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**Running head:**

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# Past and present mercury accumulation in the Lake Baikal seal: Temporal trends, effects of life history, and toxicological implications

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## Abstract

Despite global efforts to reduce anthropogenic mercury (Hg) emissions, the timescale and degree to which Hg concentrations in the environment and biota respond to decreased emissions remains challenging to assess or predict. Here we characterize long-term trends, life-history patterns in Hg accumulation and toxicological implications of Hg contamination for a freshwater seal from one of the world's largest lakes (Lake Baikal, Siberia) using contemporary tissues and archival teeth. Stable isotope analysis and Hg analyses of soft tissues (muscle, liver, kidney, blood, brain, heart) and teeth from 22 contemporary seals revealed rapid changes in diet and Hg accumulation in the first year of life with a stable diet and increase in tissue Hg throughout the rest of life. Although maternal transfer of Hg was an important source of Hg to seal pups, reproduction and lactation by female seals did not appear to result in sex-related differences in Hg concentrations or age-related accumulation in adult seals. Based on Hg analysis of archival teeth (n=114), and reconstructed values for soft tissues, we also assessed temporal trends in seal Hg between the years 1960 and 2013. Seal Hg concentrations in hard (teeth) and soft tissues (e.g., muscle, liver), were highest in the 1960s and 1970s, followed by a decrease. The decline in seal Hg concentrations in recent decades was most likely driven by a reduction in Hg inputs to the lake, suggesting that global and regional efforts to reduce Hg emissions have been successful at reducing ecosystem and human health risks posed by Hg in Lake Baikal.

**Keywords**

Mercury. Lake Baikal. Seals. Life-history. Bioaccumulation. Temporal trends.

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# INTRODUCTION

Human activities have led to the increased release of Hg to the environment, resulting in widespread contamination of ecosystems both near local and regional sources of Hg emissions, as well as in remote regions (Jackson 1997). In the environment, Hg is subject to complex biogeochemical cycling, including transformation to methyl mercury (MeHg), which is a highly bioaccumulative and potent neurotoxin (Bloom 1992). In the aquatic environment, Hg poses health risks to wildlife as well as humans who rely on aquatic resources, with consumption of fish and other aquatic organisms representing the dominant source of human exposure to MeHg (Harris et al. 2003; Li et al. 2014). For mammals, MeHg exposure can lead to adverse health effects, including neurological, reproductive, endocrine and immune effects (Wolfe et al. 1998; Mergler et al. 2007; Dietz et al. 2013). Global efforts in recent decades to reduce anthropogenic Hg emissions and associated risks to ecosystems and human health have led to reductions in the release of Hg to the environment, particularly in Europe and North America (Zhang et al. 2016). Meanwhile, the Minamata Convention, a multilateral legally-binding treaty formally adopted in 2013 and signed by 128 countries, takes a comprehensive approach to controlling and reducing global anthropogenic Hg releases (Kessler et al. 2013; Gustin et al. 2016). However, there is still a great deal of uncertainty regarding the degree to which (and the speed at which) environmental Hg concentrations and associated ecosystem risk respond to emissions reductions (Mason et al. 2005; Gustin et al. 2016).

Lake Baikal, one of the world's largest lakes, is home to a large number of endemic species, including the Baikal seal (*Pusa sibirica*). These are the world's only true freshwater seals, and in recent decades they have faced an increasing number of threats, including climate change (Moore et al. 2009), a virus-related mass mortality event (in 1987/88; Mamaev et al.

1996), and contamination with persistent organic pollutants (POPs) and heavy metals. In fact, one of the key hypotheses regarding the significant mortality (~8000 seals) in 1987/88 was that the seals may have been immuno-compromised due to exposure to environmental contaminants (Tsydenova et al. 2004; Ishibashi et al. 2008). The Baikal seal is particularly vulnerable to inputs of bioaccumulative contaminants, including Hg, due to its long life-span (> 50 years) and position at the top of the food web. The seals are also an important traditional food source for indigenous communities in the Lake Baikal region (Nomokonova et al. 2013; 2015), and as such, elevated contaminant concentrations in these seals may pose a human health risk.

Major sources of contaminants to Lake Baikal include regional anthropogenic activity, and long-range atmospheric transport (Mamontov et al. 2000; Ok et al. 2013; Ozersky et al. 2017). POPs have been of particular concern in Lake Baikal, where several studies have revealed elevated concentrations of organochlorine contaminants in seal tissues, including PCBs and DDTs (Kucklick et al. 1994; Kucklick et al. 1996; Tsydenova et al. 2004) as well as dioxins and furans (Tarasova et al. 1997). Hg concentrations in seal tissue from Lake Baikal were also reported for seals collected in 1992 and 2001 (Watanabe et al. 1996; Ciesielski et al. 2010; Perrot et al. 2012), revealing concentrations that are typically lower than those observed in other more contaminated areas and in Arctic coastal populations (Ciesielski et al. 2010), but that still often exceeded the WHO guideline level for safe consumption (0.5 $\mu$g/g wet weight; FAO/WHO 2011).

Although temporal trends of POP concentrations in the Baikal seals have been reported (Tsydenova et al. 2004; Ishibashi et al. 2008; Isobe et al. 2009), there is insufficient temporal resolution in the available Hg data for such a temporal analysis. Also, Hg emissions in Asia are rising while point-source releases of Hg in North America and Europe have decreased due to emission regulations (Sundseth et al. 2017). Furthermore, climate change has the potential to increase mobilization and release of previously deposited contaminants to aquatic ecosystems. In particular, thawing of permafrost in the Lake Baikal catchment may represent an important future source of remobilized Hg (Leitch et al. 2007; Moore et al. 2009). To assess current drivers and the potential for future Hg contamination of Lake Baikal, as well as future risks to ecosystem and human health, there is a need for information on the historical temporal trends of Hg concentrations in the lake and its biota.

