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Author contributions

Arnemo: Conceptualization; **Fuchs, Thiel:** Methodology; **Rodushkin:** Resources**; Arnemo, Boesen, Evans, Fuchs, Græsli, Hydeskov, Rodushkin, Thiel:** Investigation; **Fuchs, Thiel:** Formal analysis; **Fuchs:** Writing – Original Draft; **Arnemo, Brown, Evans, Fuchs, Græsli, Hydeskov, Kindberg, Rodushkin, Thiel, Zedrosser:** Writing – Review & Editing; **Arnemo, Zedrosser:** Supervision; **Arnemo, Kindberg:** Project administration; **Arnemo, Kindberg:** Funding acquisition

High concentrations of lead (Pb) in blood and milk of free-ranging brown bears (*Ursus arctos*) in Scandinavia

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Abstract

Exposure to lead (Pb) is a global health problem for both humans and wildlife. Despite a dramatic decline in human Pb exposure following restrictions of leaded gasoline and industry and thereby an overall reduction of Pb entering the environment, Pb exposure continues to be a problem for wildlife species. Literature on scavenging terrestrial mammals, including interactions between Pb exposure and life history, is however limited.

We quantified Pb concentration in 153 blood samples from 110 free-ranging Scandinavian brown bears *(Ursus arctos)*, 1-25 years old, using inductively coupled plasma sector field mass spectrometry. We used generalized linear models to test effects of age, body mass, reproduction status and spatial distribution on the blood Pb concentrations of 56 female bears. We sampled 28 females together with 56 dependent cubs and paired their blood Pb concentrations. From 20 lactating females, we measured the Pb concentration in milk.

The mean blood Pb concentration was 96.6 µg/L (range: 38.7.0-220.5 µg/L). Both the mean and range are well above established threshold concentrations for developmental neurotoxicity (12 µg/L), increased systolic blood pressure (36 µg/L) and prevalence of kidney disease in humans (15 µg/L). Lactating females had higher Pb blood concentrations compared to younger, non-lactating females. Blood Pb concentrations of dependent cubs were correlated with their mother's blood Pb concentration, which in turn was correlated with the Pb concentration in the milk.

Life-long Pb exposure in Scandinavian brown bears may have adverse effects both on individual and population levels. The high blood Pb concentrations found in brown bears contrast the general reduction in environmental Pb contamination over the past decades in Scandinavia and more research is needed to identify the sources and pathways of Pb exposure in the brown bears.

Keywords

Lead, Blood, Milk, *Ursus arctos*, Carnivora

Introduction

Lead (Pb) is a highly toxic element without any known biological functions, that negatively affects multiple physiological systems in vertebrates (Bellinger et al., 2013). In a risk assessment, the European Food Safety Authority (EFSA) established benchmark dose levels of blood Pb concentrations for developmental neurotoxicity in children (12 µg/L) as well as for increased systolic blood pressure (36 µg/L) and prevalence of kidney disease (15 µg/L) in adults. The EFSA further states that there is "no evidence for a threshold of lead-induced effects" and defines tolerable intake levels as not appropriate (EFSA, 2013).

Pb exposure in terrestrial mammals results from ingestion or inhalation. In humans ingested or inhaled Pb initially increases blood Pb concentration with a relatively short half-life of approximately 35 days (Rabinowitz et al., 1976). The organism treats Pb as a substitute for calcium and transfers it to soft tissues and bones (Rabinowitz et al., 1976). In mammals, over 90% of the total body Pb is stored in bones and teeth with a half-life of 10-30 years (Andreani et al., 2019; Rabinowitz et al., 1976). During periods of nutritional stress, such as pregnancy and lactation, Pb stored in bones and soft tissue can become an endogenous source resulting in increased blood Pb concentrations (Silbergeld, 1991). Increased calcium demands during skeletal development of the fetus as well as during lactation lead to increased calcium turnover during pregnancy and lactation in the mother. As a result, Pb is released from bones into the blood and into the milk (Ettinger et al., 2014), which may have detrimental effects on both the mother and her offspring.

In Europe, Pb has been mined, refined and used for more than 2,000 years, resulting in widespread airborne pollution from smelting processes and, since the mid-20th century, from Pb as gasoline additive (Settle and Patterson, 1980). In northern European lake sediments, the current environmental Pb concentrations are up to 1000 times higher than the natural background concentrations (Renberg et al., 2001; Settle and Patterson, 1980). Top soils are contaminated with Pb globally, and the Pb uptake in plants growing in these soils pose a risk for consumers (Khalid et al., 2017). Global environmental and health concerns have led to a gradual phasing out of leaded gasoline since the 1970s, which accelerated in the mid-1980s, when European Union member states started to reduce the allowed Pb limits in gasoline (von Storch et al., 2003). Consequently, atmospheric deposition of Pb decreased, especially in northern Europe (Lind et al., 2006; von Storch et al., 2003). For example, Danielsson and Karlsson (2015) reported that the mean Pb concentration in mosses had decreased by 96% in Sweden from 1975 to 2015. Blood Pb concentrations of children in southern Sweden decreased from 60 µg/L in 1978 to 11 µg/L in 2007 (Skerfving et al., 2015; Strömberg et al., 2008) and to 8.5 µg/L in 2019 (data available at Karolinska Institutet, 2020). Liver Pb concentration of bank voles (*Myodes glareolus*) in central Sweden measured in 2017 had decreased by two thirds compared to 2001 (Ecke et al., 2020). This general decrease in exposure suggests a direct link between aerial Pb pollution and Pb exposure in humans and the environment.

