Inland Norway
University of
Applied Sciences

Faculty of Applied Ecology, Agricultural Sciences and Biotechnology

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Lead (Pb) exposure and source tracing in Scandinavian brown bears *(Ursus arctos)*

PhD in Applied Ecology and Biotechnology
2024

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Sammendrag

Bly (Pb) er et giftig tungmetall som er blitt fjernet fra de fleste hverdagsprodukter, inkludert bensin og maling. Som et resultat har blynivåene hos mennesker og i miljøet blitt drastisk redusert. Brunbjørner (*Ursus arctos*) i Skandinavia har blynivåer som motsier denne generelle utviklingen. Ved å sammenligne blykonsentrasjoner i blodet hos brunbjørn med andre vilter og de viktigste matkildene, hadde denne studien som mål å kartlegge nivåer av blyeksponering i populasjonen og å spore blykilder. Blykonsentrasjon ble målt med induktivt koblet plasmamassespektrometri i blodprøver samlet mellom 2010 og 2023 i Skandinavia og Alaska.

Gjennomsnittlig (SD) blykonsentrasjon i blodet hos de undersøkte bjørnene var 89 (36) µg/L, med et spenn på 20-221 µg/L. Dette er høyere enn hos elger (*Alces alces*) [7 (3) µg/L] og ulver (*Canis lupus*) [3,5 (4) µg/L], men lavere enn hos ravner (*Corvus corax*) [192 (132) µg/L]. Eksponeringen for bly hos brunbjørner i store nasjonalparker i Alaska var betydelig lavere enn hos skandinaviske brunbjørner og den laveste rapporterte for noen brunbjørnpopulasjon. Laktasjon var assosiert med økt blykonsentrasjon i blodet og melken hos binner, med påfølgende høy blyeksponering hos diende unger. Den isotopiske sammensetningen av bly i bjørnenes blod var lik den som er i miljøet og blyholdig jaktammunisjon. Blykonsentrasjonen i blodet hos bjørner var høyere i områder med høye blykonsentrasjoner i miljøet og en større tetthet av slakteplasser fra elgjakt.

Skandinaviske brunbjørner eksponeres for bly fra tidlig alder, med blodnivåer som anses som en helsefare hos mennesker. Sammensetningen av blyisotoper i blodet skiller seg romlig i forskjellige grupper av bjørner og sannsynligvis er dette fra bly som tas opp når bjørner eter maur og materiale fra maurtuer. Blykonsentrasjon i skogsbær, maur og maurtuer er ikke høyere enn i matjord og kan ikke forklare de høye konsentrasjonene målt hos bjørner. Ravn og bjørn er åtseletere og slakterester fra dyr skutt med blyholdig kan sannsynligvis forklare de høye blykonsentrasjonene i blodet hos disse artene.

Abstract

Lead (Pb) is a toxic heavy metal that has been eliminated from most everyday products including gasoline and paint. As a result, Pb exposure levels in humans and the general environment have dramatically declined. Brown bears (*Ursus arctos*) in Scandinavia have Pb exposure levels that contradict this general development. Using blood Pb concentrations of brown bears, sympatric wildlife and concentrations in food items, this study aims to map exposure levels within the population and trace sources of Pb exposure. Whole blood samples were collected between 2010 and 2024 in south-central Scandinavia and Alaska and Pb concentrations measured using inductively coupled plasma mass spectrometry.

The mean (SD) blood Pb concentration in the studied Scandinavian bears was 89 µg/L (36), with a range of 20 – 221 µg/L. Higher as compared to sympatric moose (*Alces alces*) (7 µg/L [3]) and wolves (*Canis lupus*) (3.5 µg/L [4]) but less than in common ravens (*Corvus corax*) (192 [132]). Exposure to Pb in brown bears in large national parks in Alaska were significantly lower than in Scandinavian brown bears and the lowest reported for any brown bear population. Lactation was associated with increased blood Pb concentration which in turn was reflected in the milk, resulting in high exposure in nursing cubs. The isotopic composition of blood Pb in bears was similar to that of environmental background and Pb-based hunting ammunition. Blood Pb concentration in bears was higher in areas with higher environmental background Pb concentrations, and in areas with a denser distribution of hunter-killed moose.

Scandinavian brown bears are exposed to Pb from early life, at levels considered a health concern in humans. Environmental background Pb, likely taken up when bears unintentionally ingest ant nest material, influences Pb isotopic compositions in spatially different groups. Pb concentrations in food items and ant nest material are not higher than in topsoil and unlikely to explain the high concentrations measured in bears. Scavenging on hunting remains of animals shot with Pb-based ammunition is a source with high exposure potential, likely contributing to the high blood Pb concentrations in bears and ravens.

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List of Papers

Paper 1

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Paper 2

Brown, L., Fuchs, B., Arnemo, J. M., Kindberg, J., Rodushkin, I., Zedrosser, A., & Pelletier, F. (2023). **Lead exposure in brown bears is linked to environmental levels and the distribution of moose kills**. *Science of The Total Environment*, 162099. https://doi.org/10.1016/j.scitotenv.2023.162099

Paper 3

Fuchs, B., Joly, K., Hilderbrand, G. V., Evans, A. L., Rodushkin, I., Mangipane, L. S., Mangipane, B. A., Gustine, D. D., Zedrosser, A., Brown, L., & Arnemo, J. M. (2023). **Toxic elements in arctic and sub-arctic brown bears: Blood concentrations of As, Cd, Hg and Pb in relation to diet, age, and human footprint**. *Environmental Research*, *229*, 115952. https://doi.org/10.1016/j.envres.2023.115952

Paper 4

Fuchs, B., Thiel, A., Rodushkin, I., Zedrosser, A., Støen, O.G., Nordli, K.T., Zimmermann, B., Wabakken, P., Langvall, O., Kindberg, J., Friebe, A., Græsli, A.R., Stenbacka, F., Evans A.L., Ericsson, G., Arnemo, J.M. **Contamination from leaded gasoline combustion and hunting ammunition exposes brown bears *Ursus arctos* and other wildlife to lead (Pb) in Scandinavia**. (Manuscript)

Introduction

The magnitude of human impact on Earth's geology, climate, and ecosystems has led to the proposal of a new geological epoch called the Anthropocene. Throughout human history, it has been virtually impossible for any society to exist without impacting the environment. Contamination with both natural chemicals and xenobiotics is a key contributor to the environmental impacts that define the Anthropocene epoch (Crutzen, 2006). The environment possesses a certain buffer capacity, but excessive contamination leads to biological damage, resulting in irreversible pollution (Chapman, 2007). Every form of contamination incurs a cost, yet for every cost, there is an alternative. With knowledge, we can choose whether to bear the cost of contamination or the cost of reducing it.

Lead (Pb) is a non-essential element, toxic to all living organisms (ATSDR, 2020). For humans, 450 mg/kg body mass (36g / 80kg) is considered a lethal dose (CDC, 1994). Exposure to even relatively low Pb concentrations has been linked to detrimental effects on various organ systems, including the cardiovascular, renal, skeletal neurological, haematological, and endocrine systems (ATSDR, 2020; Bergdahl and Skerfving, 2022). Non-lethal acute symptoms of Pb poisoning are nonspecific, including headaches, fatigue and, increased systolic and asystolic blood pressure. Long term effects include decreased cognitive function, altered mood, behavior and neuromotor functions and chronic kidney disease. The developing central nervous system is at particular risk for Pb toxicity, already in the fetal status and Pb exposure results in irreversible damage (ATSDR, 2020). The European Food Safety Authority (EFSA) has set Pb blood concentration benchmark dose levels (BMDL) for increased systolic blood pressure and chronic kidney disease in adults and developmental neurotoxicity in children, at 36, 15 and 12 µg/L, respectively (EFSA, 2013). However, there is to date no established safe level of Pb exposure (Bergdahl and Skerfving, 2022).

Sources of lead in the environment

Pb is naturally present in all environments, concentrations vary but are generally low. These natural background concentrations have been substantially increased by anthropogenic activities globally (AMAP, 2005; Khalid et al., 2017; von Storch et al., 2003). In Scandinavia, natural concentrations measured in sediments and peat bogs dated to 1 000 years before common era (BCE), are close to zero (Renberg et al., 2001). In Europe, Pb has been mined and refined for more than 2 000 years. Annual production of Pb around year 0 is estimated to 80 000 metric tons (Settle and Patterson, 1980). Primitive smelting technology produced high losses of Pb by evaporation causing widespread pollution measurable as increased Pb concentrations in sediments (Renberg et al., 2001). The use of Pb increased during the industrialization period and accelerated further because of its use as antiknock additive in gasoline. During peak use in the early 1970s, 375 000 metric tons of Pb were used in gasoline annually (Nriagu, 1990). Inhalation of evaporated Pb, increasing Pb blood concentrations in humans, became a major public health concern (Rabinowitz et al., 1973). Industry interests denied scientific evidence and successfully lobbied legislation. For example, the European Union prohibited member states´ gas stations that exclusively offered unleaded fuels until 1986. With time, public opinion shifted and the contamination of the air from industry exhaust and private traffic became a major political topic and resulted in a gradual phase out of Pb in gasoline (Nriagu, 1990; von Storch et al., 2003). By 1994, the USA and European countries had reduced maximal permitted Pb concentration in gasoline dramatically (Nriagu, 1990). As a result, atmospheric depositions of Pb declined in northern Europe (Lind et al., 2006; von Storch et al., 2003). In mosses, serving as bioindicator of air pollution, Pb concentrations decreased by 96% in the period from 1975 to 2015 in Sweden (Danielsson and Karlsson, 2015). In southern Sweden, the blood Pb levels of children decreased from 60 µg/L in 1978 to 11 µg/L in 2007 (Skerfving et al., 2015; Strömberg et al., 2003), and to 8.5 µg/L by 2019 (Karolinska Institutet, 2020). However, Pb is persistent, up to 99 % of the Pb in the top 10 to 15 cm in Norwegian forest soils, is classified as contaminant Pb (Steinnes et al., 2005).

Lead in hunting ammunition

While the use of Pb additives in gasoline and paint have been phased out, Pb-based hunting ammunition is a remaining source of environmental pollution (Arnemo et al., 2016; Bellinger et al., 2013; Byrne, 2023). For small game, hunters use predominantly shot, shells loaded with pellets fired from a shotgun at relatively low velocities (Stokke et al., 2018). Alternatively, hunters use bullets, a single projectile fired from a rifle at high velocity. Rifle bullets for large game expand upon impact and most hunting bullets are designed to fragment (McTee et al., 2023; Stokke et al., 2018). Expansion and fragmentation maximize transformation of the kinetic energy from the projectile to the game, increasing tissue trauma and blood loss (Stokke et al., 2018, 2017). While alternative materials for both shot and bullets with similar performance are available on the market for a decade (Pierce et al., 2015; Thomas, 2013), most hunters use Pb-based ammunition. In Sweden, 98% of all moose are shot with high velocity, expanding Pb-based rifle bullets (Stokke et al., 2017). Fragments may have volumes from several mm^3 down to nanoparticles, which are able to cross intact cell membranes (Hunt et al., 2009; Kollander et al., 2017; McTee et al., 2023). Bioavailability, the fraction of ingested Pb that is absorbed into the blood stream, is higher for smaller fragments due to a higher surface to volume ratio (ATSDR, 2020). Retrieved Pb-based bullets from shot moose, including bullets designed not to fragment, had an average weight loss of 2.8 g (Stokke et al., 2017). Despite a broad scientific agreement on the health risks for both humans and wildlife, a phaseout or reduction of Pb-based ammunition is challenged by hunting, shooting and ammunition industry organizations in Europe and the USA (Arnemo et al., 2016; Byrne, 2023). Restrictions on the use of Pb shot in wetlands in USA and northern Europe have been in place since the early nineties and have been recently consolidated and extended to all EU member states (Thomas and Kanstrup, 2023). Total bans on any Pb-based hunting ammunition are in place for some federal states in Germany and the State of California (Mateo and Kanstrup, 2019; Schulz et al., 2023). Denmark as the first country that announced a total ban on all Pb based ammunition used for hunting from 2024 (Sonne et al., 2022).

Lead source tracing

Pb consists of four stable isotopes (^{204}Pb, ^{206}b, ^{207}Pb, ^{208}Pb). Of these, three are radiogenic i.e. end members of radioactive chains, starting with uranium isotopes (^{238}U and ^{235}U end in ^{206}Pb, ^{207}Pb) and thorium (^{232}Th) ends in ^{208}Pb (Hansmann and Köppel, 2000). All ^{204}Pb isotopes on earth are older than the solar system i.e. they are entirely primordial. Radioactive decay is happening at a constant rate resulting in a linearly increasing proportion of the radiogenic isotopes in relation to the primordial isotope. The atomic ratios between ^{206}Pb, ^{207}Pb and ^{208}Pb depends on the U/Pb and Th/Pb concentration ratios of the mother material (Gulson, 2008). Ores used to produce industrial Pb are characterized by a low proportion of radiogenic isotopes and an ore-specific U/Pb and Th/Pb concentration ratio. Natural background Pb from weathered bedrock, however, has a high proportion of radiogenic Pb (Hansmann and Köppel, 2000). Since most Pb ores have a specific U/Pb and

Th/Pb ratio and in addition have been formed at different times, they also have a specific isotopic composition, and isotope ratios can be used to trace ore derived Pb back to its origin.

The isotopic composition of Pb has been used for contamination source tracking for several decades (Gulson, 2008). Due to the proliferation of the world Pb market in combination with increasing recycling activities, the isotopic composition of modern industrial Pb becomes an industrial mix and it has become more difficult to differentiate industrial sources (Komárek et al., 2008; van den Heever et al., 2023). Methods to quantify Pb isotopes, based on multi-collector plasma mass spectrometry (MC-ICP-MS) at relatively low Pb concentrations, reveal high measurement uncertainty, especially for ^{204}Pb because of the low total abundance (Gulson et al., 2018). In current environmental exposure studies, source attribution based on stable Pb isotopes is usually coupled with other data. For example, spatial movement behavior in vultures (Arrondo et al., 2020; van den Heever et al., 2023) or temporal progression of the available biomass from hunting remains in ravens (Legagneux et al., 2014).

Lead in wildlife

Ingestion is the primary pathway of environmental Pb exposure in wildlife (Hydeskov et al., 2024; Ma, 2011). Inhalation of aerosols and dust are important pathways for humans, but less is known about this pathway for wildlife. Especially denning mammals might be exposed to Pb from dust. Ingestion of soil, either intentionally for example at mineral licks, or unintentional when consuming items that are in contact with soil, for example carrion, may be the most important exposure route for wildlife of Pb today (Cartró-Sabaté et al., 2019). In humans, bioavailability of Pb from soil is estimated 60 times higher than Pb in food (ATSDR, 2020). Mercury (Hg), another heavy metal with an anthropogenic global scale contamination history, is known to biomagnify in food webs, concentrations in organisms increase along the trophic level (Scheuhammer et al., 2007). This is not the case for Pb. Concentrations are usually lower in the consumer as compared to the source (Ali and Khan, 2019; with exemptions e.g.: Rubio-Franchini and Rico-Martínez, 2011). However, Pb like Hg, bioaccumulates, i.e., the accumulation of the substance in tissue over time (Ali and Khan, 2019). For bioaccumulation to happen, the uptake must be at a higher rate than the elimination from the tissues. Ingested or inhaled Pb is taken up into the red blood cells, via the gastric system or the lungs (ATSDR, 2020; Collin et al., 2022). In humans, Pb follows a three-compartment kinetic model. In blood, the first compartment, Pb has a half-life of three to four weeks, from the blood the Pb is distributed to and accumulating in the second compartment, the internal organs, especially the liver and kidneys, from where some of the Pb is excreted via urine and bile (Rabinowitz et al., 1976). The third compartment is the bones, Pb in bones has a half-life of 30 years. In adult humans, 94% of the total body burden of Pb is stored in bones (ATSDR, 2020; Collin et al., 2022). The kinetic process is dynamic, and concentrations probably depend on each other, eventually reaching an equilibrium. Bone Pb can be reabsorbed and become an endogenous source elevating the blood Pb concentration (Silbergeld, 1991). Due to the different half-life times in the three compartments, blood has been used to track recent Pb exposure, bones for long term exposure (Ma, 2011). However, in chronically exposed organisms, blood Pb concentration represents a mix of recent and long-term exposure, due to the constant transfer between the three compartments (Gulson et al., 1995). The elimination time from a tissue might vary substantially with exposure history, in chronically exposed humans for example, the time to half the blood Pb concentration can be up to two years (ATSDR, 2020).

