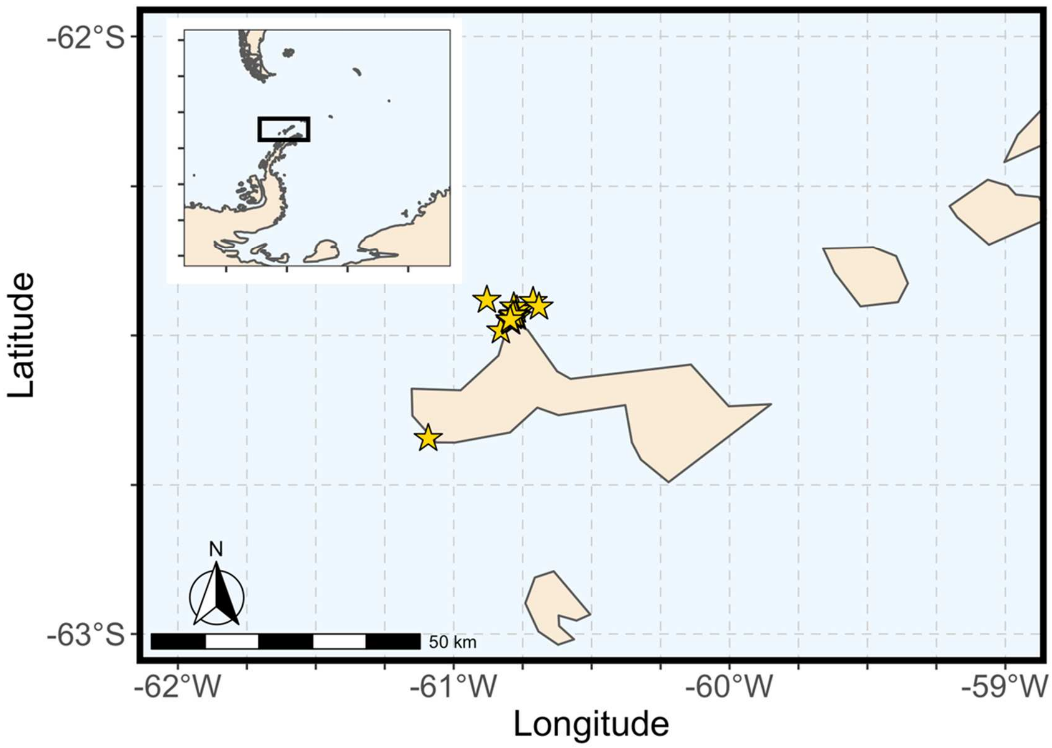

Fig. 1: Map of leopard seal whisker collection sites in Cape Shirreff, Antarctica. Inset map depicts Western Antarctic Peninsula with select area (black rectangle) showing Cape Shirreff (enlarged map) and capture locations (gold stars) of individual seals (n = 18 seals).