Previous studies have used mammalian teeth as a bioindicator of metal (including Hg) exposure and to assess temporal trends in Hg in several mammalian species (e.g. polar bears, humans, belugas, ringed seals; Dietz et al. 2009; Aubail et al. 2012). Metals are incorporated during mineralization of tooth tissues, primarily reflecting metal concentrations in blood at the time of incorporation, and with limited remobilization once deposited (Dietz et al. 2009). These properties, together with the long-term stability of stored archival hard tissues, make teeth a good matrix for reconstruction of past Hg exposure and contamination of mammals such as seals. This study builds on our previous work, where laser ablation-ICP-MS (LA-ICP-MS) was used to determine levels of several metals (V, Cu, Zn, Cd, Hg, Tl, Pb, U) in Lake Baikal seal tooth tissues representing the first years of life (Ozersky et al. 2017).

Here, we carry out a detailed analysis of temporal trends, life-history effects and toxicological implications of past and present Hg accumulation in the Lake Baikal seal. We used seal teeth (n=114) collected over more than 50 years (1960–2013) to reconstruct long-term trends in Hg exposure and accumulation in the Baikal seal, and have paired these data with analyses of contemporary tissue samples to gain insight into life-history related patterns in Hg accumulation, and the relationship between tooth and soft tissue Hg concentrations.

The main objectives of this study were to: 1) describe age and sex-specific patterns in diet and Hg contamination of Baikal seals; 2) characterize past Hg accumulation in Baikal seals using archival teeth; and 3) reconstruct past Hg concentrations in soft tissues, and 4) evaluate current and historical toxicological risks for seals, and their human consumers.

## MATERIALS AND METHODS

### *Contemporary and historical samples*

In spring, 2013, 22 seals were collected from southern and central Lake Baikal by M. Pastukhov (scientific-collection permit issued by the Russian Federal Fisheries Agency; permit number: 032013031058). Subsamples of soft tissues (muscle, liver, kidney, heart, brain, blood) were collected from each seal in the field and frozen (-20ºC) for stable isotope and Hg analysis. Upper canine teeth were also collected from all seals. For reconstruction of historical Hg concentrations, we used a collection of seal skulls collected between 1960 and 1989 (histogram showing distribution of seal collection years in Figure S1). These skulls were predominantly collected in central Lake Baikal by Russian researchers working in cooperation with commercial seal hunters. We removed upper canine teeth from 114 skulls of seals ranging in age from juvenile (<1 year old) to 33 years, including both males (n=44) and females (n=70). Two teeth were collected for each seal, one for whole tooth Hg analysis (this study), and one for laser ablation ICP-MS analysis and carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope analysis of individual dentine layers (for a companion study: Ozersky et al. 2017). For details on cleaning of seal teeth and determination of seal age, see Ozersky at al. (2017). Although specific locations for seal collection are not available, there is evidence that Lake Baikal seals tend to move extensively throughout the lake (Stewart et al. 1996), supporting the use of seal Hg concentrations as an indicator for lake-wide Hg contamination.

*Stable isotope and Hg analysis of soft tissues*

Soft tissue samples from contemporary seals were lyophilized and homogenized prior to stable isotope and Hg analysis. Moisture content was determined by weighing tissue samples before and after lyophilisation. Stable carbon and nitrogen isotope analysis was performed at the University of California Davis Stable Isotope Facility using an EA-IRMS. Batch-specific standard deviations for standard reference materials (SRMs) were 0.03 ‰ for $\delta^{13}$C and 0.1 ‰ for $\delta^{15}$N of bovine liver, and 0.08 ‰ for $\delta^{13}$C and 0.07 ‰ for $\delta^{15}$N of USGS-41 glutamic acid.

Determination of total Hg (TotHg) in soft tissues was conducted at the Norwegian Institute for Water Research (NIVA) by thermal decomposition and direct cold-vapour atomic absorption spectrometry (CV-AAS) using a Lumex Hg analyser (RA915+) with a PYRO-915 attachment (Lumex Ltd., St. Petersburg, Russia; Braaten et al. 2014a). Soft tissue MeHg analysis was also conducted at NIVA, using GC-CVAFS (Braaten et al. 2014b). SRMs were included in all sample runs (DORM-3 and DORM-4 for TotHg; and TORT-2 for MeHg), and at least one in every 10 samples was run in duplicate. SRM recoveries for TotHg (89–103 % for DORM-3 (n=13) and 96–101 % for DORM-4 (n=3)) and MeHg (93–113 % for TORT-2 (n=6) and MeHg sample matrix spikes (89–102 %; n=7) were within expected ranges, and the relative percent difference between duplicate samples ranged from 0–13 % for TotHg (n=17) and 0.6–7.7 % for MeHg (n=9). Batch-specific quality assurance/quality control information for sample runs (n=2 runs for TotHg and n=3 runs for MeHg) are included in the supporting information.