Health effects in wildlife

Scientific evaluations of Pb related health effects in wildlife have mainly focused on exposure in birds, especially waterfowl and scavenging raptors (Buekers et al., 2009; Green et al., 2022; Hydeskov et al., 2024; Pain et al., 2019). Both groups are susceptible to Pb from ammunition. Many waterfowl and gallinaceous species take up Pb shot as grit, whereas avian scavengers ingest Pb-based ammunition fragments in hunting remains (Bellrose, 1959; Byrne, 2023; Mateo, 2009; Pain et al., 2019). In Europe, large scavenging raptors, especially golden and white-tailed eagles (*Aquila chrysaetos, Heliaeetus albicilla*) are susceptible to scavenging related Pb exposure. In these species, up to 25% of the known mortality is attributed to acute Pb poisoning resulting in an estimated adult population reduction of 12 to 14% (Bassi et al., 2021; Green et al., 2022; Helander et al., 2009; Meyer et al., 2022). High mortality rates, reduced reproduction and subsequent population declines in hunted waterfowl species are the background for a complete ban of Pb shot in wetlands in the European Union including Norway and Iceland, implemented in 2023 (Thomas and Kanstrup, 2023). While it is relatively straight forward to estimate toxic effects on individual performance in experimental studies, it is notoriously difficult to apply this knowledge to a population in a real-life situation. One of the major complicators is density dependence, characterized by a negative relation between population growth rate and population density, potentially compensating for mortalities or decreased reproduction by increased survival in the remaining population (Grant, 1998; Noël, et al., 2006; Pain et al., 2019). Recovering populations with a low intrinsic growth rate, i.e., older age at first reproduction, small litters or brood size and long nursing time, are most susceptible to Pb related losses (Green et al., 2022; Meyer et al., 2022; Pain et al., 2019). The fact that populations replace losses due to poisoning via density dependent mechanisms, do not relieve from the ethical consideration of impairing health or death on an individual level.

Sublethal effects in birds include anemia due to decreased δ-aminolaevulinic acid dehydratase (ALAD) activity, increased oxidative stress, immune system disruption and lipid metabolism alterations (Martinez-Haro et al., 2011; Sato et al., 2016). In general, birds seem to have higher thresholds for displaying clinical signs from Pb exposure compared to mammals but for both groups decreased ALAD activity is expected at blood Pb concentrations below 100 µg/L (Buekers et al., 2009). Buekers et al. (2009) suggested 100 to 250 µg/L and 260 to 1 160 µg/L for mammals and birds, respectively, as the 5th percentile of no effect concentrations for impaired growth or reproduction.

Lead exposure in scavengers

Scavengers are particularly exposed to ammunition Pb. Scavenging birds, such as vultures, eagles and corvids, are specialized in locating carcasses (Green et al., 2022; Pain et al., 2019). In addition, avian scavengers have high uptake rates of ingested Pb due to the high HCl concentration in their stomach (Golden et al., 2016). This is relevant independently of the Pb source and recent studies suggest that soil Pb contributes substantially to Pb exposure in vultures (Arrondo et al., 2020; van den Heever et al., 2023). Reports on Pb exposure in free-ranging scavenging mammals are scarce and often based on single observations or exposure potential rather than data (Chiverton et al., 2022; Hydeskov et al., 2024; Rodríguez-Jorquera et al., 2017).

Lead exposure in bears

Brown bears (*Ursus arctos*) in Scandinavia combine risk factors for Pb exposure, accumulation, and health impairment. Brown bears scavenge (Ordiz et al., 2020), have long lifespans of up to 27 years in the wild (Brasington et al., 2023) and display slow life-histories with the first reproduction at four years of age and a one - two years long period of nursing offspring (Bischof et al., 2018; Van de Walle et al., 2018). Brown bears from the greater Yellowstone area, USA, have been reported to have elevated mean Pb blood concentrations (55 µg/L), higher than sympatric black bears (19 µg/L) (*Ursus americanus*), while wolves (*Canis lupus*) had blood Pb concentrations < 14 µg/L (Rogers et al., 2012). A similar picture is reported by Lazarus et al. (2017) where kidney Pb concentrations were double in brown bears as compared to wolves. Rogers et al. (2012) found that bears further away from the Yellowstone National Park have higher blood Pb concentrations, the highest concentrations (> 100 µg/L) were measured mostly in late summer/fall and before the hunting season started. The lack of spikes or a general increase of blood Pb during hunting season, as for example in ravens (Craighead and Bedrosian, 2008; Legagneux et al., 2014), was not expected by Rogers et al. (2012) and they speculate on alternative sources such as roots and mushrooms. They also point out that ravens might be exposed to higher doses when exposed to similar amount or size of fragments compared to the much larger bears, possibly resulting in seasonal peaks in blood Pb concentration in ravens but not in bears (Rogers et al., 2012). Brown et al. (2022) found that the long-term Pb exposure of Canadian black bears, measured as Pb concentration in teeth, was related to hunter kill densities in males but not in females. In Croatian brown bears, Pb concentrations increased when bears reached sexual maturity but there were no sex differences in muscle, liver, or kidney Pb concentrations (Lazarus et al., 2018). Kidney Pb concentrations in cubs-of-the-year was higher than in yearlings but similar to their mothers, suggesting maternal embryotoxic and lactational Pb exposure (Lazarus et al., 2018). The highest blood Pb concentrations in brown bears were measured in Scandinavia, ranging from 33 to 173 µg/L with median 83 µg/L (Boesen et al., 2019).

Bears in Scandinavia

In 2023, the bear population in Scandinavia was estimated between 2 500 and 3 000 individuals. The population has previously been around 3 000 bears but is decreasing due to the increased license hunting quota in Sweden (Brøseth et al., 2023; Sköld and Åsbrink, 2023). In Gävleborg county in central Sweden, bear density was 0.08/km^2, the highest in Scandinavia and brown bears were hunted here at a rate of 0.008/km^2 in 2023

(Länsstyrelserna, 2023). In Sweden hunting starts 21st August and lasts until 15th October or until the license quota is filled. Most hunters use pursuing or baying dogs, and most bears are shot within the first three days of the hunt (Brown et al., 2023; Le Grand et al., 2019).

Brown bears display highly adaptive feeding behaviors both on individual and population levels, ranging from highly carnivorous to almost herbivorous (Hertel et al., 2018; Hilderbrand et al., 1999; Koike et al., 2012). In Scandinavia, bears rely on berries (*Vaccinium* ssp. and *Empetrum nigrum*) during the hyperphagic period in later summer and autumn when they gain fat reserves for hibernation (Hertel et al., 2018; Stenset et al., 2016; Van de Walle et al., 2018). An important food resource for the entire active phase are ants (mainly ants of the genus *Formica* and *Camponotus*). The estimated dietary energy content (EDEC) from fecal samples covered by ants is 20, 28 and 11% for spring, summer and fall respectively (Stenset et al., 2016). Bears in Scandinavia predate on neonate moose in late spring, otherwise scavenging is the dominant pathway for meat consumption (Ordiz et al., 2020; Rauset et al., 2012; Swenson et al., 2007). During the hyperphagic period, meat from vertebrates constituted 13% of the EDEC, while in spring and summer this was 61% and 62%, respectively (Stenset et al., 2016).

Brown bears in Scandinavia hibernate for up to six months, beginning in late October (Evans et al., 2016; Friebe et al., 2001; Thiel et al., 2022). During hibernation they do not feed, drink, urinate or defecate (Nelson et al., 1973). Breeding season peaks in early June but implantation is delayed until November/December, after the female has entered the den. During the gestation period female bears display active level metabolism and maintain a stable normal body temperature (Evans et al., 2016; Friebe et al., 2014; Lemière et al., 2022; Tsubota et al., 1987). Fifty-six to 60 days after implantation, the female gives birth to one to three cubs and her metabolism drops again to hibernation levels while cubs are nursing (Friebe et al., 2014; Lemière et al., 2022; Tsubota et al., 1987). Offspring remain with their mother for 1.5 to 2.5 years and separation often occurs during the breeding season (Van de Walle et al., 2018).

Objectives

The aim of the study was to assess Pb exposure levels and to trace the source of the Pb exposure in the Scandinavian brown bear population. In the first step (Paper 1) I investigated the wide range of blood Pb concentrations suggested by Boesen et al. (2019), and whether intrinsic or geographic factors may explain variation within the population. I hypothesized that blood Pb concentration would increase with lactation and age. The results provided information to include in further analyses and point out spatial or social groups of particular interest.

The object of Paper 2 was to identify the exposure to environmental background Pb and Pb-based hunting ammunition of the Scandinavian brown bears, using a spatially explicit comparison of the blood Pb concentration in Scandinavian brown bears in relation to the Pb concentration in plant roots and the density of hunter-killed moose. Plant roots are used as a quantitative measure of environmental background Pb, i.e., the sum of Pb naturally present in the environment and added by human activities. Moose kill distribution serves as an indicator of hunting activity and subsequent Pb exposure through ingested ammunition fragments. The results correlated sources to exposure and can be an indication of a quantitative contribution of each.

In central Scandinavia, large aerial Pb deposits have increased the environmental background Pb. Additionally, relatively high harvest densities of moose, provide hunting remains from animals shot with Pb-based rifle ammunition to scavengers. The object of Paper 3 was to compare Pb exposure in the Scandinavian brown bears to conspecifics in large Alaskan national parks. The Alaskan bears are less exposed to industrial Pb as there is no hunting and historically much less aerial Pb depositions. I hypothesize that Pb exposure in all Alaskan areas is considerably lower as compared to Scandinavia. Differences in feeding behavior among the three Alaskan and the Scandinavian populations allowed to relate exposure to Pb and other heavy metals to different diets. The Alaskan bears function as a low exposure comparison, and I could document very low blood concentrations in these brown bears.

The object of Paper 4 was to identify and quantify sources that contribute to the blood Pb in Scandinavian brown bears. I compare Pb blood concentrations of the Scandinavian bears to other sympatric wildlife species, hypothesizing that the scavenging bears, ravens and wolverines have higher blood Pb concentrations due to exposure via hunting remains. In contrast to carnivorous but non-scavenging wolves and herbivorous moose, that only are exposed to environmental background Pb. In a second step I use Pb isotopes and relate the isotopic composition of the Pb in bear blood to potential sources including ants, ant nest material, Pb-based rifle ammunition, leaded gasoline and soil. I also relate the Pb isotopic composition in bears to spatial, and annual variation.

Methods and Results

Study area

Data from Scandinavian brown bears were collected within the study area of an ongoing long-term monitoring and research project in south central Scandinavia (SBBRP 2020), (Figure1). The area is covered with intensively managed coniferous forest (*Pinus sylvestris* and *Picea abies*), interspersed with bogs and a few agricultural fields in the west. Human density is below 9/km^2, and in large parts below 2/km^2. Paved and gravel roads, are spread through the area at a density of 0.3 and 0.7 km/ km^2, respectively. 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. Snow covers the area typically from December to April in the east and from October to early May in the west (Thiel et al., 2022).

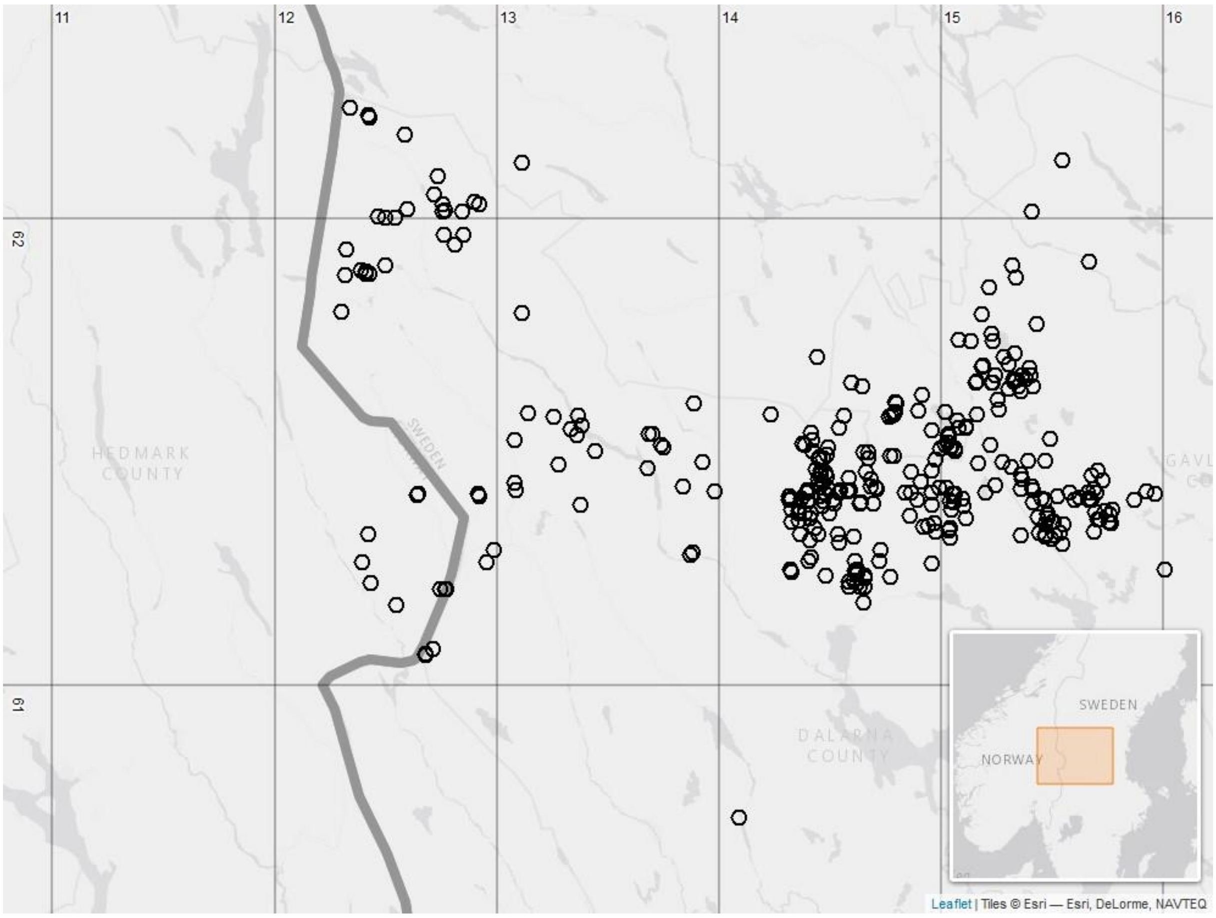

Figure 1: Locations of blood sampling of individual Scandinavian brown bears (*Ursus arctos*) used in this study (n=376 blood samples from 166 individual bears) in southcentral Scandinavia between 2010 and 2023.