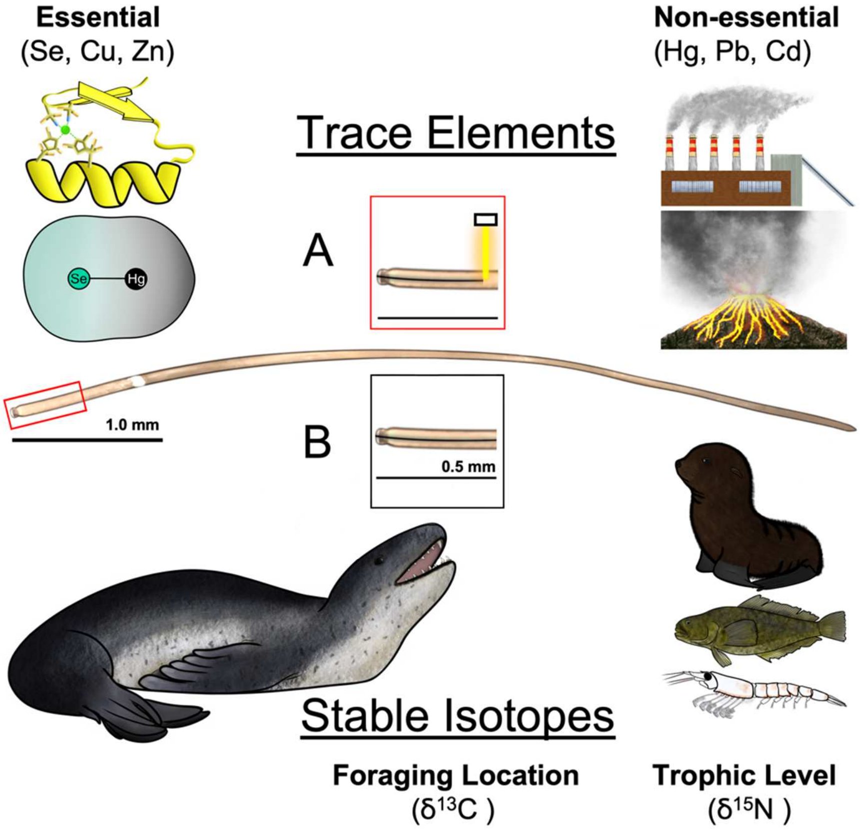

Fig. 2: Conceptual illustration of sampling design of leopard seal whiskers to obtain paired trace element and stable isotope data. Whiskers were first sampled beginning at the root (left red rectangle) for LA-ICP-MS analysis to obtain essential and non-essential trace elements of whisker sections (top zoomed in rectangle) by ablating surface of whiskers (dark line in middle of section within top red rectangle) using a laser (yellow line within top red rectangle) (A). Examples of human (coal mining) and natural (volcano) sources of non-essential and functions (e.g., Zn finger and Se:Hg bound molecule) of essential elements are provided for reference. After trace element analysis, ablated whiskers were sectioned for stable isotope analysis (B). Sections of various lengths (e.g., 0.50 mm near base) were submitted for stable isotope analysis including $\delta^{15}N$ which provides information on leopard seal trophic level (e.g., krill, benthic fish, and Antarctic fur seal pup) and $\delta^{13}C$ values giving insight into foraging location (e.g., offshore vs. inshore). Trace element data were averaged across the respective lengths of sections submitted for stable isotope analysis to acquire paired trace element and stable isotope data. All illustrations done by A. W. Kirkpatrick.