In high-income countries, most sources of Pb emission are strictly regulated today. An exemption is Pb used in hunting ammunition, which presents a significant source of exposure for both humans and wildlife (Arnemo et al., 2016; Bellinger et al., 2013). For example, Pb from hunting ammunition is an important source of morbidity and mortality in avian scavengers, such as golden eagles (*Aquila chrysaetos*) (Ecke et al., 2017) and white-tailed eagles (*Haliaeetus albicilla*) (Helander et al., 2009). Increased Pb concentrations in birds also result in behavioral alterations, lower reproductive success, and physiological changes (Berglund et al., 2010; Ecke et al., 2017; Finkelstein et al., 2012; Kelly and Kelly, 2005). Generally, the extent of Pb exposure depends on a species' feeding ecology and can vary within a population, depending on individual spatio-temporal movement patterns and resource specialization (Arrondo et al., 2020; Brown et al., 2019; Nadjafzadeh et al., 2013). Periods of nutritional stress, such as pregnancy or incubation, may mobilize Pb from endogenous sources and increase the risk for clinical effects of Pb concentration in wildlife (Lam et al., 2020).

Scientific evaluations of Pb exposure in wild-living terrestrial mammals commonly use a screening approach, typically sampling of soft tissues, with human food safety or biomonitoring as the main motivation (eg. Chiari et al., 2015; Morales et al., 2011). Studies including other variables of the investigated populations, such as sex and age, vary in results. For example, female European roe deer (*Capreolus capreolus*) in Spain had higher Pb concentrations than males in kidney and muscle tissue, but the same concentrations in liver tissue (García et al., 2011). Higher Pb concentrations with increasing age were found in male roe deer liver and muscle tissue in Spain (García et al., 2011), while no age effect was found in bone or teeth in Poland. In red deer (*Cervus elaphus*) bone Pb concentrations increase with age in Croatia (Lazarus et al., 2008), however, no such increase was found in Spain (Rodríguez-Estival et al., 2013). Lazarus et al. (2018a) found no sex differences in Pb concentrations in the femoral bones or in liver and kidney tissues (Lazarus et al., 2018b) of brown bears (*Ursus arctos*) in Croatia, but animals ≥4 years had higher Pb concentrations compared to younger individuals. Brown bear cubs-of-the-year (< 1 year old) had higher Pb concentrations in soft tissue compared to yearlings, which indicates a transfer of Pb during pregnancy and lactation.

We used a brown bear population in Scandinavia as a sentinel for environmental Pb exposure, and evaluated blood Pb concentrations in relation to life-history traits (age, body mass). Studies on free-ranging brown bears in the USA, south-eastern Europe, and Scandinavia have reported mean blood Pb concentrations of 55 μg/L (Rogers et al., 2012), 61 μg/L (Lazarus et al., 2020), and 88 μg/L (Boesen et al., 2019), respectively. These high Pb concentrations suggest that brown bears may act as a good sentinel species. Brown bears are omnivorous and their diet typically consists of vegetation and berries, but they also kill or scavenge on ungulates and feed on insects, (Bojarska and Selva, 2012; Dahle et al., 1998; Rauset et al., 2012; Stenset et al., 2016; Swenson et al., 2007b), and, thus, represent the cumulative burden of different potential Pb sources. Scandinavian brown bears hibernate up to 6 months between October and April (Evans et al., 2016). Females mate in June and delay implantation until the onset of hibernation in fall, and give birth in the winter (Friebe et al., 2014; Tsubota et al., 1987). Females exhibit active-state body temperatures during gestation, give birth after 56 days and then decrease their metabolic rate back to hibernation levels (Friebe et al., 2014). In Sweden, offspring remain with their mothers for 1-2 years (Van de Walle et al., 2018).

The aims of this study were to screen blood Pb concentrations in the brown bear population in south-central Scandinavia and to evaluate if Pb concentrations are correlated with life-history traits and lactation. We predicted that blood Pb concentrations in female brown bears increase with age, body mass, and during lactation. We also tested for spatial correlations, because exposure to Pb may vary in space and spatial clustering may affect life history traits differently. We further hypothesized that brown bear offspring are exposed to Pb from lactation, and predicted that variations in milk Pb concentration are related to a female's blood Pb concentration, and that the offspring's blood Pb concentration is positively correlated with the mother's blood Pb concentration.

Methods

Study area

This study is part of a long-term individual-based research project on brown bears in south-central Sweden and south-eastern Norway (~61°N, 15°E) (Scandinavian Brown Bear Research Project, 2020). The size of the study area is approximately 13,000 km^2, predominantly covered with intensively managed coniferous forests in stands of different ages, ranging from recent clear cuts to 90-100-year-old stands (Martin et al., 2010; Swenson et al., 1999). The rolling landscape is interspersed with lakes and bogs, and with agricultural fields towards the east. The altitude gradually increases from ≈150 m above sea level in the east to 850 m above sea level in the west, which is also the approximate tree line. Human settlements are concentrated in the north and south, with only few high-traffic roads (0.14km/km^2). However, isolated houses (mainly cabins) and both paved and gravel roads with low traffic volume are distributed throughout the study area (0.3 cabins/km^2 and 0.7 km low-traffic roads/km^2) (Martin et al., 2010).

Capture and sampling

All brown bear captures and sampling were carried out according to an established protocol (Arnemo and Evans, 2017) approved by the Swedish Ethical Committee on Animal Research (Uppsala, Sweden; Dnr 5.8.18-03376/2020), the Swedish Environmental Protection Agency (NV-00741-18), the Swedish Board of Agriculture (#31-11102/12), the Norwegian Food Safety Authority (FOTS ID 19368) and the Norwegian Environment Agency (2018/3346). All bears were darted from a helicopter in the spring (April-May; 2010-2019), sex-determined and weighed using a digital spring scale. Because the captures mainly focused on known females and their dependent yearling offspring, the age of most captured individuals was known. Bears captured the first time as adults were aged by counting the cementum layers of a vestigial first premolar (Mattson, 1993). All captured bears were tattooed and microchipped for individual recognition.

Blood and milk sampling

Blood was collected from the jugular vein in 4 mL evacuated K3EDTA tubes (EDTA, n=118) (Vacuette, Greiner Bio-One International GmbH, Kremsmünster, Austria) and in 6 mL evacuated heparin trace element tubes (TE, n=54) (Vacuette). Mammary glands of adult females were palpated to visually confirm lactation. We administrated 10 IU oxytocin (Vetocin 10 IU/mL, Bela-Pharm GmbH & Co. Kg, Vechtra, Germany) to lactating females and collected approximately 1 mL milk in a 10 mL non-collared screw cap tube (Sarstedt, Nümbrecht, Germany). Tubes with samples were frozen the same day and kept at -20° C during storage and shipment to the laboratory (ALS Scandinavia AB, Luleå, Sweden).