Sampling

All bears were captured and sampled according to an established protocol (Arnemo and Evans, 2017), 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). Bears were darted from a helicopter and immobilized using a combination of tiletamine-zolazepam and medetomidine (Arnemo and Evans, 2017). Blood was collected from the jugular vein in 6mL evacuated heparin trace element tubes (Vacuette, Greiner Bio-One International GmbH, Kremsmünster, Austria) and in 4 mL evacuated K3EDTA tubes (Vacuette). Blood tubes were frozen at -20° C until shipment to the laboratory. Concentrations of 72 elements and ratios of Pb isotopes were analyzed using high-resolution inductively-coupled plasma sector field mass spectrometry (ICP_SFMS, ELEMENT XR, ThermoScientific, Bremen, Germany).

Statistical analysis and Results Paper 1-4

Paper 1

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

I summarized a data set of 153 blood samples from 110 free-ranging Scandinavian brown bears collected between 2010 and 2019. The mean blood Pb concentration of solitary males was (104 µg/L, 42 – 175 µg/L, n=15) as compared to solitary females (78 µg/L, 41 -132 µg/L, n= 27). Using a candidate set of GLMs, I estimated the effects of age, body mass, reproductive status and spatial distribution on the blood Pb concentration of a subsample of the whole data set, only comprising sexual mature, female brown bears (n = 56, 4 - 6 years of age). I selected models using the Akaike Information Criterion (AIC) (Mazerolle, 2019). Model predicted blood Pb concentration for lactating bears was 1.3 to 1.6 times higher than in non-lactating females (Figure 2). Blood Pb concentrations were higher in the west of the study area. There was no linear decrease from west to east, spatial differences were only evident when samples were grouped into clusters. For 28 female bears, we obtained paired blood samples from 56 dependent cubs, and from 20 of these lactating females, we measured the Pb concentration in their milk. 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.

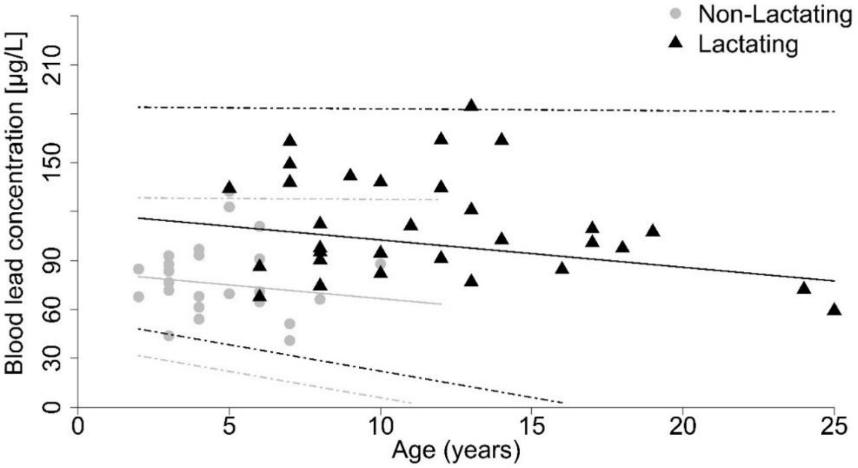

Figure 2: Blood lead (Pb) concentrations of lactating (black triangle) and nonlactating (grey dot) adult female brown bears (*Ursus arctos*) in Scandinavia in relation to their age. Model predictions for a lactating bear (black solid line) with 95% confidence interval (CI; black dash-dotted lines) and non-lactating bears (grey solid line, 95% CI grey dash-dotted lines) in the central study area. Data collected in 2010, 2013, and 2017–2019.

Paper 2

Lead exposure in brown bears is linked to environmental lead levels and the distribution of moose kills

Using linear regression models, we related the blood Pb concentration in female Scandinavian brown bears to environmental Pb, estimated from plant root concentrations and hunter-killed moose distribution during the previous fall. Additionally, the lactational status and the sampling year were added as variables. Based on AIC, the full model, including all tested variables was selected over sub models including either environmental Pb, or moose kill distribution. Both, environmental Pb concentrations as well as distribution of moose kills was positively related to the bears blood Pb concentration (Figure 3). The Pb concentration in plant roots was only available for the central and eastern parts of the Scandinavian brown bear research project study area. These results suggest that both environmental background Pb and the ingestion of Pb-based ammunition fragments contribute to the Pb exposure in Scandinavian brown bears.

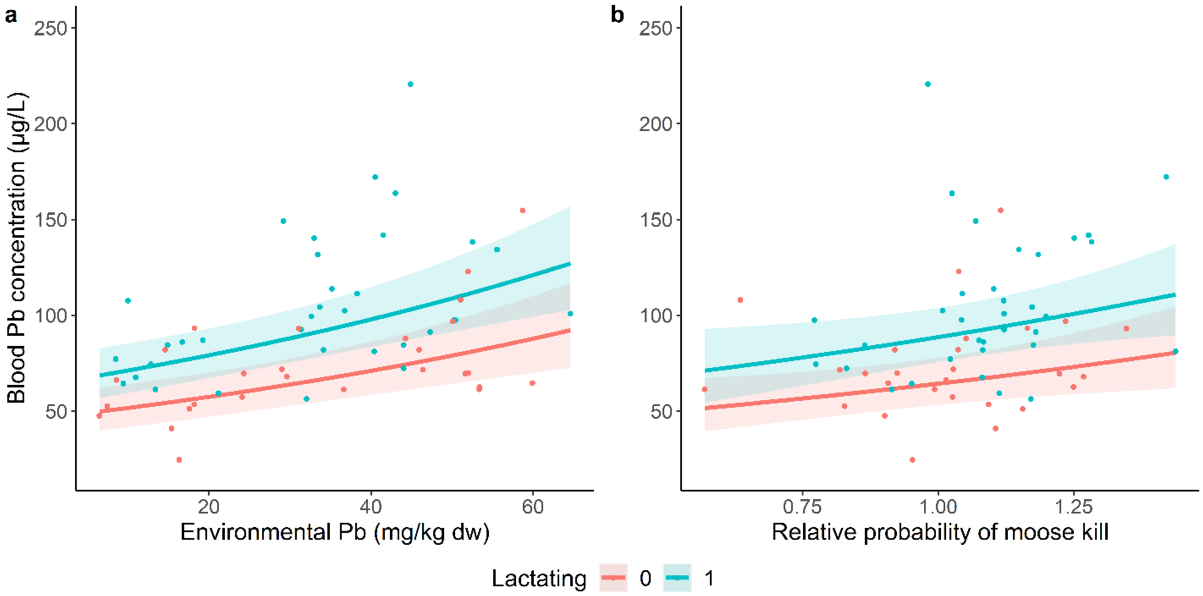

Figure 3: Predicted blood Pb concentrations (µg/L) in female brown bears (*Ursus arctos*) (n = 34 individuals; n = 61 samples) in relation to a) environmental Pb concentrations (mg/kg dry weight) and b) the relative probability of moose kill during the previous hunting season (t - 1) around the capture locations (2 km buffer) in south-central Sweden during 2017-2020. Lines show predictions and shaded polygons represent 95% confidence intervals of the models, whereas the points represent raw data. Blue points/lines indicate that the female was lactating (lactating = 1), whereas red lines/points indicates that they were not (lactating = 0).

Paper 3

Toxic elements in arctic and sub-arctic brown bears: Blood concentrations of As, Cd, Hg and Pb in relation to diet, age, and human footprint

We quantified blood concentrations of arsenic (As), cadmium (Cd), mercury (Hg) and Pb in brown bears (n = 72) four years and older in Scandinavia and three national parks in Alaska, USA. Bears in Lake Clark and Katmai National Parks, rely on salmon (*Oncorhynchus* spp.) during their hyperphagic period while bears in the Gates of the Arctic National Park either feed on salmon, or like the Scandinavian bears, on vegetation (berries) as primary diet in late summer and fall. We associated the study area as a proxy of the typical population level diet and human footprint, as well as age and sex of the bears, to their heavy metal concentration. Bears in Alaska that consumed salmon had higher Hg blood concentrations than bears feeding on berries, ants and moose. The concentrations of Cd and Pb were higher in

Scandinavian bears than in the Alaskan bears (Figure 4). Blood concentrations of As were comparable. Within the Gates of the Arctic National Park, only bears that primarily fed on vegetation had elevated Pb blood concentrations. These results show that while the remoteness of the Alaskan bears does not protect them from anthropogenic Hg pollution, their Pb blood concentration was the lowest so far reported in a bear population. This makes it very unlikely that the elevated Pb blood concentration measured in some bear populations are a result of potential different kinetics in bears related to their physiology but rather reflects actual exposure.

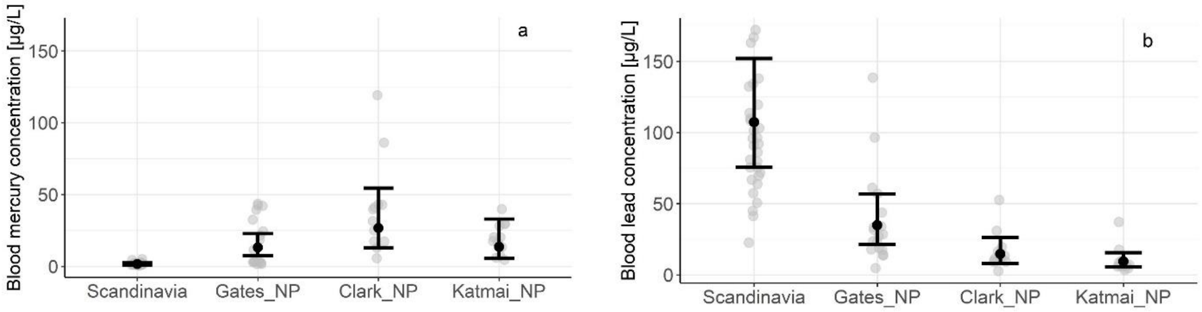

Figure 4: Concentrations of mercury (a) and lead (b) in blood from brown bears *(Ursus arctos)* (µg/L, black dots) in south-central Scandinavia and three national park and preserves (NP) in Alaska, USA, predicted by generalized linear models including sample area, sex, and age of the bears as fixed factors. Error bars indicate 95% confidence intervals for the predictions. The raw data, collected between 2010 and 2021, are displayed as grey dots.

Paper 4

Contamination from leaded gasoline combustion and hunting ammunition exposes brown bears (*Ursus arctos*) and other wildlife to lead (Pb) in Scandinavia

Paper four was divided in two steps. First, I descriptively present Pb concentrations and isotopic compositions from bear blood, sympatric wildlife species and potential sources. I found that the median Pb blood concentrations were highest in bears (84 µg/L) and ravens (172 µg/L), which are scavengers (Table 1). Mean Pb blood concentrations in moose and wolves were very low (< 10 µg/L). In wolverines, the mean Pb blood concentration was also low (10 µg/L). Wolverines are known to scavenge and are likely exposed to ammunition Pb. The highest source potential for environmental Pb was found in ant nesting material, which is accidently ingested by bears when consuming ants. To put this in perspective, to ingest 1 g of Pb, a bear would have to consume 250 kg of ant nest material, 3 937 kg of ants and 125 000 kg of berries, all at median dry weight concentrations. An expanding Pb-based hunting bullet has an average weight loss of 2.8 g when retrieved from a shot moose, fragments of varying size partly in the offal commonly left for scavengers such as bears, ravens and wolverines.

Table 1: Mean, median, standard deviation (Sd), range and sample size for blood lead (Pb) concentrations in brown bears (*Ursus arctos*), moose (*Alces alces*), common raven (*Corvus corax*), wolf (*Canis lupus*) and wolverine (*Gulo gulo*) sampled in Scandinavia between 2018 and 2023.

Species	Mean µg/L	Median µg/L	Sd µg/L	Range µg/L	n
Brown Bear	89.1	83.8	35.5	20.4 – 220.5	355
Moose	7.0	6.2	3.4	2.0 – 14.0	31
Raven	192.1	171.8	131.8	48.3 – 849.3	45
Wolf	3.5	2.1	4.0	0.8 – 20.0	31
Wolverine	10.1	6.5	11.8	2.7 – 54.5	18

The Pb isotopic composition in bear blood overlapped with expected values from modern industrial Pb as used in ammunition. However, due to the mixing geometry (Figure 5), it is also possible that Pb in bears is a mix of natural background Pb and Pb from aerial depositions during the leaded gasoline era.

In a second step, I related the bears blood Pb isotopic ratio (^{207}Pb/^{206}Pb) with the spatial origin and tested for annual variation using regression models. We found that the bears from different areas also differed in their Pb ratio. We interpreted that environmental background Pb may be a relevant contributor. Annual variations in Pb isotopic ratios were not linked to bilberry production.

In conclusion, bears in Scandinavia are exposed to anthropogenic Pb sourcing from aerial depositions from past use of leaded gasoline and current use of Pb-based hunting ammunition. Quantifying the contribution of these two sources was not possible.

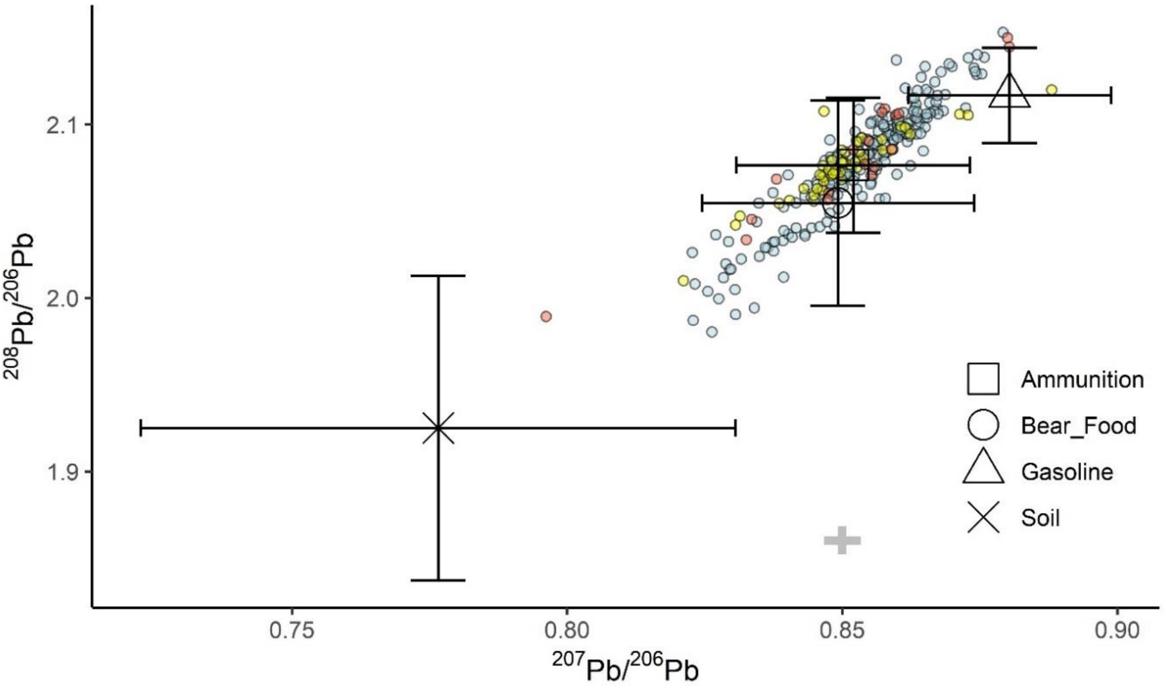

Figure 5. Three-isotope plot ($^{208}Pb/^{206}Pb$ vs $^{207}Pb/^{206}Pb$) in whole blood samples from brown bears (*Ursus arctos*) (blue dots), common ravens (*Corvus corax*) (yellow dots) and wolverines (*Gulo gulo*) (red dots) sampled in Scandinavia between 2019 and 2022. As well as the mean and standard deviation of agricultural soils in the study area (cross), Pb-based rifle ammunition used for hunting (square) and European leaded gasoline (triangle). Ants (*Formica* ssp.), ant nest material and berries (*Vaccinium* ssp., *Empetrum nigrum*) representing bear food are grouped together for better visibility (circle). Isotope data from soil is from the geochemical atlas Europe (“GEMAS,” 2021), ammunition is joined data from this study and Arrondo et al., 2020. Isotopic composition of European leaded gasoline is reported by Hansmann and Köppel, 2000; Hopper et al., 1991; Monna et al., 1997; and Resongles et al., 2021. The grey cross indicates measurement uncertainty of the Pb isotopic composition in blood.