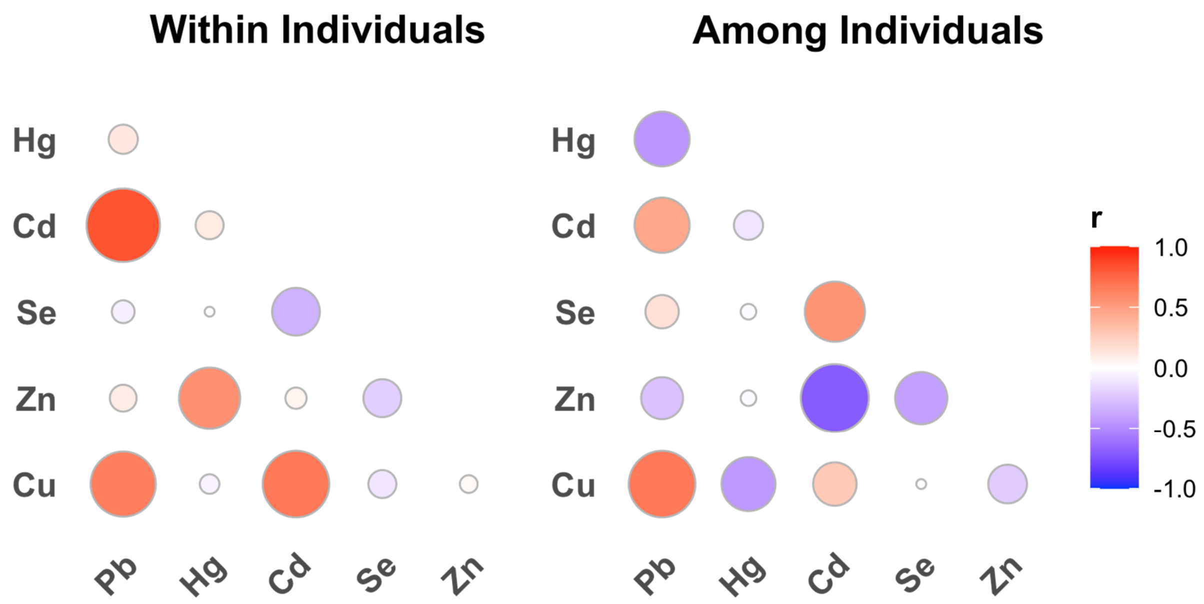

Fig. 3: Pearson's correlation plots for trace elements within and among leopard seal whiskers (n = 18 seals). Bubble size is representative of the correlation coefficient value (r) and the darkness of the color inside represents the strength of the correlation (-1.0 = dark blue, 1.0 = dark red). Mean correlation coefficients calculated from an individual's respective whisker segment trace element data and then averaged across individuals are on the left ("Within Individuals") and correlations of whole whisker trace elements among individuals is on the right ("Among Individuals"). Numeric values for Pearson correlations are found in Table S4.