Pb analysis

At the laboratory, blood and milk samples were prepared for analysis by closed vessel MicroWave-assisted acid digestion. Pb concentration in digests was measured by high-resolution inductively-coupled plasma sector field mass spectrometry (ICP-SFMS, ELEMENT XR, ThermoScientific, Bremen, Germany) using a combination of internal standardization and external calibration. Quality assurance and quality control (QA/QC) included a set of preparation blanks and matrix-matched control specimens (Seronorm Trace Elements Whole Blood Levels 1 and 2 from SERO AS, Norway) prepared and analyzed with each analytical batch of blood samples. Contribution from preparation blanks was less than 0.2 µg/L and thus negligible for Pb concentrations found in milk and blood samples. Differences between found and target Pb concentrations for the controls were under 6%, the relative standard deviation (RSD) and of the same magnitude as typical instrumental precision (in the range 3%-5% RSD). Further details on analytical methods can be found in Rodushkin et al., 2000.

Sampled brown bears

We analyzed a total of 172 blood samples for Pb concentrations. The samples were collected on 153 sample events (2010: 13, 2013: 9, 2017: 31, 2018: 46, 2019: 37, and 2020: 17). Nineteen samples were analyzed in pairs (collected at the same sample event). Sampled animals were comprised of bears sampled during family group captures, i.e. mothers (N = 28) captured with 1-3 dependent offspring (N = 56), family group capture attempts with either only the mother (N = 16) or only offspring (N = 11) captured, and captures of single bears (N = 42). A total of 67 dependent offspring were sampled, 55 yearling olds and 12 two-year olds. Mean age for both independent females and males was 8.7 years (range: 3-25 years). We collected milk samples from 20 females; nine of those had cubs of the year, eight had yearlings and three two-year old offspring. Individual bears were sampled up to four times during the study period, a total of 110 individual bears are included in the study.

Statistical analysis

To investigate possible contamination by the blood sampling tubes, we tested Pb concentrations in 19 brown bears using both TE and EDTA tubes. We tested whether the differences between Pb concentrations of the TE compared to the EDTA values were lower or greater than 0 with a paired Wilcoxon rank test. We fitted a linear regression model with the TE value as the response and the EDTA value as the predictor value and forced the intercept trough zero. Pb concentrations of blood collected in EDTA tubes were significantly lower than from samples collected in TE tubes ($W = 151$; $P = 0.01$), with a median difference of 2.51 µg/l (SD= 2.46; range: 0.03 - 9.28 µg/L). We then used the regression coefficient $\beta = 1.013$ to correct all EDTA based Pb concentrations in the data set; after this correction, the newly calculated EDTA values were not significantly different from TE values ($W = 113$; $P = 0.49$) and were used for further analysis. We evaluated the correlation between milk Pb concentration and the blood Pb concentration with Spearman's rho. We further tested if milk Pb concentration was related to the lactation period in years (i.e., the offspring's age) with a Kruskal-Wallis test. We used R version 3.6.3 for all data handling and analyses (R Core Team, 2019).

Blood Pb variation in relation to life history and spatial correlation in female bears

To investigate spatial correlation of Pb values in relation to life history traits, we fitted a variogram model with age and reproductive status as explanatory variables, using Pb concentration as the identifier and 200 km as the cutoff value (i.e., the distance from the eastern to the western edge of the study area). A second variogram was fitted and the result split by the four cardinal directions. Variograms were fitted using the variogram function of the *gstat* library (Pebesma, 2018).

To test if spatially defined groups of sampled bears within the study area differed in their Pb concentrations, we performed a hierarchical cluster analysis of the Euclidian distance between capture locations using the *hclust* function and the complete method to find similar clusters from the *stats* library in R. We plotted the cluster dendrogram and visually determined a reasonable cutoff value (Zuur et al., 2009). We added the cluster ID to each observation in the data set.

We used a generalized linear model (GLM) to investigate whether blood Pb concentration of independent (i.e. weaned from their mother) sub adult and adult females is affected by life history traits, i.e. age (in years), reproductive status (lactating vs non-lactating), and the spatial cluster ID. Because age and body mass are highly correlated in brown bears (Bartareau et al., 2011; Swenson et al., 2007a; Zedrosser et al., 2006), we only used age as the explanatory variable. We fitted the GLM with a gamma distribution and an identity link function to avoid estimation of negative Pb concentrations. Despite repeated measurements, we decided not to include the bear ID as a random variable due to non-convergence issues caused by too few replicates per bear ID (one to three measurements per bear). The cluster ID variable was retained in all candidate models, except the Null model. Then we fitted a set of candidate models comparing all possible variable combinations as well as a Null model and carried out model selection based on the Akaike Information Criterion corrected for small sample size (AICc). We averaged models within a cut off value of $\Delta AICc \leq 2$ with the *AICcmodavg* package (Mazerolle, 2019). The mean gamma dispersion parameter from the two models was used for model averaging. The age was known for only nine of the 15 solitary males and we therefore decided to exclude males from this analysis.

Blood Pb correlation between mothers and offspring

We used generalized linear mixed models (GLMM) with a Gaussian distribution and maximum likelihood estimation (ML) to evaluate the blood Pb concentration of dependent offspring in relation to the blood Pb concentration of their mothers. We used the offspring's Pb concentration as the response variable, and the mother's Pb concentration as well as categories for sex and age of the offspring as the explanatory variables. Because several offspring were captured as part of a family group, we added the ID of the mother as a random intercept. We used a model with the blood Pb concentration of the mother as a fixed variable as the base model and compared it to models containing different combinations of the variables age and sex. We performed model selection based on the lowest AICc value and averaged models within a $\Delta AICc \leq 2$ (Mazerolle, 2019).