Discussion

Blood Pb concentration in Scandinavian bears was on average 27 times higher compared to sympatric non-scavenging wolves, and half of that of ravens (Paper 4). The Scandinavian brown bears blood Pb concentrations were significantly higher than in Alaskan brown bears in large, protected areas with no hunting and a lower human footprint (Paper 3). Scandinavian brown bears in areas with higher environmental background Pb concentrations and a denser distribution of moose kills, had higher blood Pb concentrations (Paper 2). The isotopic composition of ants, ant nest material and berries, all food items consumed by bears, are highly influenced by aerial depositions from gasoline combustion. The Pb isotopic composition in the blood from bears overlapped with both natural food items and industrial Pb used to manufacture hunting ammunition. Ant nest material had the highest potential for Pb exposure among natural food items, concentrations in berries were very low (Paper 4). Bears in Scandinavia are highly exposed to Pb from industrial sources, on a level likely to impair individual health, but less as compared to specialized avian scavengers (Paper 1 & 4). The Pb isotopic composition of ammunition Pb and natural food items, particularly that of ant nest material overlaps to such an extent that it is not possible to quantify source contribution using isotopic mixing models (Paper 4). Mean (range) blood Pb concentration in all Scandinavian bears included in this study was 89 µg/L (20 – 221 µg/L). Lactating females and nursing offspring are particularly exposed due to remobilization of Pb from bone during lactation and from exposure to Pb in early life through milk (Paper 1).

Sources of lead exposure in bears

Legacy Pb from the combustion of leaded gasoline substantially increased the environmental background Pb in Scandinavia (Renberg et al., 2001; von Storch et al., 2003). Environmental background Pb is an important contributor to the Pb exposure in Scandinavian brown bears: 1.) Blood Pb concentrations in bears increased with the background concentration, 2.) The ^{207}Pb/^{206}Pb ratio in the bears blood differed between spatial clusters of sampling locations, likely reflecting spatial differences in the composition of the background Pb. This was further confirmed by ^{207}Pb/^{206}Pb ratios of moose from the eastern part of the study area, these were similar to bears but differed from moose in the western part of the study area. 3.) The isotopic composition of ant nest material overlapped with that of the bears' blood, and the

Pb concentration in bears was 10 times higher than in ants and > 400 higher than in berries (all dry weight concentrations). Pb from soil, which I assume is the source of Pb within ant nests, is 60 times more bioavailable than Pb in food or water (ATSDR, 2020). Soil is also considered to be an important Pb source in vultures (Arrondo et al., 2020; van den Heever et al., 2023). The Alaskan bears live in areas that have been less exposed to aerial depositions resulting in very low blood Pb concentrations, with a few exceptions of bears living in the Gates of the Arctic National Park. Based on the $\delta^{13}C$ and $\delta^{15}N$ stable isotopes in the hair of these individuals, they predominantly feed on vegetation, potentially ingesting soil with elevated background Pb.

In Scandinavia bears often excavated large ant mounts as dens for hibernation (Friebe et al., 2001), this potentially results in dust inhalation as an additional potential exposure route for soil Pb. Even though the Pb concentration in ant nest material is much higher than in other foods ingested by bears, they are lower than most agricultural soils in Sweden. The highest concentration measured in ant nest material was 17 600 µg/kg, similar to the median concentration in agricultural soil in Sweden (Eriksson et al., 2010). Wild boars (*Sus scrofa*) are ingesting agricultural soil in Sweden. Malmsten et al. (2021) reports a mean liver tissue concentration of 140 µg/kg ww (recalculated from 0.14 mg/kg ww) in wild boar in southern Sweden. As comparison, bears from Croatia had a much higher Pb kidney concentration of 1 075 µg/kg ww than the wild boars, but mean blood Pb concentration in Croatian bears (different individuals) was 61 µg/L, less than in Scandinavian bears (Lazarus et al., 2020, 2018). In conclusion, environmental Pb from soil ingestion contributes to the blood Pb in Scandinavian brown bears, but it is unlikely to be the only source.

Another source of Pb exposure in brown bears is Pb-based hunting ammunition: 1.) The bear blood Pb concentration correlated with the moose harvest distribution, and an increasing probability of a hunter-killed moose within a 4 km buffer around the sampling location was related with a higher Pb concentration in the blood sample from the bear. Increasing blood Pb concentrations along harvest density gradients are also found in black bear males, and avian scavengers (Brown et al., 2022; Singh et al., 2021; West et al., 2017). However, bears in the Scandinavian study area are not actively searching for hunting remains during the moose hunt and scavenging these seem mostly opportunistic (Brown et al., 2023). 2.) The isotopic composition of Pb in bear blood was very similar to that of Pb ammunition and ravens.

However, the isotopic composition of ant nest material and ammunition overlap completely, and it is not possible to reliably distinguish these sources based on Pb isotopes through mixing models. 3.) Compared to bears, mean blood Pb concentration in wolves and moose were 96 % and 92 % lower, respectively, while that of ravens was twice as high. Sampled wolves were either territorial adults or pups within the natal territory, both scavenge rarely and are very reluctant to visit hunting remains (Wikenros et al., 2023, 2013). These findings fit with the very low median blood Pb concentration of 2 µg/L in wolves. Thus, the scavenging species blood Pb concentrations are 13 to 54 times higher compared to a strict herbivore and a non-scavenging carnivore. Wolverines, however, do scavenge, and hunters may actively use hunting remains on baits to attract wolverines, but the mean blood Pb concentration was low (10 µg/L) and similar to that of moose (7 µg/L). Exposure to Pb in wolverines appears to be sporadic.

Lead exposure in bears

The mean blood Pb concentration in dependent offspring (92 µg/L), was correlated to the blood Pb concentration of the mother and exceeded the EFSA threshold for developmental neurotoxicity in children (12 µg/L) by a factor of eight (EFSA, 2013). The blood Pb concentration of the mother was correlated with the Pb concentration in her milk, indicating the exposure route for nursing offspring. Increased maternal blood Pb concentrations are the result of calcium turnover during lactation and pregnancy remobilizing Pb from bones (Gulson et al., 1998; Silbergeld, 1991). Pb-related health effects are related to blood Pb concentrations, it is the blood transporting Pb to the target tissues such as liver, kidney, and brain (Collin et al., 2022). One of the first measurable effects is decreased ALAD activity, Pb is displacing essential zinc (Zn) bound to the enzyme, impairing the heme biosynthetic reaction (Heinemann et al., 2008; Montenegro et al., 2006). This has been observed in free-ranging mallards and golden eagles at blood Pb concentrations < 60 µg/L (Martinez-Haro et al., 2011). Skerfving et al. (2015) found blood Pb concentrations < 50 µg/L during childhood negatively correlated to the cognitive ability at age 16 to 18. Stressors can potentiate adverse Pb effects, laboratory rodents at blood Pb concentrations of 150 µg/L showed similar effects on central nervous system (CNS) functions as the group at 50 µg/L exposed to a stressor in addition (Virgolini et al., 2008). Compared to other brown bear populations, mean blood Pb concentrations in Scandinavian bears are higher than in Alaskan bears (10 to

38 µg/L, depending on the area), or as compared to brown bears in Yellowstone (55 µg/L), Poland and Croatia (61 µg/L) (Lazarus et al., 2020; Rogers et al., 2012). However, giant pandas (*Ailuropoda melanoleuca*) in the captive breeding program in the Shaanxi Wild Animal Research Center in China, are reported with a mean Pb blood concentration of 188 µg/L attributed to high aerial Pb depositions and subsequent contamination of bamboo, their primary food (Chen et al., 2018).

In this study I relate blood Pb concentrations to long term factors such as lactational status, spatial distribution and the distribution of moose kills six months before the measurement. However, Pb has a rapid turn-over rate in blood. For example domestic pigs fed with minced game meat, contaminated with ammunition fragments and had increased blood Pb from less than 10 µg/L to above 20 µg/L within two days and back to pre-exposure concentrations after nine days (Hunt et al., 2009). However, repeated measurements in individual bears in different years did not signal significant annual differences, indicating that Pb blood concentrations were rather stable and with relatively small short-term variation. This doesn't imply that the half-life of elimination rate of Pb in blood is different in bears compared to other mammals, but that, as in humans, the time to half the concentration of Pb in the blood probably depends on the body burden, the remobilization rate from bones, exposure history and age (ATSDR, 2020; Specht et al., 2019, 2016). Blood is a very useful non-destructive tissue to measure Pb exposure, especially in chronic exposed populations.

Lead in ravens

Ravens are carcass specialists, breeding pairs are territorial and defend food sources against non-breeding "vagrants". These non-breeders build larger flocks to outnumber the territorial birds in order to compete for carcasses. They scout for carcasses alone or in small flocks, communicate their findings at the common nocturnal roost and fly into the carcass in large groups at dawn the following morning (Heinrich, 2011; Heinrich and Marzluff, 1995). Ravens are long-lived (10 to 15 years in the wild), and have shown cognitive learning, long term social memory and complex social behaviors, both in free-ranging as well as in experimental captive situations (eg. Boeckle and Bugnyar, 2012; Cibulski et al., 2014; Heinrich, 2011 states that > 1 400 articles are published on ravens). Considering the irreversible effects of Pb on the developing CNS, paired with long term health effects, I assume ravens and other corvids to be particularly impaired by Pb exposure. Ravens are very efficient in finding hunting remains and are the species that consumes the largest proportion of the available biomass in areas with no bears present (Gomo et al., 2017). This behavior exposes ravens to Pb fragments in hunting remains and elevated blood Pb concentrations (Craighead and Bedrosian, 2008; Legagneux et al., 2014; West et al., 2017).

Background concentration

In soil, “geochemical background”, has been defined as the natural content of a substance, resulting from geological processes, excluding any anthropogenic input (Bini et al., 2011). In wildlife studies, definitions of background concentrations or exposure thresholds, are less consistent. In ravens and white-backed vultures (*Gyps africanus*), 100 µg/L has been used as background concentration, without clear definition (Craighead and Bedrosian, 2008; Legagneux et al., 2014; van den Heever et al., 2023; West et al., 2017). Arrondo et al. (2020) uses 200 µg/L as background concentration, citing Pain et al. (2019), which uses 200 µg/L as cut off between subclinical and clinical poisoning. Buekers et al. (2009) reviewed repeated dose toxicity studies. They presented background blood Pb concentrations ranging from 10 to 700 µg/L, for birds, at a mean of 120 µg/L (n = 15), with a standard deviation (SD) of 190 µg/L, and for mammals 70 µg/L (n=13, SD= 70 µg/L) (Buekers et al., 2009). Mean concentration in moose and wolves are 10 % and 5 %, as compared to the 70 µg/L suggested as background concentration by Buekers et al (2009). The background concentration in tissues of wildlife may be defined as the inevitable exposure level, at a given environmental background, for a given species. Individuals deviating towards a higher exposure level may then be exposed to an additional source. Such a definition requires knowledge of the “inevitable” exposure level, ideally from a controlled situation, individuals that for sure are not exposed to the additional source, but from a habitat with similar environmental background. Such information is lacking in most wildlife studies. The comparison of sympatric wildlife species may be an alternative approach. Wolves in our study with a mean blood Pb concentration of 3.5 µg/L indicate that most bears in our study have Pb exposure levels above what would be expected inevitable background.

Lead isotopes

The isotopic compositions revealed a fundamental model of isotope mixing, identifying atmospheric Pb from gasoline emissions as the non-radiogenic endpoint and the local geological background (soil) as the radiogenic endpoint. Bullet fragments as well as all the biological samples, including all blood samples, berries, ants and ant nesting materials fall in between. Matrices that are not influenced by ammunition contain a mixture of the environmental background. Thus, for scavenging bears and ravens, based on only the isotopic mixture in their blood, it was not possible to distinguish between the environmental background and ammunition fragments. Similar, in South African white-backed vultures the isotopic ratio of the environmental background overlapped with that of ammunition and interpretation was only possible in combination with feeding ecology (van den Heever et al., 2023).

I have done extensive testing in fitting Bayesian mixing models (MixSIAR, Stock et al., 2018) to this data set. The output of these models are quantitative estimates of how much a source, for example ammunition contributes to the Pb in a mixture, for example bear blood (eg. Arrondo et al., 2020). Due to the high correlation of sources, the uncertainty of these estimates was so high, that I considered interpretation or publication was not appropriate (Longman et al., 2018; Stock et al., 2018).

Conclusions

Phasing out leaded gasoline is a global one health success story, that decreased aerial Pb depositions by over 90% in Scandinavia. Indeed, Pb exposure in non-scavenging wildlife, such as moose and wolves, is very low today. Despite this success, Pb exposure in brown bears and ravens remain on high levels that implicate health impairments. Scavenging on hunting remains, shot with Pb-based ammunition, is an exposure source specific for bears and ravens compared to wolves and moose. An additional exposure source of Pb for bears might be the unintentional uptake of ant nest material when consuming ants. The isotopic composition of the bears' blood Pb, overlaps well with industrial Pb used to manufacture hunting ammunition and is within the isotopic mixture of industrial Pb from gasoline combustion and natural Pb, that I found in food items of bears. Large parts of the study area are covered by intensively managed forest plantations, but human impact relative to other parts in Europe is very low. Despite the remoteness, large amounts of historical aerial Pb depositions are a persistent contamination risk. The ongoing use of Pb-based ammunition for hunting should be replaced by the well-established, non-lead alternatives, that do not pose a health risk for game meat consumers and scavengers.

Future work

A weakness of this study is that I lack data on how often bears encounter and consume hunting remains. Anecdotally, bears are very quick in finding fresh gut piles and visit these often already in the first night. During spring, we use known dumping sites for slaughter remains to find unmarked bears for capture. By visiting GPS positions from bears during the moose hunt and in early spring an encounter rate could be related to Pb blood concentrations.

The comparison of Pb exposure with other species should include mammals that are likely to ingest soil, for example wild boars or Eurasian badgers (*Meles meles*). The latter is of particular interest as they undergo pronounced hypometabolism during winter. Such a comparison might be helpful to establish environmental background levels for bears.

There is an increasing use of Bayesian mixing models to quantify sources of Pb-based on stable Pb isotopes. In some publications, basic assumptions of these models are obviously unintentional violated. Whether Pb isotopic mixtures that include modern industrial Pb can meet these model assumptions is unclear. There is an urgent need for a validation of this method using experimental Pb mixtures with known endpoints.