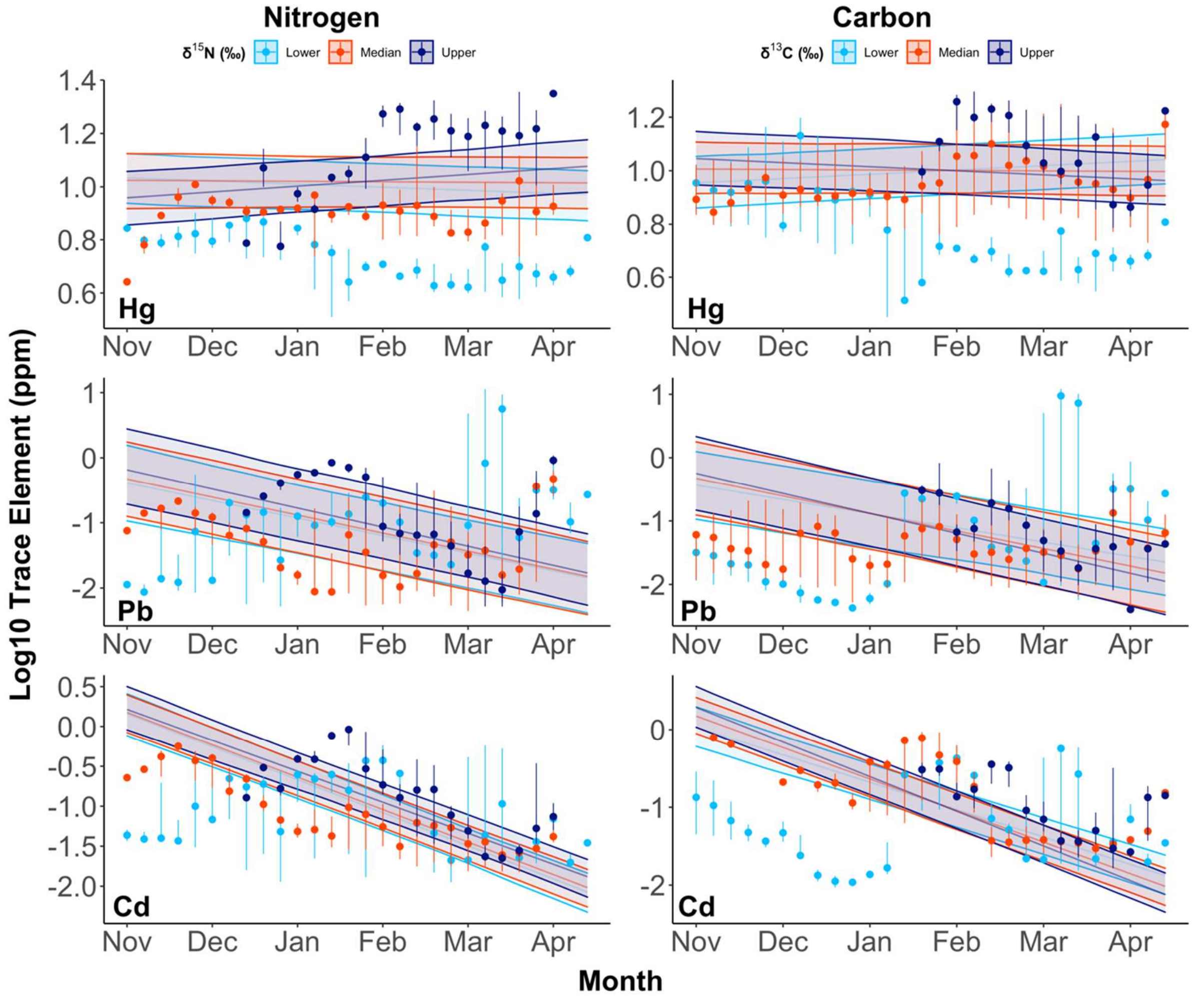

Fig. 4: Median ± interquartile range of log10 transformed non-essential (Hg [top], Pb [middle], and Cd [lower]) trace element concentrations (ppm) for select adult leopard seal whisker segments plotted by week. Seals that exhibited "lower" $\delta^{15}N$ (n = 4 seals) or $\delta^{13}C$ (n = 4 seals) values between the 0 and 1 quartiles (8.37 – 10.56 ‰ for $\delta^{15}N$ and -23.26 - -22.28 ‰ for $\delta^{13}C$, light blue circles), "median" $\delta^{15}N$ (n = 4 seals) or $\delta^{13}C$ (n = 4 seals) values between 1 and 3 quartiles (10.56 – 11.60 ‰ for $\delta^{15}N$ and -22.28 - -21.48 ‰ for $\delta^{13}C$, orange circles) and "upper" $\delta^{15}N$ (n = 4 seals) or $\delta^{13}C$ (n = 4 seals) values between 3 and 4 quartiles (11.60 – 13.14 ‰ for $\delta^{15}N$ and -21.48 - -20.74 ‰ for $\delta^{13}C$, purple circles) are plotted by week. Lines represent predictions from linear mixed models (LMMs, n = 17 seals, n = 834 segments), and shaded ribbons represent the 95% confidence intervals of fitted values. $\delta^{15}N$ for model predictions (left panel) were held constant at 10.15 ‰ for "lower", 10.83 ‰ for "median", and 12.47 ‰ for "upper." For $\delta^{13}C$ model predictions (right panel), values were held constant at -22.74 ‰ ("lower"), -21.98 ‰ ("median"), and -21.41 ‰ ("upper"). For Hg, SBM index was held constant at 404 kg (median value of dataset).