Results

Sampling

All 153 blood samples contained measurable concentrations of Pb. The overall mean blood Pb concentration was 96.6 µg/L ± 35.6 µg/L (SD) (Table 1), with a range from 38.7 µg/L measured in a two-year-old male to 220.5 µg/L in an adult female.

We excluded two milk samples due to concerns by the laboratory over the validity of the values because of very low collected volume and associated contamination risk. The Pb concentration of the remaining milk samples (N = 18) ranged from 21.4 µg/L to 103.6 µg/L, with a mean of 42.9 µg/L ± 21.1 µg/L. Females with higher blood Pb concentration also had significantly higher Pb concentration in their milk (N = 18, rho=0.60, p = 0.011). The milk Pb concentration did not differ between lactating females accompanied by cubs-of-the-year, yearlings, or two-year-old offspring ($\chi^2 = 3.2$, df = 2, p = 0.20).

Blood Pb variation in relation to life history of female bears

Age and reproductive status were available for 56 blood samples from 34 independent sub adult and adult females (1-3 samples/individual). Thirty-one bears (age: 5-25 years) were lactating, and 25 individuals (age: 2-10 years) were non-lactating. The plotted output of the variograms displayed an approximately horizontal line over the entire distance tested and a lack of spatial correlation (Figure S1). Splitting the variogram into the four cardinal directions revealed no clear spatial patterns in any direction (Figure S2).

Based on the dendrogram of the cluster analysis, we chose a cut off value of 60 km resulting in four different clusters named after their geographic location (from west to east: Fulufjell, Älvdalen, Noppikosiki (Noppi) West and Noppi East; Figure 1 and Figure 2). Two models fell within ΔAICc ≤ 2; the model with the lowest AICc contained reproductive status, age and the cluster ID as fixed terms, and the second-best model contained the variables reproductive status and cluster ID as fixed terms (Table 2). We used the model-averaged estimates of these two models for further interpretation (Table 3). Compared to non-lactating females, the top model predicted a 1.3 to 1.6 times higher blood Pb concentration in a lactating 10-year-old female bear, depending on cluster (Figure 3). Blood Pb concentrations were highest in the cluster west from the study area center and decreased towards the eastern cluster. Pb blood concentrations were also lower in older individuals, however, with overlapping confidence intervals (CI`s) and small effect size; e.g. the predicted Pb blood concentration of a 15-year-old lactating female in the eastern-most cluster was 83.7 µg/L (95% CI: 60.3-107.0 µg/L) compared to 92.0 µg/L (95% CI: 76.5-107.6 µg/L) for 10-year-olds (Table 3).

Blood Pb correlation between mothers and offspring

Two GLMMs explaining blood Pb concentrations in offspring were within ΔAICc ≤ 2: the most supported model contained only the maternal blood Pb concentration as explanatory variable, the second model contained the offspring's age in addition. Further interpretation is based on the averaged model output of these two models. Blood Pb concentrations of offspring increased significantly (0.77 µg/L; 95% CI: 0.6-1.0 µg/L) with increasing maternal blood Pb concentration (Figure 4). For example, the model estimated a blood Pb concentration of 70.4 µg/L (95% CI = 54.2-86.6 µg/L) for a yearling offspring if the mother has a blood Pb concentration of 82.0 µg/L (1st quantile of maternal Pb concentrations). If a mother has a blood Pb concentration of 138 µg/L (3rd quantile of maternal Pb concentrations), her yearling offspring has an estimated blood Pb concentration of 113.6 µg/L (95% CI = 86.1-140.6µg/L).

Discussion

We found that lactating females had significantly higher blood Pb concentrations compared to non-lactating females (supporting prediction *i*). We also found support for the hypothesis that offspring are exposed to Pb due to suckling, i.e. maternal Pb blood concentrations are significantly and positively correlated with milk Pb concentration (supporting prediction *ii*) as well as with the offspring's blood Pb concentrations (supporting prediction *iii*). The mean blood Pb concentration (92.3 µg/L) of dependent offspring with a developing neurosystem exceeded the EFSA thresholds of 12 µg/L for developmental neurotoxicity in children by a factor of eight, and all analyzed blood Pb concentrations where above the threshold for increased systolic blood pressure of 36 µg/L in humans (EFSA, 2013). The blood Pb concentrations in Scandinavian brown bears (mean 96.6 µg/L) were also higher than in brown bear populations of the greater Yellowstone area (mean 55 µg/L) and south-eastern Europe (mean 61 µg/L) (Lazarus et al., 2020; Rogers et al., 2012). Our findings strongly indicate high Pb exposure in the south-central Scandinavian brown bear population. Most likely, bears are exposed to Pb throughout life, starting during the fetal period and continuing for their entire life-spans at levels, considered a health risk in humans.

Lactation increases calcium turnover, and Pb stored in bones can be reabsorbed to the blood and become an endogenous source for blood Pb (Silbergeld, 1991). In experimental studies on laboratory mice, Keller and Doherty (1980) showed that the milk to plasma ratio is similar for calcium and Pb. In humans, the relationship between maternal blood Pb concentration and milk Pb concentration follows a linear relationship (Ettinger et al., 2014; Gulson et al., 1998). Mice with intravenously injected Pb, transferred approximately 25% of the injected Pb dose to the suckling pups (Keller and Doherty, 1980). This suggests that also lactating brown bears mobilize Pb from their bones, resulting in increased blood Pb concentrations and in transfer to milk and excretion of Pb from their bodies. A confounding factor in our study is that age and lactation are not independent. Almost all females above 10 years of age were lactating at the time of sampling whereas only three out of 23 females younger than 7 years old were lactating. Data exploration suggested an increasing blood Pb concentration with increasing age (Figure 3). However, once we accounted for lactation, the effect of age disappeared, indicating no correlation between blood Pb concentration and increasing age.