In case of a complete and enforced ban on Pb-based hunting ammunition, the monitoring of Pb exposure in scavengers in Scandinavia and elsewhere could be used to track effects of such a ban. Due to the long life-spans and exposure history in these species, blood Pb concentrations may take several years to significantly decrease.

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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: 38.7–220.5 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.

1. 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

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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 2000 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.

2. Methods

2.1. 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 (0.14 km/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).

2.2. 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.

2.3. 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).

2.4. 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, Thermo-Scientific, 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).

2.5. 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 yearlings 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.

2.6. 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. 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 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. We 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).

2.7. 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.

2.8. 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 ΔAICc ≤ 2 (Mazerolle, 2019).

3. Results

3.1. 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).

3.2. 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 (Fig. S1). Splitting the variogram into the four cardinal directions revealed no clear spatial patterns in any direction (Fig. 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; Fig. 1 and Fig. 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 (Fig. 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).

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

3.3. 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 (Fig. 4) (Table 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 = 86.1–140.6 μg/L) (Table 4).

4. 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 (Fig. 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

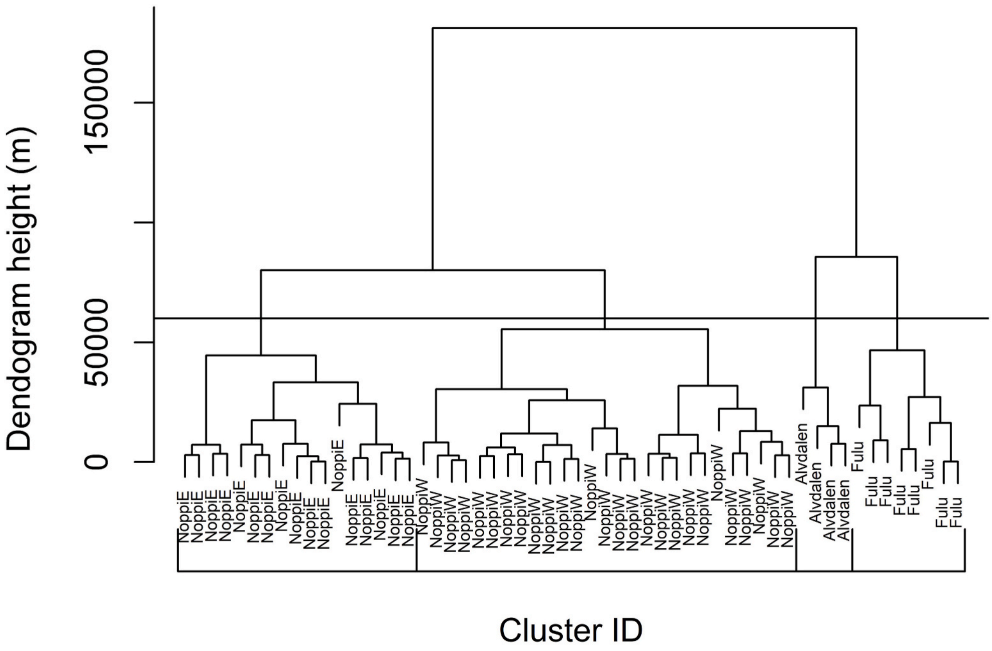

Fig. 1. Dendrogram of the hierarchical cluster analysis based on brown bear (*Ursus arctos*) sampling locations from Scandinavia in 2010, 2013, and 2017–2019. Locations grouped below the cutoff value of 60,000 m (solid horizontal line) build a spatial cluster. The cluster ID is added to the dataset and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

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 months 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 to 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 68–266 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 (Fig. 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 2–4 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 2–3 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 7–8 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

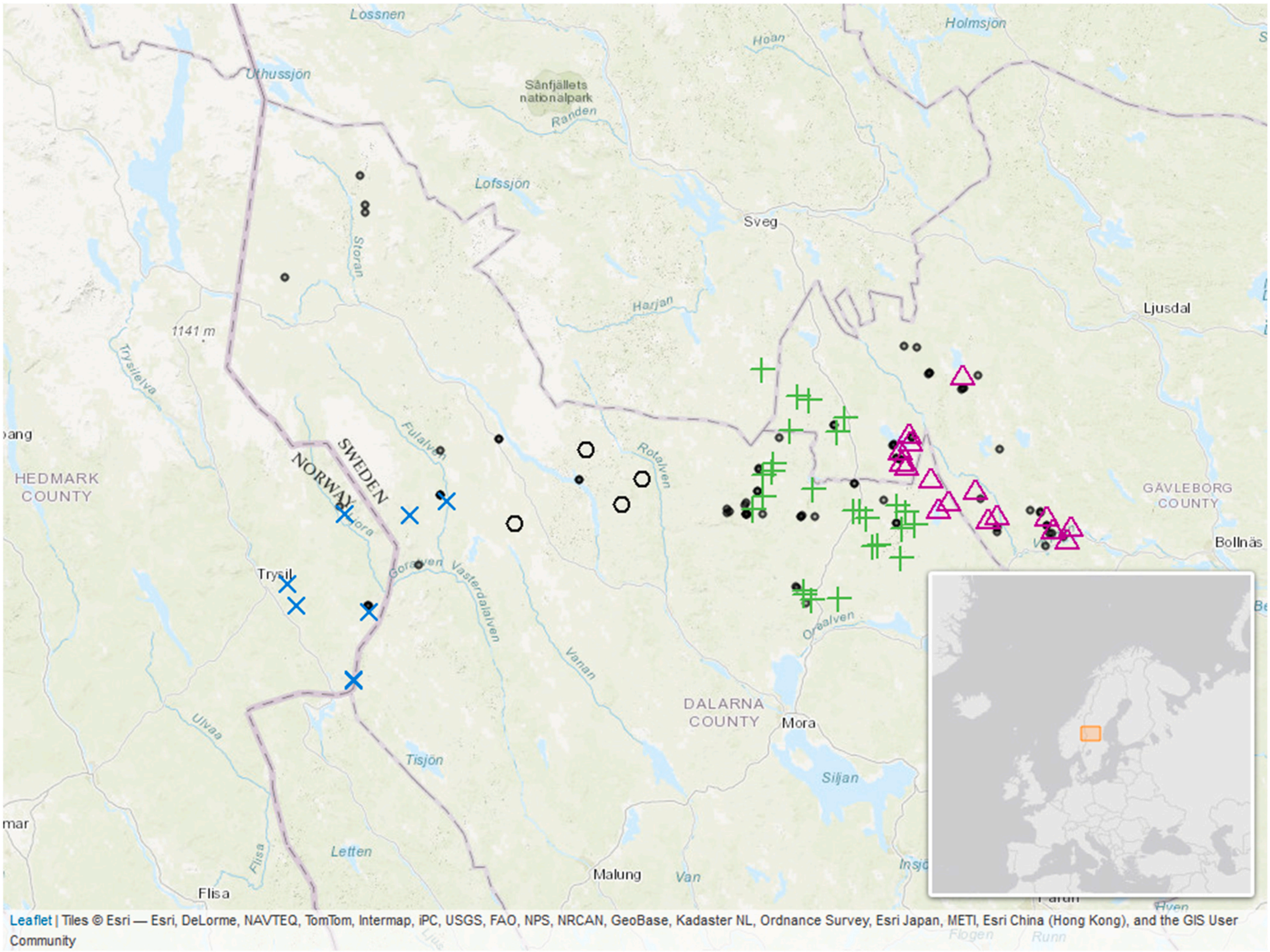

Fig. 2. Sampling locations of Pb concentrations in blood and milk from Scandinavian brown bears (*Ursus arctos*) collected in 2010, 2013, and 2017–2019. Adult females included in the life history model are divided in four spatial clusters; from West to East: Fulufjell (blue X), Älvdalen (open black circles), 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.

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

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

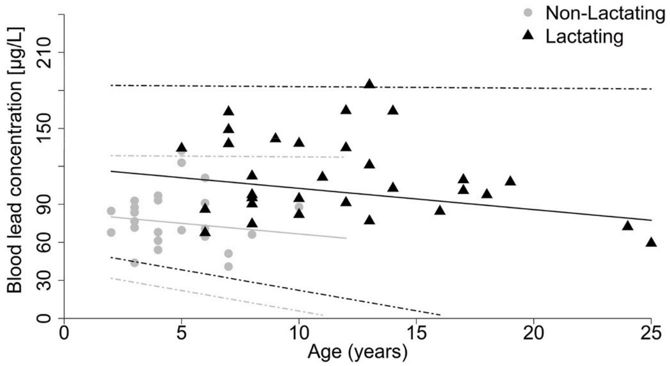

Fig. 3. Blood lead (Pb) concentrations of lactating (black triangle) and non-lactating (grey dot) adult female brown bears (*Ursus arctos*) in Scandinavia in relation to their age. Model predictions for a lactating bear (black solid line) with 95% confidence interval (CI; black dash-dotted lines) and 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.

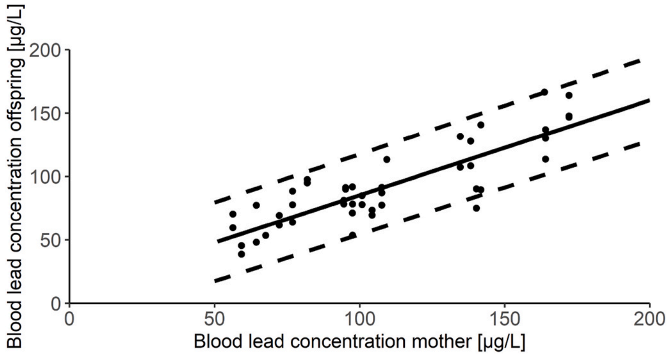

Fig. 4. Blood lead (Pb) concentrations of Scandinavian brown bear *(Ursus arctos)* offspring in relation to the blood lead concentration of their mother (points) and predicted values for female offspring from a linear mixed model (solid line) with 95% confidence intervals (dashed lines). Data collected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

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

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).

5. 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.

Declaration of competing interest

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.

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Lead exposure in brown bears is linked to environmental levels and the distribution of moose kills

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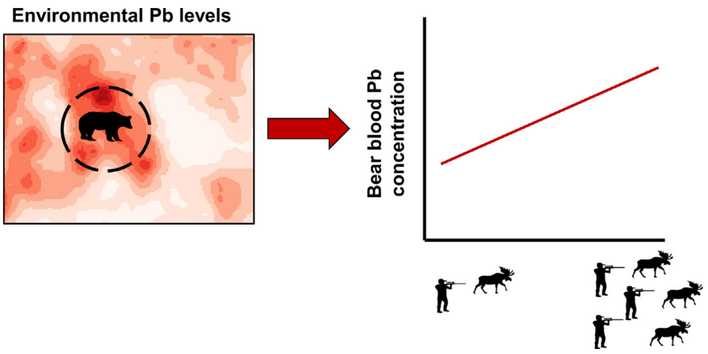
HIGHLIGHTS

- We used spatially explicit models to predict sources of Pb exposure in brown bears.
- The distribution of moose harvest was estimated with a resource selection function.
- Environmental Pb concentration was the main predictor of blood Pb levels in bears.
- Moose harvest distribution was an additional source of Pb exposure in bears.
- Scavenging on slaughter remains can be an additional source of Pb exposure in bears.

GRAPHICAL ABSTRACT



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ABSTRACT

Lead (Pb) is heterogeneously distributed in the environment and multiple sources like Pb ammunition and fossil fuel combustion can increase the risk of exposure in wildlife. Brown bears (*Ursus arctos*) in Sweden have higher blood Pb levels compared to bears from other populations, but the sources and routes of exposure are unknown. The objective of this study was to quantify the contribution of two potential sources of Pb exposure in female brown bears ($n = 34$ individuals; $n = 61$ samples). We used multiple linear regressions to determine the contribution of both environmental Pb levels estimated from plant roots and moose (*Alces alces*) kills to blood Pb concentrations in female brown bears. We found positive relationships between blood Pb concentrations in bears and both the distribution of moose kills by hunters and environmental Pb levels around capture locations. Our results suggest that the consumption of slaughter remains discarded by moose hunters is a likely significant pathway of Pb exposure and this exposure is additive to environmental Pb exposure in female brown bears in Sweden. We suggest that spatially explicit models, incorporating habitat selection analyses of harvest data, may prove useful in predicting Pb exposure in scavengers.

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1. Introduction

Lead (Pb) is a naturally occurring trace element that is heterogeneously distributed in the environment and its geochemical cycle has been affected by human activities (Arnemo et al., 2022; Komárek et al., 2008). Atmospheric Pb emissions declined in most countries following the ban on leaded gasoline during the late-20th century (Danielsson and Karlsson, 2015; Nriagu, 1990; Strömberg et al., 2008), but unleaded gasoline and smelter emissions may still influence environmental Pb levels (Chételat et al., 2022; Chrastný et al., 2018; Widory et al., 2018). Pb also has a long residence time in soils and certain areas with high historic Pb depositions still contain high levels (Berglund et al., 2009). Thus, organisms inhabiting areas with high Pb levels are at greater risk of Pb exposure either by direct soil ingestion or by foraging on soil organisms or plants (Berglund et al., 2009; Scheifler et al., 2006). For instance, passerines sampled in urban environments, where the soils are contaminated by vehicle emissions, have higher blood Pb concentrations when compared to birds sampled in rural environments (Chatelain et al., 2021; McClelland et al., 2019; Roux and Marra, 2007).

Several types of human activities, such as hunting with Pb-based ammunition, can increase the level of Pb found in the environment. Bullets used in hunting rifles are designed to expand upon penetration and shed metal fragments in tissues (Green et al., 2022; Hunt et al., 2006; Kollander et al., 2017; Leontowich et al., 2022; Menozzi et al., 2019; Stokke et al., 2017). Carcasses and gut piles discarded during the hunting season have high numbers of embedded bullet fragments and animals that scavenge on this food resource can be exposed to high Pb levels (Fisher et al., 2006; Helander et al., 2021; Legagneux et al., 2014); and the resulting risk is not uniform in space because it is intrinsically linked to the distribution of hunters on the landscape.

Spatially explicit models can be used to predict the risk of Pb exposure from multiple sources in wildlife (Mateo-Tomás et al., 2016). These models are known for being sensitive to scale (Johnson et al., 2021); yet we are currently lacking information on the fine-scale variations in risk of Pb exposure from bullet fragments embedded in discarded slaughter remains. Many studies have investigated the relationship between hunting and Pb exposure in scavengers without considering the spatial variation (Craighead and Bedrosian, 2008; Ecke et al., 2017; Legagneux et al., 2014), or only consider this aspect at coarser spatial scales (Kelly and Johnson, 2011; Singh et al., 2021), which may limit the identification of exposure sources, especially when the variations in environmental Pb levels are recorded at scales larger than the area used by model organisms (Johnson et al., 2021). In this study, we first modelled the fine-scale distribution of moose (*Alces alces*) harvest in Sweden by analysing moose harvest locations with habitat selection analysis, which originally has been developed to analyse data from GPS-collared animals (Northrup et al., 2022). Second, we determine whether variations in blood Pb levels in brown bears (*Ursus arctos*) were related to the distribution of harvested moose. Most studies that have investigated Pb exposure from bullet fragments embedded in slaughter remains in scavengers were on birds, but mammalian scavengers are also likely at risk of increased Pb exposure when feeding on slaughter remains (Brown et al., 2022; Chiverton et al., 2022; Kelly et al., 2021).