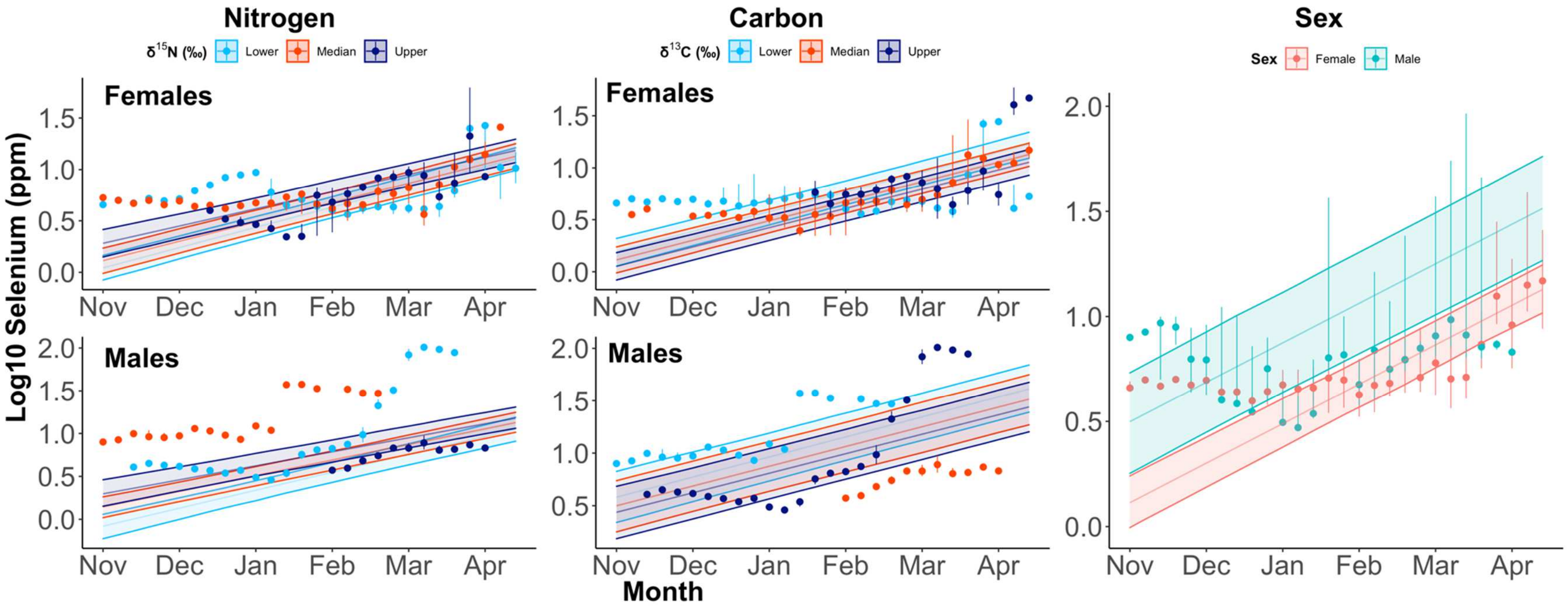

Fig. 5: Predictive modeling results of the top candidate linear mixed model for Se. Median ± interquartile range of log10 transformed Se concentrations (ppm) for adult male (n = 3) and female (n = 14) leopard seal whisker segments plotted by week ("Sex", far right panel). Median ± interquartile range of log10 transformed Se concentrations for adult male and female leopard seal whisker segments based on $\delta^{15}N$ ("Nitrogen", far left panels) and $\delta^{13}C$ ("Carbon", middle panels) values by week. Seals that exhibited "lower" $\delta^{15}N$ (n = 4 females and n = 1 male) or $\delta^{13}C$ (n = 4 females and n = 1 male) values between the 0 and 1 quartiles (8.37 – 10.56 ‰ for $\delta^{15}N$ and -23.26 - -22.28 ‰ for $\delta^{13}C$, light blue circles), "median" $\delta^{15}N$ (n = 4 females and n = 1 male) or $\delta^{13}C$ (n = 4 females and n = 1 male) values between 1 and 3 quartiles (10.56 – 11.60 ‰ for $\delta^{15}N$ and -22.28 - -21.48 ‰ for $\delta^{13}C$, orange circles) and "upper" $\delta^{15}N$ (n = 4 females and n = 1 male) or $\delta^{13}C$ (n = 4 females and n = 1 male) values between 3 and 4 quartiles (11.60 – 13.14 ‰ for $\delta^{15}N$ and -21.48 - -20.74 ‰ for $\delta^{13}C$, purple circles) are plotted by week. Since low sample sizes for males in stable isotope categories (n = 1 per category), only weekly datum points are plotted. Lines represent predictions from linear mixed models (LMMs, n = 17 seals, n = 834 segments), and shaded ribbons represent the 95% confidence intervals of fitted values. $\delta^{15}N$ for model predictions (left panel) were held constant at 10.15 ‰ for "lower", 10.83 ‰ for "median", and 12.47 ‰ for "upper." For $\delta^{13}C$ model predictions (right panel), values were held constant at -22.74 ‰ ("lower"), -21.98 ‰ ("median"), and -21.41 ‰ ("upper").