More than one third of the offspring in the study area wean from their mothers during their third year of life (Van de Walle et al., 2018) and we found that females with 2-year-old offspring were still lactating (N=3). Although Pb concentration in milk decreases during the lactation period in other mammals (Antunovic et al., 2005; Ettinger et al., 2014), female brown bears with 2-year-olds had milk with similar Pb concentrations as females with cubs-of-the-year. We conclude that offspring are exposed to Pb in the milk for the entire lactation period, i.e. up to 28 in our study population. Lazarus et al. (2018b) found that renal Pb concentrations were higher in cubs-of-the-year compared to yearling bears, which suggests a high initial Pb absorption capacity of younger offspring that decreases with increasing age. We lack data from cubs-of-the-year, but similar to Lazarus et al. (2018b), we found a high correlation between the mother's and the offspring's Pb concentrations. In addition to Pb ingestion via milk, both the mother and her dependent offspring feed together on the same resources and at the same locations for up to two years and have thus a similar environmental Pb exposure.

Laboratory experiments on rats showed neurobehavioral effects in offspring of mothers with blood Pb concentrations of 110 µg/L (Virgolini et al., 2008). The blood Pb concentrations of bear offspring in our study ranged from 38.7-166.5 µg/L, i.e. Pb concentrations related to behavioural changes in other mammalian species (Ma, 2011).

Blood Pb concentrations have been used as an indicator of recent Pb exposure due to an estimated half-life of 35 days in humans (Rabinowitz et al., 1976). Due to the reabsorption from bones and tissue and the equilibrium between various body compartments, however, the actual blood Pb concentration might take much longer to halve. In domestic cattle exposed to a high initial dose of Pb, blood concentrations halved after 68 to 266 days, and in chronically exposed humans after a median of 619 days (Hryhorczuk et al., 1985; Miranda et al., 2006). Increased blood Pb concentrations measured at the population level, as in brown bears in Scandinavia, Eastern Europe and Yellowstone, are most likely due to chronic Pb exposure.

Much of the Pb in the environment of the Scandinavian brown bears is from aerial depositions originating in emissions from leaded gasoline and smelters from entire Europe (Renberg et al., 2001; von Storch et al., 2003). A possible exposure pathway are major food resources, such as bilberries and lingonberries that take up Pb from the soil. According to Welch et al. (1997), a bear of 80 kg body mass needs 0.925 kg wet weight daily intake of bilberries to maintain body mass and has a maximum digestive capacity of 28.6 kg wet weight per day. Rodushkin et al. (1999) reported background Pb concentration of 3.4 µg/kg wet weight in bilberries. For a brown bear of 80 kg the daily Pb ingestion from berries would be 0.04 µg/kg to maintain body mass and 1.22 µg/kg body mass for a bear consuming berries at the digestive capacity.

Experimental studies evaluating the amount of ingested Pb in relation to blood Pb concentration focus on exposure, i.e. subjects are exposed to contaminated food, water or air/dust with the total ingested dose unknown. For example, in laboratory rats, a 2-month intake of drinking water with a Pb concentration of 50 µg/L resulted in a blood Pb concentration of about 110 µg/L (Cory-Slechta et al., 1983). In comparison, the mean Pb concentration in bear milk in our study is 44.9 µg/L and the mean blood concentration of dependent offspring is 93.2 µg/L, suggesting similar exposure compared to the laboratory rats from Cory-Slechta et al., 1983. In laboratory mice, a 3-month exposure to food with 200 µg Pb/kg dry mass led to a blood Pb concentration of 70 µg/L (Iavicoli et al., 2003). In a pilot study (Fuchs, unpublished), we sampled berries inside the most eastern cluster (Figure 1), and found mean dry weight concentrations of 9.0, 6.7 and 30.8 µg/kg for bilberries, lingonberries and crowberries, respectively. The mean blood Pb concentration of 96.6 µg/L in bears is similar to concentrations measured in laboratory rodents, however, exposure from berries is likely much lower.

Rabinowitz at al. (1976) exposed five humans to a daily dietary Pb ingestion ranging from 2.4 µg to 5.1 µg Pb/kg body mass and were able to maintain each subject’s pre-study Pb concentrations ranging between 170 µg/L and 250 µg/L blood. Reported ingestion in Rabinowitz’s study was 2 to 4 times higher than Pb ingestion of a bear with a berry intake at the digestive capacity, similar to the human subject’s blood Pb concentrations which were 2 to 3 times higher compared to the mean blood concentration of bears in this study. However, this assumes similar uptake rates and Pb kinetics between humans and bears. In addition, while Rabinowitz et al. (1976) sampled during ingestion, the bears were sampled after hibernation and 7 to 8 months after the peak hyperphagic berry consumption.

Bears that consume vegetation and insects, such as ants, commonly ingest significant amounts of soil, humus and other debris (Stenset et al., 2016). Bears might hide larger prey with vegetation and soil, probably ingesting residues when resume feeding. Salminen et al. (2005) reported increased Pb concentrations in humus in relation to sub and top-soils due to aerial depositions for our entire study area. Further, the increased blood Pb concentration predicted west in the study area for female bears coincides with increased Pb concentrations in sub soil in the same area (Salminen et al., 2005). It remains unclear if and how, hibernation affects Pb kinetics and blood Pb concentration in bears. During hibernation, there is no ingestion of Pb and excretion of Pb is likely very low because bears do not eat or drink during hibernation, their urine production is very low and bile excretion entirely absent (Nelson et al., 1973).

Large game hunting with Pb-based ammunition poses an additional potential source for high blood and milk Pb concentrations of bears. In Sweden, 90% of bullets used to harvest moose are Pb based and these bullets normally fragment into inner organs that are left for scavengers (Stokke et al., 2017). The moose-hunting season in central Sweden starts in early September and lasts until the end of January. While most bears start hibernating in late October (Evans et al., 2016), pregnant females may enter their dens between late September and late October (Friebe et al., 2014, 2001; Manchi and Swenson, 2005). Of the total moose harvest in our study area, 75% are shot during October and consequently the available moose-based biomass for scavengers is estimated to be 15 fold higher compared to before the hunting season (Länsstyrelserna, 2019; Wikenros et al., 2013). We lack data on the frequency of use of gut piles by brown bears but individuals might have reduced mobility or hibernate through parts of the peak hunting season. During spring in Scandinavia, after hibernation, moose is a major proportion of the bears estimated dietary energy content (Stenset et al., 2016). Winter mortality, traffic kills and, in areas with sympatric wolf (*Canis lupus*) presence, wolf-killed moose compose the available biomass in spring (Ordiz et al., 2020; Wikenros et al., 2013). A potential source of Pb exposure during spring are slaughter remains that are discarded in the forest by hunters and visited by bears. Common ravens (*Corvus corax*) in Wyoming USA, showed sharp increases in blood Pb concentrations by the start of the moose hunt and slightly elevated concentrations during the snow melt in spring when hunting remains from the fall reappear (Craighead and Bedrosian, 2008). Gut piles from the fall however, are commonly rapidly consumed and only rarely available to bears in the spring (Gomo et al., 2017).