*Hg analysis of seal teeth*

TotHg concentrations in contemporary and archival tooth samples were determined through $HNO_3$ digestion (65% $HNO_3$ at 90 ºC for 1.5 hours), followed by oxidation, purge and trap and CVAFS (based on USEPA Method 1631). Tooth samples were fully dissolved after acid digestion. SRMs used included bone ash (NIST-1400), TORT-2, and a custom SRM prepared (as in Aubail et al. 2010) to mimic the sample matrix. The custom SRM was needed because there were no appropriate SRMs available, with both certified values for TotHg and a matrix that was similar to our tooth samples. Our custom SRM was a mix of NIST-1400 (which is prepared at high temperatures and therefore contains very little mercury) and TORT-2 with a TotHg concentration of approximately 10 ng/g dry weight. TotHg recovery for SRMs ranged from 94–103 % for TORT-2 (n=6) and 91–100 % (n=6) for the custom SRM. TotHg concentrations in bone ash (NIST-1400) ranged from 0.66–0.82 ng/g (n=6).

*Calculations and statistical analyses*

We used analysis of variance to test for significant differences in $\delta^{13}C$, $\delta^{15}N$, TotHg, MeHg and % MeHg across tissue types, and linear regression to assess relationships between seal age-at-collection and Hg concentrations in teeth and soft tissues. We also carried out analysis of covariance to test for effects of seal sex on the age-Hg, and soft tissue-tooth Hg relationships for the various seal tissues analyzed. Shapiro-Wilk's test were used to test for normality prior to analysis of variance, linear regression and analysis of covariance, and data were log-transformed where not normal (this was the case for the Hg concentration data). Statistical analyses were carried out using the stats package in R (R Core Team 2015).

We used generalized additive modelling (GAM; Zuur et al. 2009) to identify temporal trends in seal teeth (including both archival and contemporary teeth). Two GAMs were constructed using the mgcv package (Wood 2011) in R; one with tooth Hg concentrations as the response variable, and one with tooth Hg content (i.e. pg Hg in whole teeth) as the response variable. For both models, collection year, seal age and sex were tested as potential covariates.

## RESULTS AND DISCUSSION

### *Rapid dietary shifts in early life revealed by stable isotope analysis*

Lake Baikal seals can grow up to 1.8 m, weighing up to 130 kg (Nomokonova et al. 2015). The seals are born in early spring in ice dens, wean at approximately 2.5 months, become sexually mature at 2–5 years (females) or 5–8 years (males), and are long-lived (> 50 years) (Nomokonova et al. 2013, 2015). The primary food source for the seals is pelagic sculpins, with seal diet dominated by two species of the endemic golomyanka (*Comephorus dybowskii* and *Comephorus baicalensis*), and some evidence of feeding on pelagic amphipods (*Macrohectopus branickii*; Pastukhov 1993; Yoshii et al. 1999).

Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic values are widely used as indicators of diet, with $\delta^{13}C$ values used as an indicator of dietary carbon source, and $\delta^{15}N$ values acting as an indicator of trophic level. $\delta^{13}C$ and $\delta^{15}N$ values can also be influenced by a wide range of other biological factors including: lipid content (lipids tend to be depleted in $^{13}C$; Tieszen et al. 1983), starvation (leading to increased $\delta^{15}N$ values as $^{14}N$ is preferentially metabolized), and dietary nitrogen content (lower trophic enrichment of $^{15}N$ observed with higher nitrogen food sources; Adams et al. 2000).

$\delta^{13}C$ values (Table S1) were significantly lower in brain than in any other tissue, and significantly higher (ANOVA; F-value=27.98, df=6, P<0.0001) in blood than for all tissues except for muscle. These differences between tissues were likely attributable to differences in lipid content (since lipids are typically depleted in $^{13}C$; Post et al. 2007), with the lipid-rich brain tissue having the lowest $\delta^{13}C$ values and low-lipid blood tissue having the highest values. These differences in lipid content are also demonstrated by the C:N ratios for these tissues (higher C:N ratios typically indicate higher lipid content (Post et al. 2007)), with the highest C:N ratios observed for brain tissue, and the lowest for blood tissue (Figure S2). For all soft tissues, $\delta^{13}C$ values tended to increase with age and stabilize in adulthood (Figure 1), likely due to higher lipid content (and therefore lower $\delta^{13}C$) in the lipid-rich milk diet of seal pups relative to juveniles and adults.

$\delta^{15}N$ values (Table S1) were significantly lower (ANOVA; F-value=6.47, df=6, $P<0.0001$) in blood than for any of the other tissues sampled. These data are consistent with previous results for tissue-specific isotopic fractionation in seals, where captive seals (including ringed seals) fed a constant diet of herring (*Clupea harengus*) over a two year period experienced lower mean $^{15}N$ enrichment for blood tissue (1.7‰) than for muscle, liver, heart or kidney (between 2.4 and 3.1‰; Hobson et al. 1996). $\delta^{15}N$ values exhibited a strong decline in the first year, followed by relatively stable values in adult seals (Figure 1). The strong decrease in $\delta^{15}N$ in the seals' first year of life is likely due to a decrease in the effective trophic level of the juvenile seals as they wean and begin feeding independently (after approximately 2-3 months), as mammalian fetuses and breastfeeding juveniles are typically enriched in $^{15}N$ relative to their mothers (Habran et al. 2010; Borrell et al. 2016). These results are remarkably consistent with observations for seal muscle tissue collected from Lake Baikal between 1992-1994, where seals >1 year had $\delta^{15}N$ values of ~14‰ that stayed relatively constant with age, and those under 1 year of age had $\delta^{15}N$ values 1–2‰ higher than for older seals (Yoshii et al. 1999). The stability of $\delta^{15}N$

values after age 1 is consistent with previous studies that have documented the reliance of Baikal seals on endemic pelagic sculpins as a primary food source throughout adulthood.