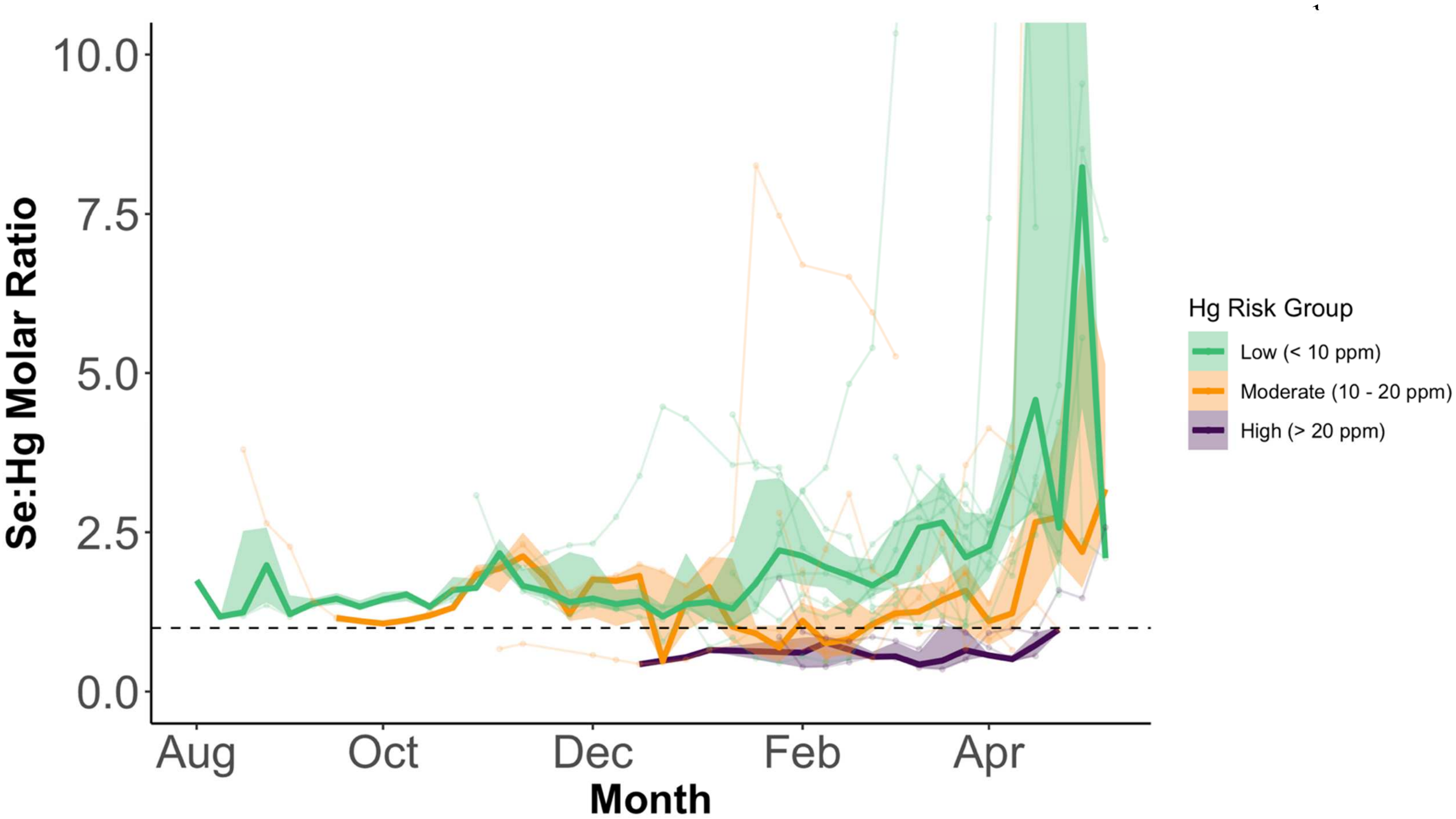

Fig. 6: Se: Hg molar ratios plotted by week for all leopard seals grouped by Hg risk. Thick lines and shaded areas represent overall weekly median ± interquartile range Se: Hg molar ratios, respectively, from all whisker segments classified as either "low" (green, n = 574), "moderate" (orange, n = 311), or "high" (purple, n = 96) Hg risk. More transparent lines with points represent individual seals' (n = 18 total) weekly median Se: Hg molar ratios colored by their Hg risk based on their whole whisker Hg concentrations (n = 11 low, n = 5 moderate, and n = 2 high). Se: Hg molar ratio equal to one is plotted for reference (dashed black line). Note: Se: Hg molar ratio limits 0 – 10 to capture overall trends. Some ratios went well above 10 during April – May, thus, the breaks at Se: Hg molar ratios = 10.

Table 1: Summary of whole whisker trace element and foraging ecology data from leopard seals. All trace element data are in ppm, carbon and nitrogen isotope ratios have units per mil (‰), and Se: Hg molar ratios and standard ellipse area corrected for small sample size (SEAc) are unitless. Whole whisker values are averages