As model organism, we used the brown bear, an opportunistic omnivore that occupies large home ranges (Dahle and Swenson, 2003; Graham and Stenhouse, 2014; Schwartz et al., 2003) and feeds across trophic levels. In Sweden, brown bears feed mostly on berries, invertebrates, such as ants, as well as vertebrates, including moose calves and ungulate carcasses when available (Bojarska and Selva, 2012; Schwartz et al., 2003; Stenset et al., 2016). Due to their habitat use and foraging behaviours, brown bears could be exposed to multiple potential sources of Pb, including fossil fuel combustion and ammunition. Brown bears in Sweden are exposed to Pb from unconfirmed sources (Fuchs et al., 2021). The mean (SD) blood Pb level of 96.6 (35.6) μg/L reported by Fuchs et al. (2021) is higher than the means of 55 (40) μg/L and 58.0 (34.7) μg/L reported in North American and other European brown bears, respectively (Lazarus et al., 2018; Rogers et al., 2012). However, none of these studies have identified sources of Pb exposure in brown bears. Due to the high toxicity of Pb for vertebrates at low concentrations (Pain et al., 2019), it is important to identify the sources and understand the route of exposure in vertebrate scavengers to implement efficient management actions aiming at reducing Pb exposure in wildlife.

The aim of this study is to build a spatially explicit model to quantify the contribution of two potential sources of Pb to the blood Pb concentrations measured in Scandinavian brown bears: Pb from plant roots (hereafter refer to environmental Pb level) and Pb from ammunition used by moose hunters. We hypothesised that the environmental Pb levels, and the distribution of moose kills influence blood Pb concentrations in brown bears. We predicted that blood Pb concentrations in Scandinavian brown bears would be positively related to both environmental Pb levels, and the probability of moose kill.

2. Material and methods

2.1. Study area

The study area was in Dalarna and Gävleborg counties, south-central Sweden (~61°N, 15°E). The landscape mainly consists of a highly managed boreal forest with stands of different age classes and interspersed by lakes and bogs (Martin et al., 2010). The canopy is mainly composed of Scots pine (*Pinus sylvestris*), Norway spruce (*Picea abies*), and birch (*Betula* spp.), whereas the underlayer mainly consists of berry shrubs (*Vaccinium* spp.), heather (*Calluna vulgaris*) and grasses with mosses and lichens covering the ground (Elfström et al., 2008; Ordiz et al., 2013; Swenson et al., 1999). The area is also characterized by a dense network of forest roads (0.7 km/km^2) and low human density (4–7 inhabitants/km^2) (Martin et al., 2010; Ordiz et al., 2013).

Moose hunting in Sweden during the study period was allowed from the first Monday of September to the end of January and, on average, 84,000 moose are harvested annually during this period. Most moose (~75 %) are harvested between September and the end of October (Wikenros et al., 2013). Brown bears in Sweden typically enter their den towards the end of October (Evans et al., 2016; Friebe et al., 2001, 2014) and thus have access to the slaughter remains discarded by hunters. Those slaughter remains likely contain Pb fragments because most hunters in Scandinavia use Pb ammunition (Stokke et al., 2017).

2.2. Environmental Pb concentrations and hunting variable

We obtained a biogeochemistry (Biogeokemi) database from the Geological Survey of Sweden (© Sveriges Geologiska Undersökning) that contains the concentrations of trace elements, including Pb, of plant roots (*Carex* spp., *Fontinalis antipyretica* or *Filipendula ulmaria)* collected in or near small streams in Sweden between 1982 and 1996. The methods used for sampling and conducting chemical analyses are described in Lax and Selinus (2005). The trace element concentrations in plant roots reflect the concentrations in the water as well as those of the surrounding soil and bedrock and thus represent reliable estimates of the amount of trace elements circulating in the environment (Lax and Selinus, 2005). Our study area in south-central Sweden was represented by a total of 2264 samples of plant roots in this database. We used these samples to predict environmental Pb concentrations on a dry weight basis across our study area by using ordinary kriging. We fitted a Matern variogram with Stein's parameterization (nugget = 0.224, psill = 0.366, kappa = 0.3, range = 0–23,594 m) with the *fit.variogram* function [*gstat* package (Gräler et al., 2016)] to predict Pb concentrations across our study area with a resolution of 500 m. The interpolated surface was generated with the *krige* function [*gstat* package, (Gräler et al., 2016)]. We log-transformed Pb concentrations and added a constant (C = 2.5) to all observations to obtain normally distributed data ($W = 0.99, p = 0.06$). We then back-transformed the predicted values to the original scale to obtain a map with predicted Pb concentrations across our study area. We used five-fold cross validation [*krige.cv* function; *gstat* package, (Gräler et al., 2016)] to assess the predictive power of the variogram.

The distribution of moose kills in south-central Sweden was estimated following the approach in Brown et al. (2023) by applying resource selection functions (RSF) to moose harvest locations during 2017–2019. Briefly, the RSF was estimated by using a logistic regression that contrasted the landscape characteristics at used (i.e., moose harvest locations provided by hunters) and available (i.e., random) locations (Fieberg et al., 2021; Manly et al., 2002). Hunters typically use areas that are located closer to roads and that have good lateral visibility (e.g., clearcuts), but avoid rugged terrain with poor visibility. The RSF included landcover types (i.e., forest composition, clearcut and bogs), distance to closest road and other variables such as elevation, and terrain ruggedness index, which may impede the movement of hunters across the landscape. The values produced by an RSF are proportional to the probability of selection (Johnson et al., 2006; Manly et al., 2002) or, in this specific example, they are proportional to the probability of hunters killing a moose at any location within our study area. The relative probability of moose kill was estimated for each year based on moose harvest locations from the previous fall$_{t-1}$. The RSF model is described and validated in Brown et al. (2023).

2.3. Capture, sample collection and chemical analyses

We carried out a total of 61 captures of adult female brown bears (n = 34 individuals), during 2017–2020. The captures were carried out as part of the Scandinavian Brown Bear Research Project, which mainly focuses on the demography of female brown bears. A total of 34 samples were collected from lactating females, whereas 27 samples were collected from females that were not lactating. Some individuals (n = 16) were captured and sampled more than once. Bears were darted from a helicopter with a remote drug delivery system (Dan-Inject, Børkop, Denmark) during the spring (April to June). See Arnemo and Evans (2017) for more details on the capture protocol. The capture location was recorded with a hand-held GPS. At each capture, 4 or 6 mL of blood were collected from the jugular vein with evacuated K3EDTA tubes (n = 26) (Vacuette, Greiner Bio-One International GmbH, Kremsmünster, Austria) or evacuated heparin trace element tubes (n = 35) (Vacuette), respectively. The samples were first stored in a cooler in the field and then frozen at -20° C until they were processed in the lab. The Pb concentrations was measured by inductively coupled plasma sector field mass spectrometry (ICP-SFMS, ELEMENT XR, Thermo-Scientific, Bremen, Germany). The blood samples collected during 2017–2019 (n = 42) were part of a previous study (Fuchs et al., 2021), which mainly aimed at determining whether blood Pb concentrations were correlated to life history traits. The Pb concentrations in digestion blanks were low ($<$ 2 μg/L) compared with sample results and the difference between measured and expected Pb concentration in the certified reference material (Seronorm Trace Elements Whole Blood Levels 1 and 2 from SERO AS, Norway) was $<$ 6 % (Fuchs et al., 2021). See Fuchs et al. (2021) for further details about sample collections and Rodushkin et al. (2000) for further details about chemical analyses.

2.4. Statistical analyses

We calculated the mean environmental Pb concentrations, and the mean relative probability of moose kill (fall $_{t-1}$) within circular buffers with a radius of 2, 4 and 6 km around the capture locations. We used multiple linear regressions [*lm* function; *stats* package (R Core Team, 2021)] to determine if the environmental Pb concentrations and the probability of moose kill around the capture locations influenced blood Pb concentrations in brown bears. We started by building a set of six candidate models (Table 1). This set contained a null model, a model in which Pb concentrations only changed according to sampling year, and another model in which blood Pb concentration in female brown bears was affected by their lactation status (Table 1). Other models within that set contained either the relative probability of moose kill, or the environmental Pb concentrations. The last model contained a combination of all the variables (Table 1). All the models (except the null model) included the year of sample collection as a variable to control for potential differences between years. We did not include the age of bears because it was previously shown that this variable was not related to blood Pb concentrations in Scandinavian brown bears (Fuchs et al., 2021).

Although our dataset contained 16 females sampled more than one year, we could not use mixed effect models with a random intercept with bear ID due to insufficient replication (bears were sampled 1.79 times on average over the study period). Model selection was conducted by Akaike's Information Criterion corrected for small sample size (AICc) by using the *aicmodavg* package (Mazerolle, 2020) in R. We considered models with a ΔAICc $<$ 2 to be equivalent. We first conducted the model selection for each scale (2, 4 and 6 km) separately and then carried out a second AICc with the top-ranked model of each scale to determine the best performing model. We used diagnostic plots to ensure that model assumptions were fulfilled. We log-transformed the response variable (i.e., blood Pb concentrations) to achieve normality of residuals. The environmental Pb concentration was not correlated to the probability of moose kill within buffers at the three scales (2 km, rho = 0.03, p = 0.79; 4 km, rho = -0.04, p = 0.76; 6 km, rho = -0.02, p = 0.87). All statistical analyses were conducted in R version 4.1.0 (R Core Team, 2021).

3. Results

Female brown bears had a mean (SD) blood concentration of 91 (36) μg/L. Lactating females (n = 34) had a mean blood Pb concentration of 104 (36) μg/L (range: 56–221 μg/L), whereas this value was 73 (36) μg/L (range: 25–155 μg/L) in non-lactating females (n = 27). Our kriging model predicted environmental Pb concentrations ranging from 4.6 to 95 mg/kg (dry weigth) on the measured scale (i.e., back-transformed; exp (x)-2.5) and showed large spatial variations with the highest Pb concentrations being in the west of the study area (Fig. 1a). The cross-validation procedure revealed that observed and predicted values of environmental Pb concentrations were positively correlated (rho = 0.60, $p < 0.001$), indicating that the model indeed predicted environmental Pb concentrations. Residuals were uncorrelated to predicted values (rho = 0.02, p = 0.45). The mean of residuals was $<$ 0.01, which also indicates that the prediction errors were small relative to predicted values.

The model that performed best at explaining blood Pb concentrations in bears was the Full model (Akaike weight = 0.86) that included effects of the lactation status, the sampling year, the environmental Pb concentration and the relative probability the moose kill (fall $_{t-1}$) extracted within 2 km buffers around the capture locations (Table 2). The second-best performing model was the Environmental Pb models (Akaike weight = 0.14), which

Table 1
The structure and biological hypotheses of candidate linear models used to predict the main source of lead (Pb) exposure in female brown bears (n = 34 individuals; n = 61 samples) from south-central Sweden, during 2017–2020.

	Model structure	Biological hypotheses
Null	log(Pb) ~ 1	Pb is constant
Year	log(Pb) ~ Year	Pb is influenced by year only
Lactation	log(Pb) ~ Lactation	Pb is influenced by lactation only
RSF hunt	log(Pb) ~ Probability of moose kill + Lactation + Year	Pb is influenced by probability of moose kill, lactation and year
Environmental Pb	log(Pb) ~ Environmental Pb + Lactation + Year	Pb is influenced by environmental Pb, lactation and year
Full	log(Pb) ~ Environmental Pb + Probability of moose kill + Lactation + Year	Pb is influenced by environmental Pb, probability of moose kill, lactation and year

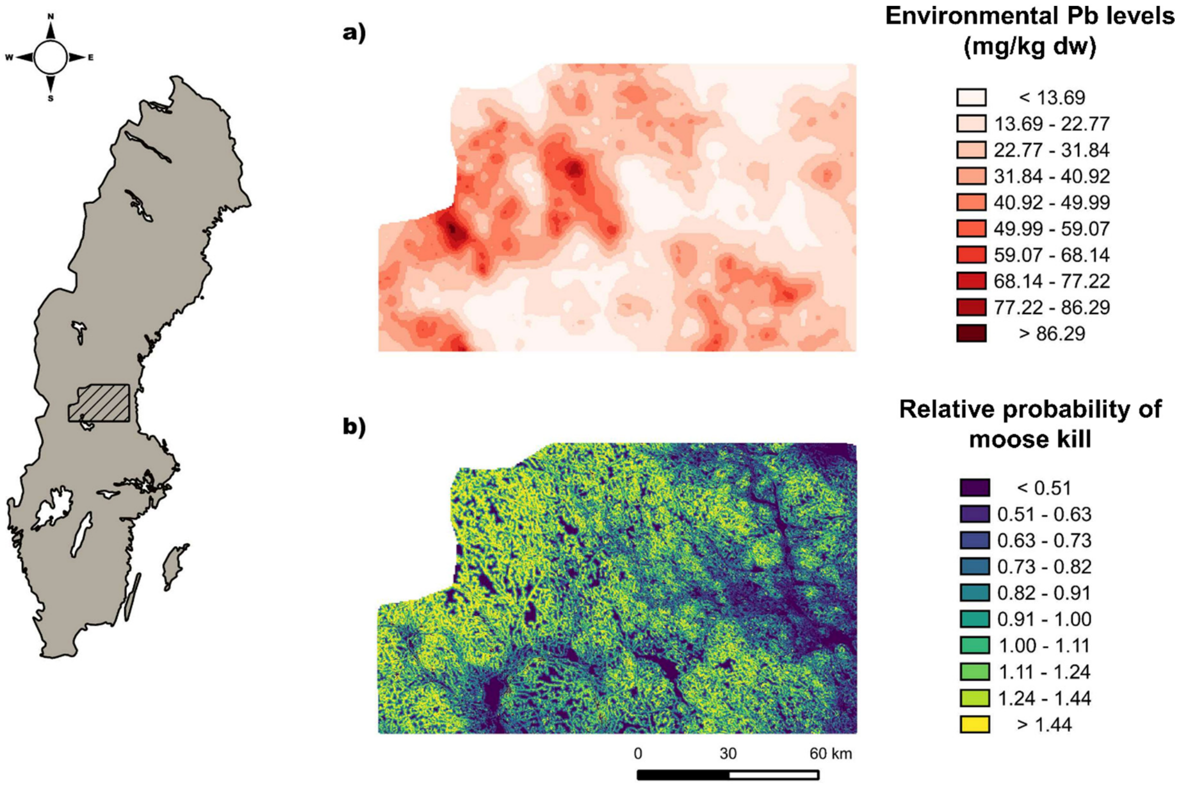

Fig. 1. a) Predicted environmental lead (Pb) concentration (mg/kg dry weight) at a 500 m resolution in the study area in south-central Sweden. The interpolated surface was generated by using ordinary kriging on Pb concentrations measured in plant roots. The data were extracted from the biogeochemistry database of the Geological Survey of Sweden (© Sveriges Geologiska Undersökning). b) Predicted probability of moose kill at a 100 m resolution in the study area. The relative probabilities were calculated from a resource selection function based on moose harvest locations that were provided by hunters [modified from Brown et al. (2023)]. See Brown et al. (2023) for more details about the resource selection function.

had a ΔAICc of 3.69 with the best model (Table 2). The other models received considerably less support as their ΔAICc were > 19 with the top-ranked model (Table 2). The results were similar across scales (Table A1), but the 2 km-scale performed best (Table A2). Thus, we only discuss the results with variables extracted at 2 km and present the other results as supporting information (Table A3).