The similarity in $\delta^{15}N$ values between the current study and samples collected 20 years earlier (Yoshii et al. 1999), and the lack of long-term directional changes observed for stable carbon and nitrogen isotope values for Baikal seal tooth dentine in a companion study to the current work (Ozersky et al. 2017)) highlight the temporal stability of the Lake Baikal food web. This implies that long-term changes in seal diet and food web structure are unlikely to be underlying drivers of temporal changes in seal Hg accumulation, suggesting that changes in seal tooth Hg concentrations may reflect long-term trends in contamination of the Lake Baikal food web.

### *Importance of maternal transfer and age for Hg accumulation and tissue distribution*

For TotHg and MeHg, concentrations in the liver and kidney were highest, while concentrations in the blood and brain were lowest (juvenile and adult seals pooled; TotHg ANOVA: F-value=3.47, df=6, $P<0.01$; MeHg ANOVA: F-value=7.57, df=5, $P<0.0001$; Figure 2, Table S2). The kidneys had the lowest proportion of Hg present as MeHg (% MeHg; 11 ± 5%), followed by the liver (30 ± 16%), while for the remaining tissues, mean % MeHg ranged from 77–88 %. These results are consistent with what has been reported for seals and other mammals, where Hg is known to selectively accumulate in liver, kidney and muscle, with higher proportions of inorganic Hg present in the kidney and liver than in other tissues, including muscle (Wagemann et al. 1998; Dietz et al. 2013).

For all tissues with the exception of blood and heart tissue, there were significant positive relationships ($P<0.05$) between seal age and both MeHg and TotHg (Figure 1, Table S3). This reflects age-related accumulation of Hg in seals (Dietz et al. 1996; Wagemann et al. 1998), particularly for tissues that have slower turnover times (e.g. muscle) and that are known to preferentially accumulate Hg (e.g. muscle and liver). The lack of a relationship between TotHg and MeHg concentrations and age for blood is likely due to blood Hg concentrations reflecting Hg content in the seal's recent diet, which would be expected to be relatively constant and independent of age, given that seals do not appear to undergo substantial dietary shifts during their adult lives (as observed in this study, by Ozersky et al. 2017, and by Yoshii et al. 1999). Furthermore, the prey that dominates the seals' diet (two species of *Comephorus* (pelagic sculpins), and, to a lesser degree, pelagic amphipods) have Hg concentrations that do not differ substantially (Ciesielski et al. 2016). Given the potential for remobilization and loss of Hg during reproduction and lactation, we expected that adult female seals may have reduced Hg compared to male seals who lack these Hg excretion pathways, however, based on analysis of covariance, we did not find a significant effect of seal sex on the Hg~Age relationship for any of the tissues sampled.

Although Hg concentrations tended to increase with age, our results also highlight the importance of maternal transfer of Hg for Lake Baikal seals. The importance of maternal transfer is particularly apparent when comparing Hg concentrations in seals that have not yet weaned (<3 months) with those of adult seals (Table S2). Mean MeHg concentrations in juveniles were between 33–43 % of adult concentrations for all tissues sampled, suggesting that Hg accumulation *in utero* and during milk-feeding accounts for substantial Hg accumulation. Similar proportions were observed for TotHg in blood, brain, heart and muscle (juvenile concentrations ranging from 29–36% of adult concentrations), with very high proportions for kidney (80%), but only 8%for liver. These results demonstrate that for most tissues, maternal transfer represents an important source of Hg, even when compared to long-term accumulation, and also highlight important tissue-specific differences in maternal transfer and long-term accumulation of Hg for kidney and liver tissue. Previous work on grey seals (*Halichoerus grypus*) has shown particularly high levels of Hg in fetal liver and kidney tissues, indicating significant *in utero* maternal transfer to these tissues (Teigen et al. 1999). This is consistent with elevated TotHg concentrations observed in kidney and liver tissues from Lake Baikal juveniles, and in particular, the very high ratio of TotHg concentrations in juvenile vs. adult kidney tissues. For liver, the tendency of Hg to accumulate preferentially in liver tissue leads to substantial long-term Hg accumulation as seals age, likely "overpowering" the maternal transfer signal.

### *Seal teeth as indicators of life-history and Hg exposure*

Tooth formation in the Lake Baikal seal begins *in utero* and, as the seals age, dentine is laid down until the root cavity is completely occluded (typically when seals reach 20–25 years of age). Hg concentrations in dentine have been shown to reflect blood Hg concentrations at the time of formation (Goodman et al. 2011), which are not expected to change substantially throughout adulthood, since blood Hg concentrations typically reflect recent dietary Hg exposure.

Hg concentrations in contemporary seal teeth ranged from 0.69–4.24 ng/g dw, and were lower than in the soft tissues sampled (Table S2), likely due to the tendency for Hg to selectively accumulate in sulphur rich tissues through binding to thiol groups (Leaner and Mason 2004). Tooth Hg concentrations were highest in juvenile seals (2.28 ± 0.88 ng/g; Table S2, Figure S3), once again highlighting the importance of maternal Hg transfer for seals. After a decline in tooth Hg concentrations between birth and age 1, concentrations were relatively stable throughout adulthood (1.12 ± 0.25 ng/g).