Conclusions

Scandinavian brown bears are highly exposed to environmental Pb despite the generally large decrease of the Pb burden on a spatio-temporal scale in Europe. In Scandinavia, sediment core Pb concentrations dated back to pre-human metallurgic activities are very low (Renberg et al., 2001) therefore we assume that the major proportion of Pb exposure in bears is related to human use of Pb. The generally high Pb blood concentrations of bears and the differences in Pb concentrations between lactating and non-lactating females indicate endogenous release of Pb stored in other body parts into the blood. The exogenous sources remain unclear, but berry consumption, ingestion of soil during foraging and Pb from large game hunting with Pb-based ammunition are most likely the major contributing factors. For suckling offspring, Pb in milk is most likely the major source. Based on the Pb blood concentrations observed the early life stages and latent exposure on the population level, we would expect to observe adverse effects at individual or population level. Our findings are in contradiction to the dramatic decrease in Pb exposure in Scandinavia over the past decades in both the environment and humans as a result of successful mitigation of Pb pollution from gasoline additives. We see a strong need to investigate the source of the Pb exposure in the bears and to investigate potential negative health effects in order to further reduce Pb pollution in the environment.

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Author contributions

Arnemo: Conceptualization; **Fuchs, Thiel:** Methodology; **Rodushkin:** Resources**; Arnemo, Boesen, Evans, Fuchs, Græsli, Hydeskov, Rodushkin, Thiel:** Investigation; **Fuchs, Thiel:** Formal analysis; **Fuchs:** Writing – Original Draft; **Arnemo, Brown, Evans, Fuchs, Græsli, Hydeskov, Kindberg, Rodushkin, Thiel, Zedrosser:** Writing – Review & Editing; **Arnemo, Zedrosser:** Supervision; **Arnemo, Kindberg:** Project administration; **Arnemo, Kindberg:** Funding acquisition

Competing interests

I. Rodushkin is employed by ALS Global AB in Luleå, Sweden.

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Table 1: Mean blood lead (Pb) concentrations (µg/L) of brown bears (*Ursus arctos*) in Scandinavia, collected in 2010, 2013, and 2017-2020. Concentrations are shown for all bears in the data set (All), solitary males and females, females accompanied by dependent offspring, and dependent offspring. N = sample size, mean = arithmetic mean, SD = standard deviation of the mean, range = minimum and maximum values observed in the data.

Group	N	Mean	SD	Range
All	153	96.6	35.6	38.7 – 220.5
Solitary	42	87.2	32.9	40.9 – 175.2
- males	15	104.2	42.4	41.9 – 175.2
- females	27	77.7	21.9	40.9 – 132.3
Females with dependent offspring	44	112.0	37.5	53.1 – 220.5
All offspring	67	92.3	33.0	38.7 – 166.5

Table 2: Model selection for estimating blood lead (Pb) concentration (µg/L) in female brown bears (*Ursus arctos*) in Scandinavia, based on the Akaike Information Criterion for small sample size (AICc). Estimated parameters are lactating or non-lactating bears (Status), the Age of the bear in years, and the ID of the spatial Cluster, the number of estimated parameters (K), the AICc, the AICc difference to the top model (ΔAICc) and the weight of evidence (W_i) for each model. Data was collected in 2010, 2013, and 2017-2019.

Model	Parameters	K	AICc	ΔAICc	W_i
Full	Status + Age + Cluster	7	518.63	0	0.71
Status	Status + Cluster	6	520.47	1.84	0.28
Age	Age + Cluster	6	535.86	17.23	0.00
Cluster	Cluster	5	536.25	17.62	0.00
Null	1	2	546.36	27.73	0.00

Table 3: Generalized linear regression model estimates predicting blood lead (Pb) concentrations (µg/L) for female brown bears (*Ursus arctos*) in Scandinavia for the study period 2010, 2013, and 2017-2019. Status Lactating: Lactating female with offspring, Age: Age of the bear in years, Cluster: ID of the four spatial clusters based on spatial cluster analysis (Cluster Fulufjell is used as intercept).

Coefficient	Estimate	Standard Error	95% Confidence intervals
Intercept	100.00	11.89	76.69 – 123.32
Status Lactating	35.98	10.01	16.35 – 55.60
Age	-1.68	0.79	-3.23 – -0.12
Cluster Älvdalen	28.94	20.77	-11.76 – 69.64
Cluster Noppi West	-16.58	11.20	-38.54 – 5.37
Cluster Noppi East	-27.17	11.39	-49.50 – -4.84

Table 4: Mixed linear regression model estimates predicting blood lead (Pb) concentrations (µg/L) for offspring brown bears *(Ursus arctos)* in Scandinavia for the study period 2010, 2013, and 2017-2020, dependent on the blood Pb concentration of the mother and whether offspring is one or two years old. The mothers ID was used as a random factor.