of respective data across the entire whisker for each individual and then summary data calculated for females (n = 15) and data from all males (n = 3) given due to low sample size.

Females (n = 15)	**Mercury (Hg)**	**Lead (Pb)**	**Cadmium (Cd)**	**Selenium (Se)**	**Copper (Cu)**	**Zinc (Zn)**	**Se:Hg Molar Ratios**	**Carbon ($\delta^{13}C$)**	**Nitrogen ($\delta^{15}N$)**	**SEAc**
Median	9.05	0.07	0.13	7.17	14.85	195.04	2.04	-22.02	11.27	1.02
SD	5.83	1.45	0.09	2.66	6.45	37.45	1.30	0.71	0.99	0.85
Min	4.32	0.02	0.05	4.08	12.35	116.28	0.71	-23.26	10.07	0.29
Max	22.98	5.71	0.35	13.84	39.25	250.23	4.91	-20.74	13.14	3.28
Males (n = 3)										
Male 1	11.54	0.09	0.24	13.21	13.97	152.09	2.97	-22.74	10.59	1.64
Male 2	6.72	1.54	0.10	6.27	20.12	263.47	2.46	-21.98	10.70	0.83
Male 3	6.48	0.26	0.16	19.36	15.80	162.00	8.09	-21.51	8.37	2.74

Table 2: Summary of monthly trace element and stable isotope ratios from all leopard seal whiskers. All trace element data are in ppm, carbon and nitrogen isotope ratios have units per mil (‰), and Se: Hg molar ratios are unitless. Monthly summary data are based on timestamps applied to each individual seals' (N) whisker segments (n).