Our observed Hg concentrations in contemporary teeth from Lake Baikal seals were lower than those previously reported for ringed seals collected between 1982 and 2006 from Greenland (2.95 ± 1.99 ng/g dw for Central Western Greenland, and 5.75 ± 6.2 ng/g dw for Central Eastern Greenland; Aubail et al. 2010) as well as seals collected in 2001–2003 from Amundsen Gulf in the Canadian Arctic (with concentrations rising from 4.4 ± 1.6 ng/g dw for 5 year old seals to 8.6 ± 3.7 ng/g dw for 25 year old seals; Outridge et al. 2009). These results are consistent with the fact that Hg concentrations in Lake Baikal seal tissues are typically lower than those observed in other more contaminated areas and in Arctic coastal populations (Ciesielski et al. 2010, this study). As in the current study, and in contrast to the results from Amundsen Gulf (Outridge et al. 2009), Aubail et al. (2010) observed higher Hg concentrations in juvenile seal teeth than in adult teeth. This likely reflects the fact that differences in exposure histories are likely to lead to regional or site-specific differences in tooth Hg-age relationships.

Whole tooth Hg content (i.e. pg Hg present in the tooth as a whole) was highly variable in juveniles (potentially reflecting variability in maternal Hg burdens, and therefore maternal transfer) and tended to increase with age (Figure S3), with a significant positive relationship observed between whole tooth Hg content and seal age ($r^2$=0.56; P<0.0001). The high variability in Hg concentrations and content for juvenile seals could also in part reflect individual-level variability in growth-related factors and tooth formation.

Although other studies have made use of seal teeth to reconstruct past Hg exposure (e.g. Outridge et al. 2009, Aubail et al. 2010), previous studies have not included any direct comparison of Hg in teeth and soft tissues. However, Hg concentrations in beluga tooth cementum have been found to be highly correlated ($r^2$ values from 0.46–0.61) with Hg concentrations in liver, muscle, kidney and blubber (Outridge et al. 2000). Several other studies have also found positive correlations between Hg in teeth and soft tissues as well as Hg in teeth and dietary Hg exposure in other mammals such as rats (e.g. Eide and Wesenberg 1993; Eide et al. 1995). For the contemporary seals in the current study, we found significant positive relationships between whole tooth Hg content (pg Hg in the whole tooth) and both MeHg and TotHg in all soft tissues except for heart, suggesting that Hg analysis of archival teeth can be used to make estimates of past Hg concentrations for soft tissues in the Baikal seal (Table 1, Table S4). For all tissues, when only adults were considered, relationships between tooth Hg content and soft tissue TotHg concentrations became stronger (Figure 3). To explore whether we could develop stronger predictive relationships between tooth Hg content and soft tissue Hg content, we also attempted to incorporate effects of seal age through multiple linear regression; however, the significant correlation between age and tooth Hg content ($r = 0.76$, $P$=0.00004) violated the assumption of independence, indicating that age should not be included in such a predictive model.

We also tested for potential sex-related effects on the relationships between tooth and tissue Hg through analysis of covariance and found no significant effects. This differs from our expectations, where we anticipated a divergence between soft tissue and tooth Hg for female seals (with lower soft tissue concentrations than males with similar tooth Hg content), since they may mobilize and release contaminants during parturition and lactation, potentially leading to lower contaminant concentrations in tissues that are more metabolically active (unlike teeth). However, it should be noted that Watanabe et al. (1996) and Ciesielski et al. (2006) both found higher Hg concentrations in muscle, liver and kidney tissues in adult female seals from Lake Baikal compared to adult male seals, suggesting that Hg losses through reproduction and lactation do not result in lower soft tissue concentrations in female than in males.

### *Long-term trends in Hg exposure and accumulation in Lake Baikal seals*

TotHg concentrations and whole tooth Hg content for archival teeth were typically higher than those observed for the teeth collected in 2013 (Figure 4, Figure S4). As observed for contemporary seals, the results from the archival teeth indicated a tendency for TotHg concentrations to be highest in juvenile seals, without strong age-related differences in seals over 1 year of age (Figure S3), and for whole tooth Hg content to increase with age (Figure 4). There were substantial differences in the age distribution of seals across collection years, with more juveniles collected in some years (e.g. 1964, 1989 and 2013) and a larger number of much older seals (>10 years of age) collected in other years (e.g. 1966 and 1975) (Figure 4), highlighting the importance of considering age-effects when assessing temporal trends in tooth Hg. When specific age-ranges of seals are considered, a clearer temporal pattern emerges, with a peak in Hg concentrations and whole tooth Hg content in the late 1960s until approximately 1980, with somewhat lower concentrations in the years prior to and after this period, and even lower concentrations in 2013 (Figure 4, Figure S4). This is particularly apparent for the 5–10 year old age class of seals, for which the most detailed data were available. The juvenile seals show a more variable pattern (Figure 4), with lower concentrations in 2013, and higher concentrations from the 1960s until 1980, but with elevated concentrations observed in the seals collected in 1989, potentially reflecting maternal transfer of Hg from adult females with high levels of Hg contamination due to exposure in the years and potentially decades prior to reproduction.

Using a GAM approach (including both archival and contemporary teeth), we were able to disentangle the temporal trends in tooth Hg concentrations and whole tooth Hg content from the effects of age on Hg accumulation. GAMs differ from linear models in that they link the predictor variables (covariates) to the response variable using non-parametric smooth functions, which allows for non-linear influence of covariates on response parameters. This approach is particularly useful for this dataset, given that the relationship between tooth Hg concentration and age is non-linear, due to elevated Hg in juvenile teeth related to maternal transfer, and since temporal trends in Hg exposure and accumulation also may not be linear. Using collection year and age as predictor variables, we explained 52.6 % of the variation in TotHg concentrations in teeth (Figure S5) and 68.8 % of the variation in whole tooth Hg content (Figure S6). Including seal sex as a covariate did not improve model fit for either model, suggesting that sex does not have a significant effect on tooth Hg concentrations or content, consistent with our expectations and results based on contemporary soft tissues and teeth. More detailed information for GAM models can be found in Table S5.