The following effect sizes and confidence intervals (95 % CI) are expressed in percent of change, and they were calculated by back-transforming the coefficients (multiplied by the standard deviation for continuous variables), subtracting 1 and multiplying the results by 100. Our models indicated that blood Pb concentration in female brown bears was positively related to the environmental Pb concentration around the capture location (Fig. 2; Table 3). For every unit-increase of 16.02 mg/kg in environmental Pb concentrations (1 unit of standard deviation), the blood Pb concentrations in female brown bears increased by 18.5 % (Lower: 10.5 %, Upper: 27.1 %). Similarly, our model predicted higher blood Pb concentrations in brown bears captured in areas where hunters were more likely to kill moose during the previous fall (Fig. 2; Table 3). Blood Pb concentrations in brown bears increased by 9.1 % (Lower: 1.5 %, Upper: 17.2 %) for every increase of 0.17 (1 unit of standard deviation) in the relative probability of moose kill. Lactating females also had blood Pb concentrations that were 37.7 % higher (Lower: 17.8 %, Upper: 61.0 %) when compared to non-lactating females.

Table 2
Model selection by Akaike information criterion corrected for small sample size (AICc). The model set was used to identify potential sources of Pb exposure in female brown bears ($n = 34$ individuals; $n = 61$ samples) with variables extracted within 2 km buffers centred around capture locations in south-central Sweden, during 2017–2020.

	K	AICc	ΔAICc	*w*	LL
Full	8	24.47	0	0.86	−2.85
Environmental Pb	7	28.16	3.69	0.14	−6.02
RSF hunt	7	44.04	19.57	0	−13.96
Lactating	6	45.89	21.42	0	−16.17
Year	5	57.32	32.85	0	−23.12
Null	2	59.36	34.89	0	−27.58

Notes: K = the number of parameters, ΔAICc = the AICc difference with the top-ranked model, *w* = Akaike weight within the set and LL = log-likelihood of the model.

4. Discussion

Our results support the hypothesis that the environmental Pb levels and moose kills jointly influenced blood Pb concentrations in Scandinavian brown bears. Our model explained 56 % of the variation in blood Pb concentrations in female brown bears, while the environmental Pb concentrations and probability of moose kill explained 20 % and 9 % of the variations in blood Pb concentrations, respectively. We found strong support for our prediction that higher environmental Pb levels and availability of moose kill by hunters are related to higher blood Pb concentrations in brown bears.

Our results indicate that blood Pb concentrations in brown bears reflect the concentration of Pb that circulates in the environment within their home range. Bears could be exposed to Pb by accidental ingestion of soil when, for example, digging their den or foraging on ants and plants (Gall et al., 2015). Berries are the main food source of bears during hyperphagia in Sweden (Stenset et al., 2016), which may explain why environmental Pb concentrations were a greater contributor to blood Pb levels in bears. We also acknowledge that the environmental Pb concentrations estimated

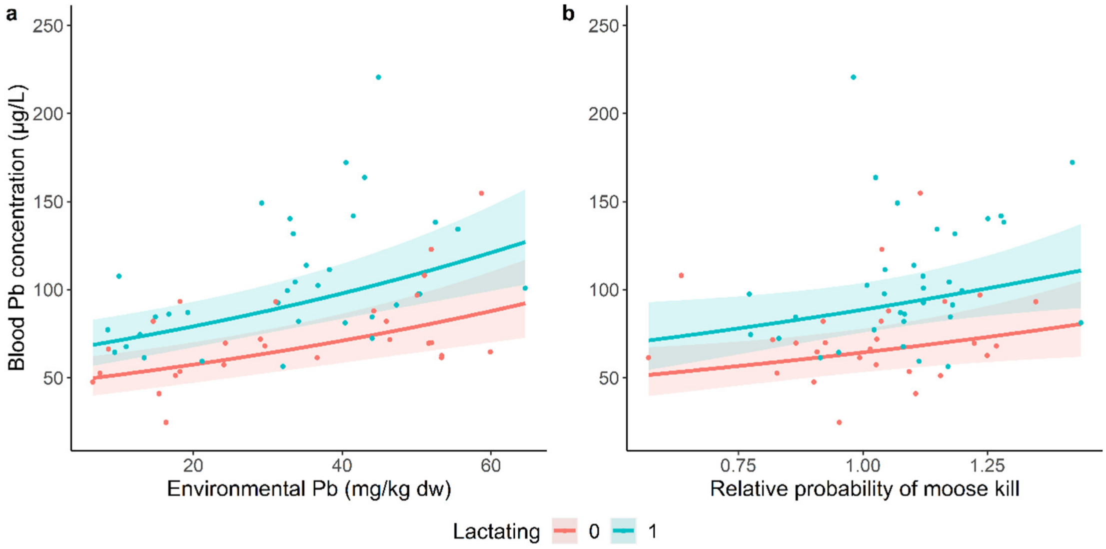

Fig. 2. Predicted blood Pb concentrations (μg/L) in female brown bears ($n = 34$ individuals; $n = 61$ samples) in relation to a) environmental Pb concentrations (mg/kg dry weight) and b) the relative probability of moose kill during the previous hunting season (t - 1) around the capture locations (2 km buffer) in south-central Sweden during 2017–2020. Lines show predictions and shaded polygons represent 95 % confidence intervals of the models, whereas the points represent raw data. Blue points/lines indicate that the female was lactating (lactating = 1), whereas red points/lines indicates that they were not (lactating = 0).

from plant roots may not be entirely representative of Pb concentrations in food items consumed by bears. Different parts of plants may incorporate Pb from different sources. Roots mainly incorporate Pb from the surrounding soil, whereas leaves and stems also incorporate Pb from atmospheric sources (Klaminder et al., 2005); berries may also be coated with dust from atmospheric depositions (Stachiw et al., 2019). Additionally, the rationale behind the use of the plant root data was not to establish a direct link with the bears' diet, but rather to estimate and compare the amount of Pb that circulates in the different regions of our study areas.

Our conservative approach with buffers of various radii centred on capture locations was sufficiently accurate to correlate blood Pb levels with the concentrations of Pb circulating in the environment around those sites. Models predicting the tissue concentration of contaminants in animals can be refined further by incorporating movement or space use parameters (Sorais et al., 2020, 2021). Adding data from GPS transmitters could have improved the performance of our model. Spatially explicit models also need to account for the physiological state of an individual (i.e., reproductive status) and failure to do so may introduce bias in the models, as evidenced by the blood Pb concentrations that were 38 % higher on average in lactating females when compared to non-lactating females. During lactation, there is remobilization of calcium (and Pb) from bones into the bloodstream and lactating females are exposed to an additional endogenous source of Pb (Fuchs et al., 2021).

Table 3

Parameters of the top-ranked model used to predict blood Pb concentrations [log (Pb μg/L)] in female brown bears ($n = 34$ individuals; $n = 61$ samples) from south-central Sweden, during 2017–2020.

Variables	Estimate	S.E.	95 % CI	
			Lower	Upper
Intercept	3.262	0.247	2.768	3.757
Environmental Pb	0.011	0.002	0.006	0.015
Hunter RSF	0.511	0.210	0.090	0.932
Lactating	0.320	0.078	0.164	0.477
Year 2018	0.035	0.106	−0.177	0.248
Year 2019	0.287	0.101	0.085	0.490
Year 2020	0.050	0.102	−0.154	0.256
Multiple $R^2 = 0.56$, Adjusted $R^2 = 0.51$				

Notes: S.E. = standard error, Hunter RSF = Relative probability of moose kill. Variables were extracted within 2 km buffers centred on capture locations.

Our results also indicate that high probabilities of moose kills are linked to higher blood Pb levels in brown bears. Some concerns have been raised previously regarding the risk of Pb exposure from bullet fragments in mammalian scavengers (Kelly et al., 2021; Legagneux et al., 2014; Rogers et al., 2012), but so far this source of Pb exposure had mostly been reported in avian scavengers (Pain et al., 2019). Pb ammunition is an important source of Pb exposure in avian scavengers and can have consequences at both the individual and population levels (Helander et al., 2021; Pain et al., 2019; Slabe et al., 2022). For instance, Pb from ammunition has been linked to increased risk of mortality (Singh et al., 2021) and altered flight behavior (Ecke et al., 2017) in avian scavengers, and has prevented the recovery of the California condor (*Gymnogyps californianus*) (Finkelstein et al., 2012).

The blood Pb levels reported in Scandinavian brown bears are in general below the 180 μg/L hazardous concentration for 5 % of mammals reported by Buekers et al. (2009). However, values below toxicity thresholds should not be labelled as "safe" because sublethal and subclinical effects of Pb can still be harmful. For instance, subclinical effects on movement behavior have been reported in golden eagles at blood Pb levels (25 μg/L) well below toxicity thresholds for birds (Ecke et al., 2017), and it has been reported that eagles with blood Pb levels above 25 μg/L were more likely to die compared to individuals below this threshold (Singh et al., 2021). Although the conclusions of these studies cannot be directly translated to brown bears, they suggest that subclinical effects of increased Pb exposure can occur at levels well below commonly reported guidelines and that some bears may be subjected to those consequences. However, we did not investigate this topic and were not able to confirm whether there were deleterious effects in bears. If subclinical or sublethal effects of Pb occur in brown bears, the consumption of slaughter remains discarded by hunters could be considered an evolutionary trap (Schlaepfer et al., 2002), because there is no reason for an opportunistic omnivore to not consume easily accessible, energy-rich and easily digestible foods when encountered (DeVault et al., 2003; Pritchard and Robbins, 1990). However, this 'easy meal' acquired at low costs may be deleterious in the long term, because it may contain high concentrations of Pb (Gremse et al., 2014; Hunt et al., 2006; Menozzi et al., 2019; Stokke et al., 2017).

A previous study on Scandinavian brown bear showed that they generally did not modify their behavior in order to gain access to slaughter remains during the fall and concluded that the scavenging behavior of bears in Sweden is mostly opportunistic (Brown et al., 2023). Despite this seemingly low exposure rate, we found a positive relationship between blood Pb levels, and the probability of moose kill around the capture locations. Studies conducted on bears from other populations in North America have shown that bears actively use areas where they are likely to find slaughter remains (Lafferty et al., 2016; Legagneux et al., 2014; Ruth et al., 2003), suggesting that bears from other populations may be at greater risk of Pb exposure than Scandinavian brown bears, especially in areas where the peak of the hunting season is earlier during their active period. The risk of increased Pb exposure should thus be evaluated in bears and other mammalian scavengers from other populations with an appropriate design.

The risk of Pb exposure in relation with the timing of the hunting season has been extensively studied in avian scavengers (Ecke et al., 2017; Fisher et al., 2006; Legagneux et al., 2014; Pain, 2009), while other studies have also looked at the risk of Pb exposure across a gradient of harvest density (Kelly and Johnson, 2011; Singh et al., 2021). The distribution of kill sites or harvest density is typically calculated at the scale of management areas by counting the number of animals that were harvested with a firearm within a specific area (Helander et al., 2021; Kelly and Johnson, 2011); however, this approach is based on the assumption that the distribution of hunter kills is uniform within the area. This assumption is inaccurate in most cases. Hunter kills are neither randomly nor uniformly distributed across the landscape, but are rather concentrated around specific features that provide accessibility, concealment, and/or visibility, depending on the hunting style (Gaynor et al., 2022; Norum et al., 2015). Ignoring the fine-scale distribution of kill sites might not matter for avian scavengers because they can efficiently travel between patches with high harvest densities and easily access slaughter remains (DeVault et al., 2003). However, mammals do not travel as efficiently as most avian scavengers and those movement constraints restrict their ability to access slaughter remains (DeVault et al., 2003), which underlines the importance of reliable estimates of the fine-scale distribution of hunter kill sites. Using an RSF-based approach on ungulate kill sites provided by hunters may be useful for predicting the fine-scale distribution of hunter kills and, by extension the increased risk of Pb exposure, within an area.

The advantage of using an RSF-based approach to predict the fine-scale distribution of harvest locations is that it only requires a subsample of the total harvest. It essentially allows to circumvent the problem of obtaining all the harvest locations within an area. A potential disadvantage is that, depending on the number of included variables, it may still require hundreds of harvest locations, and by extension, it also requires the cooperation of many hunters, which may choose to not disclose or collect information on harvest locations for research purposes. Wildlife management agencies can however obtain this information relatively easily from voluntary hunters, or by making it mandatory to disclose the harvest locations when harvested animals are registered. RSF are relatively easy to fit with widely available statistical softwares, but their results may be difficult to interpret properly. Fortunately, multiple tools are now available to facilitate the implementation of RSF and the interpretation as well as the validation of their results (Fieberg et al., 2021; Muff et al., 2020; Northrup et al., 2022; Roberts et al., 2017).

Potential limitations of our study include the relatively small sample size, the absence of males from the analyses, and the timing of blood sampling. A sample size of 34 females may be small for many species, but considering that, in 2008, the entire population of bears in Sweden was estimated at ~3300 individuals (Kindberg et al., 2011), our sample size can be considered acceptable. Due to the absence of samples from adult males, we also could not investigate exposure in this demographic group. Larger males commonly monopolize foraging locations (Ben-David et al., 2004; Zedrosser et al., 2013) and could deter females from using slaughter remains; however, it is unlikely a problem in our study because avoidance of males by females with dependent offspring is more common during the mating season (June–July) compared to the fall when slaughter remains are available (Steyaert et al., 2013). Another potential limitation of our study is that it may be difficult to relate Pb concentrations from blood samples collected during spring to moose hunting activities that occurred during the previous fall. Other studies have shown that the blood Pb concentrations in scavengers decrease during the winter and spring (Craighead and Bedrosian, 2008; Slabe et al., 2022) and our model may have underestimated the contribution of ammunition as a Pb source due to the timing of blood sampling. Nevertheless, our conclusions are similar to those reported by Arrondo et al. (2020) who also found that soil was an important source of Pb exposure in vultures from Spain. Additionally, it is also possible that bears scavenge on thawed slaughter remains after den emergence and are thus exposed to Pb during the spring, as suggested by Fuchs et al. (2021); however, no information is available on the frequency of this behavior.

An alternative explanation based on Pb kinetics is likely a better explanation for the relationship between spring blood Pb levels and the distribution of moose kills during the previous fall. We do not know if and how hibernation affects Pb kinetics, but blood half-life of elimination may be extended because bears do not urinate nor defecate during this period (Nelson et al., 1983), thereby suggesting no or minimal excretion from the body during the winter. However, we can reasonably expect a blood half-life of elimination of four to five weeks in active bears (Arnemo et al., 2022). Due to this rapid turnover rate, blood Pb concentrations typically reflect short time exposure, but this parameter also depends on the equilibrium between the different compartments of the body in which Pb is stored (Rabinowitz, 1991). For instance, Pb stored in bones, which reflects long time exposure, may be remobilized into the bloodstream during periods of nutritional stress, gestation and lactation (Arnemo et al., 2022). Bears do not eat nor drink during hibernation and Pb may be mobilized from bones in all individuals; this phenomenon was especially obvious in lactating females (Fuchs et al., 2021). Therefore, blood Pb concentrations in bears during the spring are likely the results of a mixture of recent intakes and long-term exposure from blood-organ-bone equilibrium (i.e., mobilisation of Pb from bones).

5. Conclusion

We found a link between the distribution of moose kills by hunters and the blood Pb concentrations in bears; however, the environmental Pb level was a greater contributor to bears' blood Pb concentrations. Pb from ammunition is mainly available for a few weeks during the hunting season and potentially the spring through the consumption of soft tissues with embedded metal fragments, although slaughter remains also include hides and bone dumps that may last longer. Despite that relatively short period during which Pb exposure from ammunition likely occurs in bears, it still represents a potential risk for bears and possibly other mammalian scavengers. We also propose that an RSF-based approach with harvest locations provided by hunters should be relatively easy to implement in other systems, thereby improving our capacity to better understand the risk of increased Pb exposure from bullet fragments in scavengers. This study suggests that regulations on both Pb ammunition and other anthropogenic Pb emissions are needed to reduce Pb exposure in bears.