Coefficient	Estimate	Standard Error	95% confidence interval
Intercept	7.48	11.42	-15.29 – 30.24
Blood Pb Mother	0.77	0.10	0.57 – 0.97
Offspring age: 2 years	7.41	6.22	-4.79 – 19.60

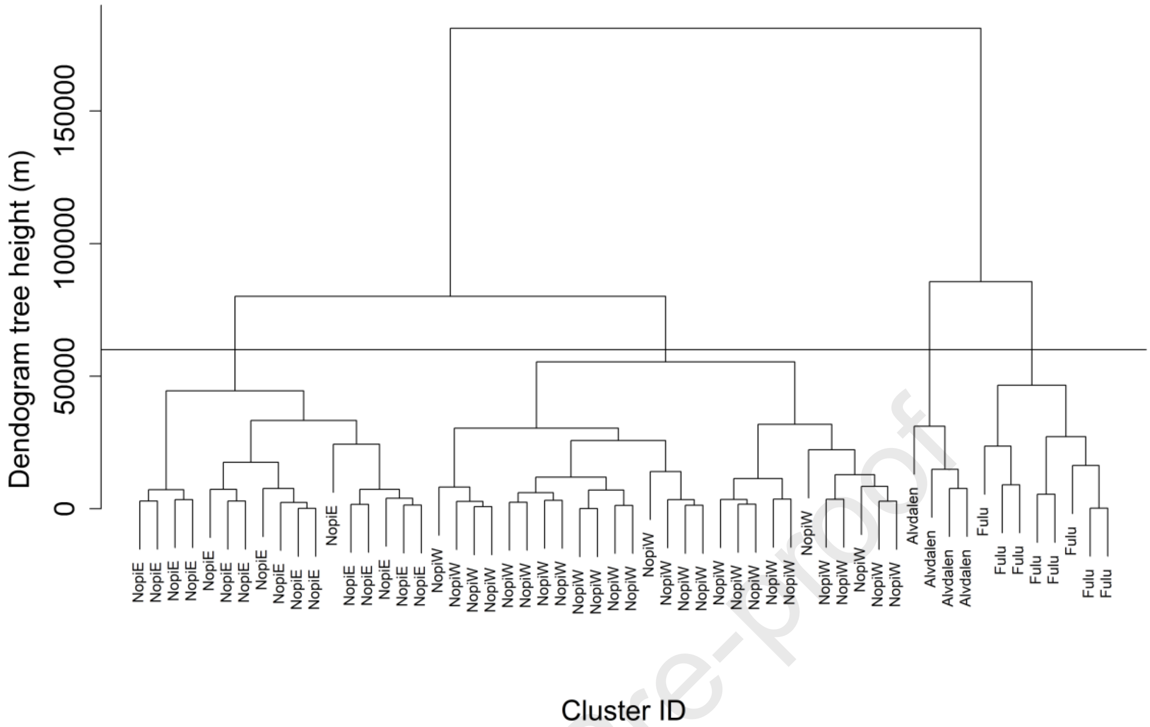

Figure 1: Dendrogram of the hierarchical cluster analysis based on brown bear (*Ursus arctos*) sampling locations from Scandinavia in 2010, 2013, and 2017-2019. Locations grouped below the cutoff value of 60,000 meters (solid horizontal line) build a spatial cluster. The cluster ID is added to the dataset and included in the linear model.

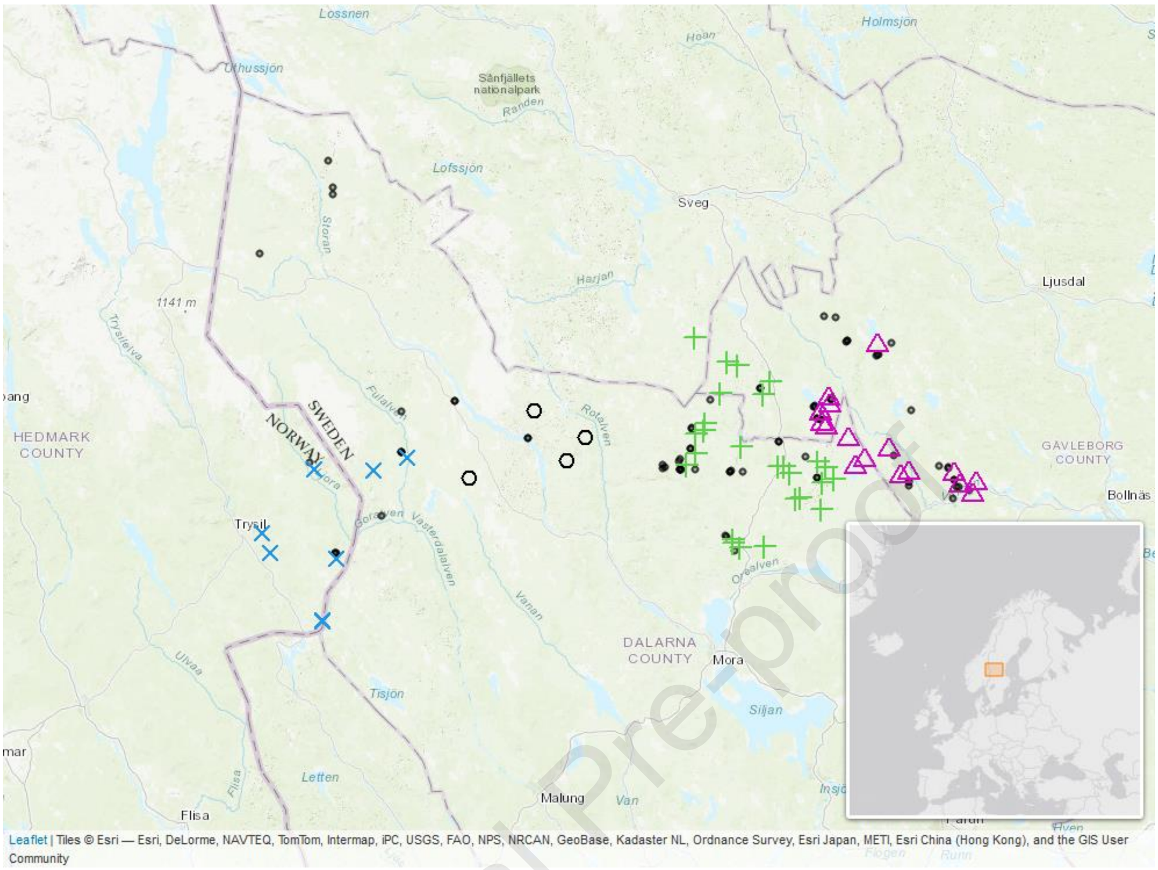

Figure 2: Sampling locations of Pb concentrations in blood and milk from Scandinavian brown bears (*Ursus arctos*) collected in 2010, 2013, and 2017-2019. Adult females included in the life history model are divided in four spatial clusters; from West to East: Fulufjell (blue X), Älvdalen (open black circles), Noppi West (green crosses) and Noppi East (pink triangles). Sampling locations of independent males, females with missing information about the age and all dependent offspring are displayed as black dots.