The GAM for whole tooth Hg content (pg Hg in whole tooth) revealed an increase with age of whole tooth Hg content, as observed for the contemporary seals (Figure S6). The smooth function linking seal collection year with TotHg content in teeth reveals a distinct peak in approximately 1970, as well as a recent decline (Figure S6). A secondary peak appeared to be present in the 1990s, but the lack of data between the 1989 and 2014 makes it impossible for us to assess whether this peak is a real feature of the temporal trend in Hg contamination of Baikal seals, or a spurious pattern driven by values before and after this data gap (especially a juvenile with extremely high Hg concentrations from 1989 (Figure 4)). The GAM for TotHg concentrations in seal teeth revealed a very similar relationship between year of collection and Hg concentrations, and the relationship between age and TotHg concentrations in teeth was very similar to the patterns observed for the contemporary seal teeth, with higher concentrations in early life, followed by a decline and then relatively stable concentrations throughout adulthood

(Figure S5). The consistency in the age-tooth Hg concentration and age-whole tooth Hg content relationships between the GAM results and our observations from the contemporary seal samples suggests that this modelling approach accurately captured the influence of age on Hg concentrations and content in seal teeth, lending strength to the temporal trends revealed by the GAM models.

These results are also consistent with the results of LA-ICP-MS analysis, where a distinct peak in Hg concentrations in the dentine of pre-natal and independently feeding seals is observed between the 1950s and 1970s, followed by a decrease in concentrations to the present (see Ozersky et al. 2017). This further supports our observation that teeth from Lake Baikal seals, and by extension seal soft tissues, and the Lake Baikal food web as a whole, had particularly high levels of Hg contamination in the second half of the 1900s followed by a decrease to present. However, it should be noted that the timing of this peak based on whole teeth is approximately 10 years later than the peak observed based on LA-ICP-MS analysis, likely reflecting the fact that the reconstructed Hg concentrations based on LA-ICP-MS focus on Hg concentrations in the first layers of tooth deposited (i.e. Hg concentrations representing year-specific exposure), while the reconstruction based on whole teeth reflects an integration of long-term Hg accumulation, and for older seals elevated exposure to Hg in previous years of life would be reflected in whole tooth Hg despite potentially reduced exposure in recent years related to Hg emissions reductions.

There are three main potential drivers for the observed changes in Hg in teeth from Lake Baikal seals: 1) changes in Hg inputs to Lake Baikal, 2) changes in Hg cycling in Lake Baikal, and 3) shifts in seal ecology (including diet). Of these three potential factors, the significant long-term changes in Hg in teeth from Lake Baikal are most likely driven by changes in deposition. Globally, a wide range of studies have shown that increases and decreases in Hg inputs can be reflected in Hg contamination of aquatic biota, both at sites impacted by point source Hg pollution, as well as at sites where Hg inputs are dominated by long-range transport, although responses to changing inputs can occur at widely varying time-scales (Munthe et al. 2007). Ozersky et al. (2017) offer a detailed analysis of local, regional and global trends of Hg emissions affecting Lake Baikal, and suggest that both local emissions and long-range emissions from Europe may be particularly important sources of Hg to the lake.

Shifts in within-lake Hg cycling, including changes in sedimentation, transformation (methylation, demethylation) and uptake at the base of the food web could also play a role in mediating Hg concentrations in Lake Baikal's food web; however, this is beyond the scope of the current study. Also, although long-term shifts in seal trophic level and/or diet could be expected to drive changes in Hg exposure and accumulation, there is no strong evidence for such changes in the Baikal seal, based on long-term stability in soft tissue stable isotope values (current study vs. Yoshii et al. 1999) as well as the lack of directional change in dentine stable isotope values over the archival seal time series (as reported by Ozersky et al. 2017). Furthermore, the pelagic food web of Lake Baikal is relatively simple, with few overlaps in the isotopic niches of the taxa present (Yoshii et al. 1999), reducing the risk for shifts in diet that are not detected through stable isotope analysis.

Taken together, these results suggest that despite being a large lake with a long water residence time, Lake Baikal appears to respond rapidly to changing atmospheric inputs of Hg, with these changes reflected even in a long-lived top predator. This may in part reflect the importance of direct atmospheric deposition of Hg to Lake Baikal (Leermakers et al. 1996), in contrast to smaller catchment-dominated lakes, where responses to decreased Hg emissions are expected to be moderated by inputs from catchments which still have considerable pools of anthropogenic Hg that have accumulated over the past centuries (Munthe et al. 2007). This highlights the importance of past emission control measures in reducing Hg contamination of Lake Baikal and its food web, and also suggests that future changes in inputs will likely be reflected in this large lake.

### *Current and reconstructed soft tissue concentrations and toxicological implications*

In the current study, our detailed analysis of Hg concentrations and speciation in a wide range of contemporary tissues provides an opportunity to assess current toxicological risks posed by Hg to the seals themselves as well as to human consumers. Meanwhile, the relationship between Hg in seal teeth and their soft tissues allows us to explore potential toxicological risks to seals and human consumers over the past 50 years using Hg data from archival teeth. Based on the relationships presented in Table 1, we generated estimates of past Hg concentrations in seal muscle, liver, and brain (Figure 5). These tissues were selected for more detailed analysis because of their importance as potential food sources (muscle and liver; Nomokonova et al. 2013), and/or because Hg is known to have toxic effects on them (liver and brain; Dietz et al. 2013, Krey et al. 2015).