Month	Sample Size (Unique Seals, Segments)	Mercury (Hg)	Lead (Pb)	Cadmium (Cd)	Selenium (Se)	Copper (Cu)	Zinc (Zn)	Se:Hg Molar Ratios	Carbon ($\delta^{13}C$)	Nitrogen ($\delta^{15}N$)
August	**(N = 2, n = 7)**									
	Median	7.15	0.20	0.52	3.58	21.69	179.52	1.26	-23.90	10.06
	SD	1.39	0.10	0.22	1.29	6.05	60.07	1.02	0.83	1.10
	Min	4.44	0.06	0.14	3.38	14.39	41.37	1.13	-24.76	8.39
	Max	8.04	0.31	0.65	6.63	33.06	204.09	3.79	-22.50	11.89
September	**(N = 2, n = 24)**									
	Median	7.24	0.07	0.19	4.10	15.00	185.10	1.34	-23.23	10.56
	SD	2.24	0.10	0.26	0.84	4.48	34.52	0.34	0.55	1.05
	Min	5.86	0.02	0.09	3.15	13.00	75.06	1.03	-24.26	8.02
	Max	13.65	0.29	0.79	5.79	27.37	201.90	2.51	-21.97	11.23
October	**(N = 3, n = 33)**									
	Median	8.15	0.05	0.10	4.92	14.07	197.02	1.39	-22.91	10.46
	SD	2.24	0.08	0.24	1.21	7.04	24.67	0.81	0.63	0.66
	Min	3.55	0.01	0.04	3.25	8.80	103.53	1.01	-23.74	9.35
	Max	12.23	0.30	0.90	8.14	52.52	220.93	5.83	-20.87	11.90
November	**(N = 5, n = 50)**									
	Median	8.10	0.05	0.16	4.99	12.96	169.61	1.82	-22.21	11.05
	SD	2.86	0.16	0.23	2.39	6.22	38.56	0.56	0.56	0.85

Table 2 cont:

	Min	4.02	0.01	0.03	2.73	8.16	57.47	0.61	-23.43	9.24
	Max	14.77	0.64	0.85	12.96	40.19	251.86	3.08	-21.30	11.89
December	**(N = 6, n = 66)**									
	Median	8.16	0.08	0.17	4.35	14.15	184.80	1.38	-22.71	9.43
	SD	4.22	0.12	0.12	2.43	3.20	58.53	0.90	1.10	1.55
	Min	4.58	0.00	0.01	2.68	8.92	38.56	0.43	-23.99	7.34
	Max	21.38	0.74	0.51	11.49	25.78	281.56	4.72	-20.68	13.10
January	**(N = 13, n = 91)**									
	Median	8.17	0.07	0.09	4.59	15.29	167.08	1.41	-22.01	11.04
	SD	4.78	0.25	0.24	5.86	6.13	57.38	1.50	0.93	1.79
	Min	2.27	0.00	0.01	1.82	8.40	37.30	0.39	-23.95	6.95
	Max	23.17	0.89	1.03	38.29	36.50	288.01	8.26	-20.24	14.13
February	**(N = 17, n = 197)**									
	Median	9.92	0.06	0.15	4.80	15.18	201.39	1.33	-22.14	11.29
	SD	6.39	0.55	0.23	5.28	5.21	56.11	1.29	0.89	1.59
	Min	3.43	0.00	0.01	1.88	5.89	52.26	0.27	-24.43	7.15
	Max	30.34	6.16	1.23	36.72	45.13	365.23	7.89	-20.42	13.87
March	**(N = 18, n = 293)**									
	Median	9.69	0.03	0.04	5.92	12.74	213.41	1.59	-21.98	11.42
	SD	6.88	2.13	0.13	10.52	11.53	59.07	5.83	0.89	1.57
	Min	2.55	0.00	0.00	2.15	4.56	40.35	0.24	-24.30	6.96
	Max	34.24	27.63	0.76	115.17	92.22	412.86	80.46	-19.83	14.05

	(N = 18, n = 205)									
April	Median	8.09	0.03	0.03	7.29	13.77	204.53	2.48	-21.59	11.58
	SD	6.07	2.28	0.15	18.34	12.28	59.94	6.36	0.79	1.04
	Min	3.08	0.00	0.00	2.36	4.85	23.48	0.39	-23.82	8.00
	Max	33.30	15.40	1.13	100.72	141.93	432.71	35.13	-20.56	13.48
	(N = 4, n = 15)									
May	Median	12.12	0.05	0.14	21.13	16.42	221.37	3.18	-21.76	11.92
	SD	5.07	0.47	0.08	34.82	7.20	72.92	17.22	0.50	0.55
	Min	4.36	0.01	0.02	3.99	9.29	63.85	1.41	-22.96	10.96
	Max	18.77	1.57	0.25	137.09	36.64	338.38	68.69	-21.11	13.11

GRAPHICAL ABSTRACT