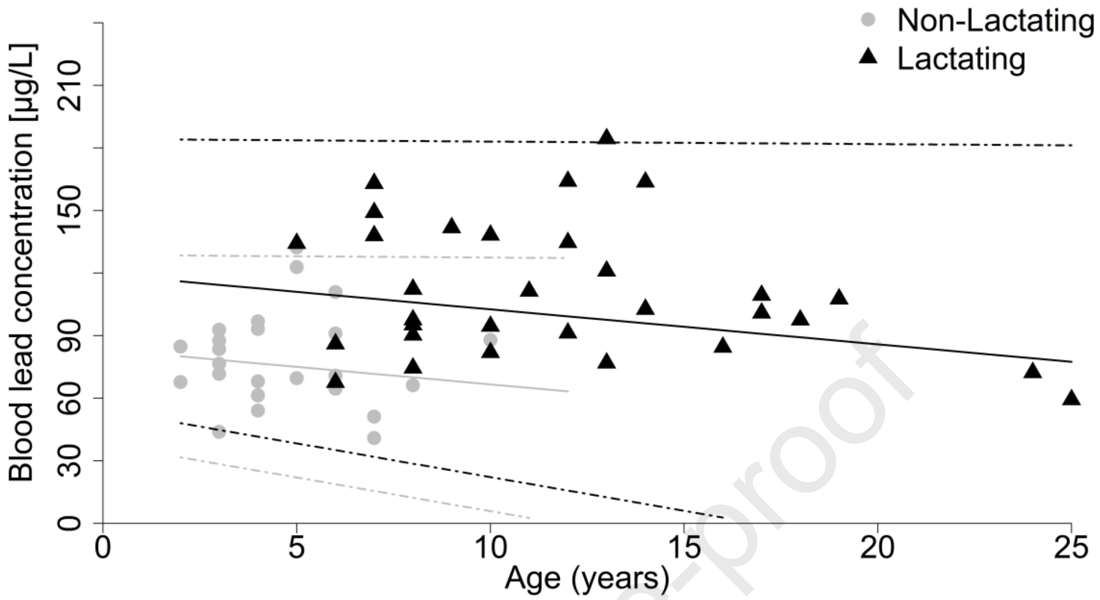

Figure 3: Blood lead (Pb) concentrations of lactating (black triangle) and non-lactating (grey dot) adult female brown bears (*Ursus arctos*) in Scandinavia in relation to their age. Model predictions for a lactating bear (black solid line) with 95% confidence interval (CI; black dash-dotted lines) and non-lactating bears (grey solid line, 95% CI grey dash-dotted lines) in the Noppi West cluster. Data collected in 2010, 2013, and 2017-2019.

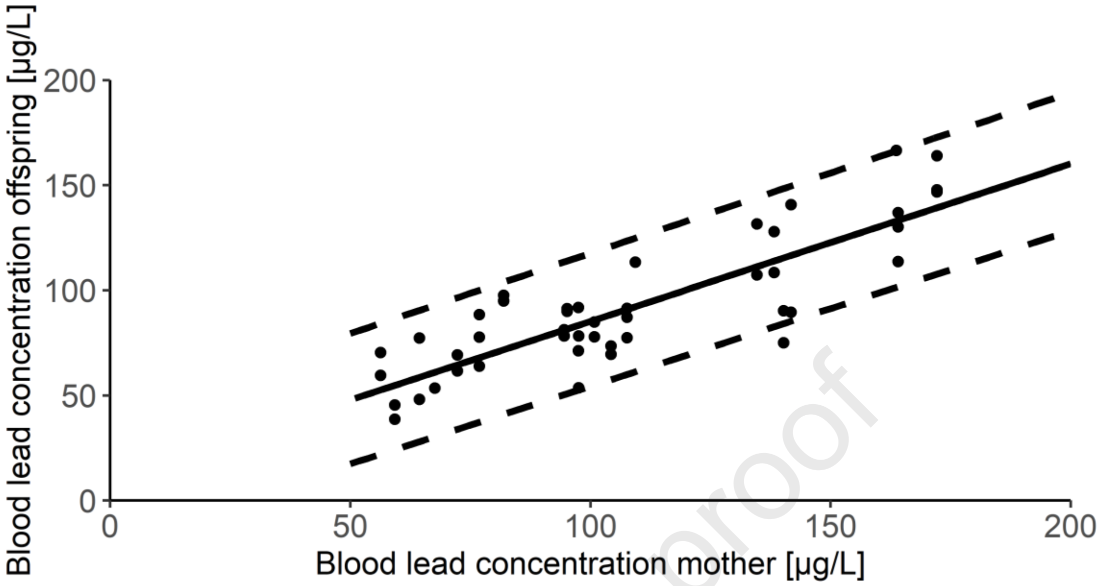

Figure 4: Blood lead (Pb) concentrations of Scandinavian brown bear *(Ursus arctos)* offspring in relation to the blood lead concentration of their mother (points) and predicted values for female offspring from a linear mixed model (solid line) with 95% confidence intervals (dashed lines). Data collected in 2010, 2013, and 2017-2020.

Highlights

- Blood and milk lead (Pb) of Scandinavian brown bears was quantified by ICP-SFMS
- In lactating female bears, milk Pb was positively correlated with blood Pb
- Blood Pb of suckling cubs was positively correlated with their mothers blood Pb
- Dependent cubs are exposed to milk Pb throughout their suckling period
- Scandinavian brown bears are highly exposed to environmental Pb

Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

I. Rodushkin is employed by ALS Global AB in Luleå, Sweden.