Reconstructed Hg concentrations in soft tissues were highest between the mid-1960s and the late 1970s (Figure 5). However, because these reconstructed concentrations are based on the relationship between tooth Hg content and soft tissue Hg concentration (Table 1), they do not account for the age of the seals, meaning that the uneven age distribution of sampled seals across years is likely to lead to skewed results, with higher values observed for years where more older seals were sampled.

We also compared tissue concentrations of Hg, both contemporary and reconstructed values, with previously published concentrations (Figure 5) for seals collected in 1992 (Watanabe et al. 1996; Watanabe et al. 1998) and 2001 (Cielsielski et al. 2006; Perrot et al. 2012). Measured muscle concentrations in 2013 were approximately two-fold lower than in 1992 for both juvenile and adult seals. In 2001, adult seals had muscle Hg concentrations comparable to those observed in 1992, while juveniles had similar concentrations to those observed in 2013. For liver tissue, the highest concentrations were observed for seals from 1992, the lowest concentrations in seals from 2001 and then intermediate concentrations in 2013 (approximately 50% higher than those observed in 2001). Brain tissue was only analyzed from two relatively old seals from 1992 (age 18 and 19 years), and as such, it is not possible to assess whether there was a decline in brain TotHg from 1992 to present. For all three tissues, concentrations in 1992 are comparable or lower than the reconstructed concentrations from the late 1980's.

To assess potential current and historical risk of human exposure to Hg through seal consumption, we compared muscle and liver TotHg concentrations with the WHO guideline level for Hg in fish (500 ng/g wet weight (FAO/WHO 2011); ~1600 ng/g dry weight), as there are no such guidelines available for seals. To allow for direct comparison between guideline values and measured values (Figure 5), we converted guideline values to a dry weight basis, using the measured tissue-specific wet/dry conversion ratios for the contemporary seals. Current TotHg (and MeHg) concentrations in muscle tissue are well below WHO guideline values, while liver concentrations were above the guideline values only for adult seals (Table S2, Figure 5). Predicted historic muscle Hg concentrations were often higher than the guideline level (particularly in the late 1960s and the 1970s), but, as for 2013, the measured concentrations in tissues collected in 1992 and 2001 were well below this level. For liver, predicted and measured

TotHg concentrations were consistently and substantially above the guideline level throughout the duration of the time series, with the exception of juvenile seals from 2001 and 2013. Indigenous peoples in the Baikal region consume both seal muscle and liver tissue, but primarily from young seals (age < 2 years; Nomokonova et al. 2013). Our data suggest that there is not currently a significant risk of Hg exposure from consumption of juvenile seal muscle or liver tissue, however, there is evidence that both muscle and especially liver consumption may have led to considerable Hg exposure in the past (particularly between 1950–1980).

With respect to potential toxicity to the seals themselves, we identified threshold values for toxic effects based on recent reviews of Hg toxicity to marine mammals. For liver, lesions have been observed at TotHg concentrations greater than 60 000 ng/g dw (Dietz et al. 2013), which is much higher than current liver tissue concentrations, but is below the reconstructed concentrations for many seals collected in the 1960s and 1970s, as well as in 1989 (these high concentrations are likely due to the large number of older seals (>10 years of age) collected in 1989, as discussed previously) (Figure 5). For the brain, we tested our data against two threshold values: 400 ng/g MeHg ww (~1700 ng/g dw), above which neurochemical disruptions have been observed, and 100 ng/g MeHg ww (~425 ng/g dw), above which changes including shifts in behaviour and immune response have been observed (Krey et al. 2015). Although these thresholds are for MeHg, we observed that for seals collected in 2013, the majority of TotHg in brain tissue was present as MeHg (Table S2). We found that contemporary and historical seals almost never exceeded the higher threshold, while seals commonly exceeded the lower threshold throughout the 1960's–1980's (Figure 5). These data suggest that although Hg likely does not pose a substantial toxicological risk to the seals at present, concentrations in the second half of the 20th century may have been high enough to pose risks to seal health. Also, given the high concentrations of POPs documented in Baikal seals, there is a potential for additive and synergistic effects of a broad mixture of contaminants (including Hg), making it difficult to assess the current (or future) toxicological risk posed to the seals (or their human consumers) by Hg alone.

When considering potential toxicological risks posed by Hg to seals and their human consumers, it is also important to consider the potential role of selenium (Se) in mitigating Hg toxicity (Ralston et al. 2007). It has been suggested that at tissue Se:Hg molar ratios exceeding 1, Se provides protection against Hg toxicity, with higher risk of Hg toxicity when Hg exceeds Se (Ralston et al. 2007). Despite low Se concentrations in Lake Baikal's waters (Leeves 2011), Se:Hg ratios have been shown to consistently exceed 1 in Lake Baikal fish and seal tissues (Leeves 2011; Li 2013), suggesting that Se may moderate the toxic risk posed by Hg to seals (and human consumers).

## *Conclusions*

Although Hg concentrations in Lake Baikal seals are relatively low, and pose apparently low toxicological risk to the seals themselves and their human consumers, our reconstruction of past Hg concentrations in Lake Baikal seals based on historical tooth samples suggests that Hg concentrations were significantly higher three to four decades ago (Figure 5). The strong changes over time in seal Hg concentrations suggests that changes in Hg inputs can have dramatic effects on contamination of the lake and its food web, and associated ecosystem and human health risk despite the lake's great size, immense depth, and long turnover time. This highlights the importance of local, regional and global efforts to reduce Hg emissions, and indicates that Lake Baikal may be vulnerable to future increases in Hg inputs due to changes in regional human activity, and climate change, including the potential for permafrost in Lake Baikal's catchment to release Hg as it thaws. However, the lake may also benefit from emission reductions resulting from regional and global efforts, including the Minamata Convention.

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## TABLES

**Table 1**. Summary of results of linear regression between soft tissue TotHg concentrations and tooth Hg content for adult seals collected in 2013.

| **Regression equation** | **n seals** | $r^2_{adj}$ | $p$ |
|---|---|---|---|
| $\log_{10}$(Muscle TotHg) = 0.57±0.61 + 0.76±0.22*log(tooth Hg content) | 12 | 0.481 | 0.005 |
| $\log_{10}$(Liver TotHg) = -1.68±1.38 + 1.86±0.49*log(tooth Hg content) | 12 | 0.528 | 0.003 |
| $\log_{10}$(Brain TotHg) = 0.46±0.60 + 0.67±0.22*log(tooth Hg content) | 11 | 0.441 | 0.011 |
| $\log_{10}$(Blood TotHg)= -0.52±0.61 + 0.95±0.21*log(tooth Hg content) | 9 | 0.676 | 0.002 |

| | | | |
|---|---|---|---|
| log$_{10}$(Kidney TotHg)= 0.73±0.64 + 0.99±0.23*log(tooth Hg content) | 11 | 0.618 | 0.001 |

FIGURE CAPTIONS

**Figure 1** Stable carbon (a) and nitrogen (b) isotope values and TotHg concentrations (c) for select soft tissues (blue: blood, red: muscle, orange: liver) for seals collected in 2013. Dashed vertical lines indicate age 3 months (approximate time of weaning: 2–3 months) and 6 years (approximate age where seals reach sexual maturity). Lines and shading represent LOESS smoothers intended to make the data easier to visualize.

**Figure 2** TotHg and MeHg concentrations and proportion of TotHg present as MeHg (% MeHg) for soft tissues from contemporary seals (n=22).

**Figure 3** Linear regressions between TotHg concentrations in select soft tissues and tooth Hg content (pg Hg in whole tooth) for adult seals collected in 2013 (age 1 and up; n=13); circles: females, triangles: males.

**Figure 4** Hg content (pg of TotHg in whole tooth) vs. collection year for contemporary and historical seal teeth. The colour of data points indicates age-at-death of seals, with categories for juveniles (<1 year), 1–2 year old seals, 2–5 year old seals, 5–10 year old seals and seals over 10 years of age.

**Figure 5** Temporal trends in reconstructed and measured soft tissue Hg concentrations in Baikal seals. Boxplots show soft tissue Hg concentrations for historical seals as predicted from tooth Hg content (outliers shown as black points). Measured tissue Hg concentrations from 1992,[21,45] 2001,[20,43] and 2013 (current study) are shown using red and blue points (for juvenile and adult seals respectively). The WHO guideline level for Hg in fish for safe consumption (500 ng/g wet weight (~1700 ng/g dry weight)) is shown as a dashed blue line for both muscle and liver, and thresholds for potential toxic effects on seals are shown as red dotted lines. All literature tissue data, consumption and toxicity thresholds were converted to a dry weight basis. Toxicity thresholds shown are based on recent reviews of effects of Hg on marine mammals and are described in the manuscript text.

## SUPPORTING INFORMATION

**Table S1** $\delta^{15}N$, $\delta^{13}C$ and C:N ratios for seals collected in 2013. Values are shown as mean ± standard deviation.

**Table S2** TotHg and MeHg concentrations, and proportion of TotHg present as MeHg for seals collected in 2013. Values are shown as mean ± standard deviation.

**Table S3** Results of linear regressions of tissue-specific TotHg and MeHg concentrations vs. seal age for all seals collected in 2013.

**Table S4** Summary of results of linear regression between soft tissue TotHg concentrations and tooth Hg content for all seals collected in 2013 (results for adult seals only are shown in Table 1 in the main manuscript).

**Table S5** Summary of generalized additive models including collection year and age-at-death as covariate. We also tested sex as a potential covariate, but it was omitted in the final models as it did not significantly improve model performance and did not have a statistically significant smooth term. For all smooth terms, the number of knots (k) was set to k=5.

**Figure S1** Histogram showing collection years for the seals included in the current study. Historical seal samples (prior to 1990) include canine teeth, while contemporary seal samples (2013) include teeth and several soft tissues.

**Figure S2** Carbon to nitrogen ratios (C:N) for soft tissues from contemporary seals.

**Figure S3** Whole tooth Hg content (pg Hg) and tooth Hg concentrations (ng/g) for seals collected in 2013. Dashed vertical lines indicate age 3 months (approximate time of weaning: 2–3 months) and 6 years (approximate age where seals reach sexual maturity). Lines and shading represent LOESS smoothers intended to make the data easier to visualize.

**Figure S4** Total Hg concentrations (ng/g) in teeth vs. collection year for contemporary and historical seal teeth. The colour of data points indicates age-at-death of seals, with categories for juveniles (<1 year), 1–2 year old seals, 2–5 year old seals, 5–10 year old seals and seals over 10 years of age.

**Figure S5** Results from GAM model of $\log_{10}$(TotHg in teeth (ng/g))~s(Year Collected) + s(Age), with the number of knots (k) set to 5 for each covariate. Model includes data for both archival and contemporary seals.

**Figure S6** Results from GAM model of $\log_{10}$(TotHg content in teeth (pg))~s(Year Collected) + s(Age), with the number of knots (k) set to 5 for each covariate. Model includes data for both archival and contemporary seals.