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CRediT authorship contribution statement

Ludovick Brown: Conceptualization, Formal analysis, Visualization, Writing – original draft. **Boris Fuchs:** Data curation, Investigation, Writing – review & editing. **Jon M. Arnemo:** Conceptualization, Investigation, Resources, Funding acquisition, Writing – review & editing. **Jonas**

Kindberg: Project administration, Writing – review & editing. **Ilia Rodushkin:** Investigation, Resources, Writing – review & editing. **Andreas Zedrosser:** Conceptualization, Supervision, Writing – review & editing. **Fanie Pelletier:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

Data availability

Data will be made available on request.

Declaration of competing interest

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.162099.

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Toxic elements in arctic and sub-arctic brown bears: Blood concentrations of As, Cd, Hg and Pb in relation to diet, age, and human footprint

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ABSTRACT

Contamination with arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb) is a global concern impairing resilience of organisms and ecosystems. Proximity to emission sources increases exposure risk but remoteness does not alleviate it. These toxic elements are transported in atmospheric and oceanic pathways and accumulate in organisms. Mercury accumulates in higher trophic levels. Brown bears (*Ursus arctos*), which often live in remote areas, are long-lived omnivores, feeding on salmon (*Oncorhynchus* spp.) and berries (*Vaccinium* spp.), resources also consumed by humans.

We measured blood concentrations of As, Cd, Hg and Pb in bears ($n = 72$) four years and older in Scandinavia and three national parks in Alaska, USA (Lake Clark, Katmai and Gates of the Arctic) using high-resolution, inductively-coupled plasma sector field mass spectrometry. Age and sex of the bears, as well as the typical population level diet was associated with blood element concentrations using generalized linear regression models.

Alaskan bears consuming salmon had higher Hg blood concentrations compared to Scandinavian bears feeding on berries, ants (*Formica* spp.) and moose (*Alces*). Cadmium and Pb blood concentrations were higher in Scandinavian bears than in Alaskan bears. Bears using marine food sources, in addition to salmon in Katmai, had higher As blood concentrations than bears in Scandinavia. Blood concentrations of Cd and Pb, as well as for As in female bears increased with age. Arsenic in males and Hg concentrations decreased with age.

We detected elevated levels of toxic elements in bears from landscapes that are among the most pristine on the planet. Sources are unknown but anthropogenic emissions are most likely involved. All study areas face upcoming change: Increasing tourism and mining in Alaska and more intensive forestry in Scandinavia, combined with global climate change in both regions. Baseline contaminant concentrations as presented here are important knowledge in our changing world.

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1. Introduction

Heavy metals/metaloids (HM), such as arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb), are non-essential toxic elements and exposure to these elements in the body from both natural and anthropogenic sources is a global One Health issue (Buttke et al., 2015). Even in areas with a minimal cumulative human impact on the ecosystem, measured as the human footprint (Williams et al., 2020), anthropogenic contaminant levels of these elements increase natural background levels substantially (AMAP, 2005; Dastoor et al., 2022; Macdonald et al., 2005; Renberg et al., 2001). Heavy metal and metalloid emissions mainly originate from fossil fuel combustion, non-ferrous metal production, waste incineration, or changes in the environment, such as the draining of wetlands, and are spread globally through atmospheric deposition (AMAP, 2005; Bjerregaard et al., 2022). In addition, contamination with Pb causing toxicity in vertebrates occurs when humans hunt with Pb-based ammunition (Arnemo et al., 2022; Bjerregaard et al., 2022).

Arsenic, Cd, Hg and Pb are known to readily bioaccumulate (i.e., concentrate in bodies over time), whereas biomagnification (i.e., increases at higher trophic levels) is more pronounced for Hg (Ali and Khan, 2019; Atwell et al., 1998; Le Bourg et al., 2019). Arctic and sub-arctic regions are susceptible to Hg contamination because snow and ice in combination with polar light conditions facilitate the formation and deposition of Hg oxides. Dietary uptake of methyl-Hg^+ (MeHg) is the major exposure pathway in terrestrial and aquatic wildlife (Scheuhammer et al., 2007).

Wildlife is especially prone to HM exposure; for example, the California condor (*Gymnogyps californianus*) was on the brink of extinction with Pb poisoning being an important accelerating factor (Finkelstein et al., 2012). Effects of exposure are often sublethal, for example, golden eagles (*Aquila chrysaetos*) are documented to change movement behavior with increasing Pb exposure (Ecke et al., 2017). Feeding ecology and habitat use are important factors of pollutant exposure. For example, coastal Arctic foxes (*Vulpes lagopus*) have higher Hg concentrations than their conspecifics further inland due to different diets (Bocharova et al., 2013), and omnivores have been found with higher concentrations of Cd and Pb than sympatric carnivores (Lazarus et al., 2017). Black bears (*Ursus americanus*) in areas with higher ungulate harvest density have higher concentrations of Pb measured in their teeth likely due to ingestion of fragments from spent Pb – based ammunition (Brown et al., 2022).

Brown bears (*U. arctos*) inhabit the lower arctic and sub-arctic regions and are susceptible to HM exposure, especially Pb, in areas with a relatively large human footprint (Boesen et al., 2019; Fuchs et al., 2021; Lazarus et al., 2017; Rogers et al., 2012). No direct links to any sources of exposure have been established yet, but there is general agreement on the dietary intake of either environmental Pb as well as the ingestion of Pb fragments from Pb-based ammunition used for hunting ungulates as major exposure routes (Fuchs et al., 2021; Lazarus et al., 2017; Rogers et al., 2012). During hibernation, bears do not defecate or urinate, both of which are major pathways to excrete HM from the body. Individual bears exhibit highly adaptive feeding behaviors, ranging from nearly herbivorous to highly carnivorous, and food sources from mainly marine to mainly terrestrial depending on the region (Hertel et al., 2018; Hilderbrand et al., 1999; Koike et al., 2012). During hyperphagia bears within a region typically facilitate the same major food source: In Scandinavia, bears mainly feed on berries (*Vaccinium* spp.) (Hertel et al., 2018), in southern and eastern Europe mainly on hard mast (i.e. nuts and seeds) (Bojarska and Selva, 2012; Kavčič et al., 2015), and in North America, some populations rely on salmon (*Oncorhynchus* spp.) (Hilderbrand et al., 1999). The enrichment of ^{13}C and ^{15}N isotopes has been used to reconstruct and link diet to HM exposure in vertebrates and invertebrates in both terrestrial and maritime systems (Larsson et al., 2007; Le Croizier et al., 2019; Noël et al., 2014; Ramos et al., 2009). For example, polar bears (*U. maritimus*) and brown bears that use maritime food sources, as measured by the enrichment of the ^{13}C isotope, have higher Hg levels compared to bears using terrestrial food sources (Cardona-Marek et al., 2009; Noël et al., 2014).

Studies on HM contamination in sub-arctic terrestrial wildlife commonly report concentrations for internal organs, such as the liver, kidneys, or bones from dead specimens (AMAP, 2005). These tissues are well suited to study exposure and accumulation of As, Cd, Hg and Pb (Tan et al., 2009); however, they often involve destructive sampling. Tissues such as blood or hair, which can be collected from live animals, are more suitable for specimens from protected areas or endangered species. Blood is often used as an indicator for recent exposure to one of these four elements. The gastrointestinal tract takes up As, Cd, Hg and Pb which are distributed by the blood stream and stored in different body compartments. Half-life of elimination in blood is relatively short, for example, in humans; As has a half-life in blood of three to 4 h, and blood is not used as a tissue to detect low exposure (i.e., population means < 1 μg/L) (ATSDR, 2020), however, blood As concentration accurately reflects levels of chronic exposure (Hall et al., 2006). For Cd, Hg and Pb, whole blood concentrations are used to detect exposure. Half-life of Cd in blood is three to four months (ATSDR, 2015). Total Hg half-life in blood is one to three weeks and in contrast to the other discussed elements, also half-time of elimination from the human body is with estimated three months much shorter (ATSDR, 2022; Yaginuma-Sakurai et al., 2012). The half-life of Pb in blood is four to five weeks in humans (Rabinowitz et al., 1976), and probably similar in bears (Arnemo et al., 2022). However, elements accumulated in organs and bones remobilize into the blood stream and concentrations reach equilibrium between the different compartments (Mattisson et al., 2010; Rabinowitz et al., 1976; Yaginuma-Sakurai et al., 2012). Thus, blood HM concentrations reflect a combination of recent uptake and total body burden. Different physiological states, such as lactation, hibernation, malnutrition, or death, might change the concentrations at which these elements are at equilibrium between the different compartments (Fuchs et al., 2021; Silbergeld, 1991; Söderberg et al., 2023).

The goal of our study was to compare total concentrations of As, Cd, Hg and Pb in whole blood samples from four brown bear populations living on different continents and subsisting on different diets, as well as to identify potential sources of exposure. Three of these populations were located in Alaska, USA, and include bears with an almost entirely marine protein-based diet consisting mainly of salmon (Hilderbrand et al., 2018), as well as a population in Scandinavia with a diet based on terrestrial proteins and vegetation (Stenset et al., 2016). To help identify potential sources for As, Cd, Hg and Pb, we paired blood metal concentrations with stable $\delta^{13}C$ and $\delta^{15}N$ isotopes in hair samples in one population (Gates of the Arctic National Park and Preserve, Alaska) where individual bears subsist on either a terrestrial vegetation-based diet or a maritime protein-based diet.

We hypothesized that *i)* HM concentrations is highest in Scandinavia associated with the magnitude of the human footprint index at the sample location; *ii)* HM concentrations reflect the major food resource of the population, and *iii*) bioaccumulation of As, Cd and Pb in target tissues with age is reflected in whole blood samples. Based on these hypotheses, we predicted that: I) HM concentrations are greater in areas with a larger human footprint; II) bears acting as apex predators (i.e., with a high proportion of salmon in their diet) have higher concentrations of HM compared to bears acting as primary consumers (i.e., with high vegetation levels in their diet); and III) older bears have higher whole blood concentrations of As, Cd and Pb than younger bears.

2. Material and Methods

2.1. Study populations

The study area for the Scandinavian brown bear population is in south-central Sweden (~61°N, 15°E). The area (~13,000 km^2) is predominantly covered with intensively managed coniferous forest (*Pinus sylvestris* and *Picea abies*). Forest stands are typically planted, thinned, and clear-cut at an age of 80–100 years, followed by scarification and replanting (Martin et al., 2010; Swenson et al., 1999). The hilly landscape is interspersed with bogs and only few agricultural fields in the east. Small settlements occur in the entire study area with a human density $<9/\text{km}^2$. Low-traffic roads used for forestry and recreational access are spread throughout the study area at a density of 0.3 km/km^2 and 0.7 km/km^2 for paved and gravel roads, respectively. Between 2010 and 2020, the annual moose (*Alces alces*) harvest rate was 0.23/km^2 (Länsstyrelserna, 2020); 98% of the moose were shot with Pb-based ammunition and their digestive tracts, organs, and slaughter remains were mostly left in the forest (Stokke et al., 2017). There are indications for, but no hard data, that more moose hunters are using Pb-free bullets in recent years. In 2022, brown bears were hunted at a rate of 0.002/km^2 within management areas 2 and 6 in Dalarna County, representing the study area (Länsstyrelserna, 2022). Based on scat sampling during the hyperphagic period, berries (*Vaccinium* spp*., Empetrum hermaphroditum*) make up 68% of the estimated dietary energy content, insects (mainly ants of the genus *Formica*) 14%, and ungulates (moose) 14%. In spring (April/May), ungulates are the most important food source (61%), followed by insects (20%), and berries (9%, mainly *V. vitis-idaea* from the previous fall) (Stenset et al., 2016). No anadromous fish species occurs in the study area and there are no indications of fish in the bears' diet. Aerial depositions are the primary source of environmental As, Cd and Pb in south-central Scandinavia (Renberg et al., 2001; von Storch et al., 2003). Blood samples were collected during the period 2010–2021, between mid-April and mid-June (Table 1). Pb concentrations from 14 of these samples have been included in Fuchs et al. (2021).

Gates of the Arctic National Park and Preserve (Gates NP) is a remote and undeveloped 34,400 km^2 protected area in the Brooks Range in northern Alaska (~68°N, 154°E), USA. This interior park is > 200 km from the coast and is covered by arctic tundra, boreal forest (with main tree species *Picea* spp*.*, *Betula neoalaskana*, P*opulus tremuloides*), lowland riparian communities, and high alpine terrain. There are no roads within the park boundary. Bears in Gates NP show high individual and moderate interannual variation in primary food intake. Based on stable carbon and nitrogen isotope analysis, protein contributes 2–96% to the late summer and fall diet in female bears (Mangipane et al., 2020). Of the protein intake, 77% is of marine origin (i.e., chum salmon (*Oncorhynchus keta*)) (Mangipane et al., 2020). Other sources of protein are terrestrial mammals, such as moose, caribou (*Rangifer tarandus*), Dall's sheep (*Ovis dalli*), and Arctic ground squirrel (*Urocitellus parryii*). Bears relying on vegetation during hyperphagia consume mainly berries (*Vaccinium* ssp*., Empetrum nigrum, Shepherdia canadensis*). Approximately 10,000 visitors travel to Gates of the Arctic NP each summer, and most visitors access the park by small aircraft (IRMA, 2022). Blood and hair samples were collected in May and June 2016 (Table 1).

Lake Clark National Park and Preserve (Clark NP) is situated along the coast of southwestern Alaska (~60°N, 153°E) and covers approximately 16,300 km^2. The area for this study was on the western side of the Chigmit Mountain Range and none of the bears collared as part of this study accessed the coastal (east) side of the park (Mangipane et al., 2018). Deciduous and coniferous forest, as well as different shrub and grass communities, cover large parts of this study area (Hilderbrand et al., 1999). Salmon originating in Bristol Bay were the primary resource for Clark NP bears, but berries and other ungulates were available. Clark NP is visited by approximately 20,000 visitors each summer and is not connected to any road system but primarily accessed by small aircraft (IRMA, 2022). Blood samples were collected in May 2016 (Table 1).

Table 1
Sample size (N), range and mean of body mass (kg), age (years), and date of blood sampling (month of the year) of brown bears (*Ursus arctos*) in Scandinavia and national parks and preserves (NP) in Alaska, USA.

Sample area	N	Body mass	Age	Sample Date
Scandinavia	30	58–197, 90	4–13, 6	April–June 2010–2021
Gates NP	18	80–251, 114	8–25, 13	May–June 2016
Clark NP	12	100–202, 159	9–30, 15	May 2016
Katmai NP	12	108–184, 146	5–19, 10	May & July 2017
Total	72	58–251, 116	4–30, 10	

The coastal Katmai National Park and Preserve (Katmai NP) covers about 16,500 km^2 in south-western Alaska (58°N, 155°E) and has one of the highest brown bear densities in the world (Ferguson and McLoughlin, 2000). Salmon and other marine resources, such as clams (*Mya arenaria, Siliqua patula*), flounder (*Platichthys stellatus*), and marine mammals were important food resources for bears (Erlenbach, J.A., 2020). However, diets have shifted during recent decades and vegetation is now the dominant food component at the population level. Bear habitat included extensive areas of shrubs (*Alnus* spp., *Salix* spp., *Betula* spp.), as well as boreal forest and alpine terrain. All bears sampled in Katmai NP generally remained on the coast and did not venture inland and thus mainly relied on marine resources from Cook Inlet (Erlenbach, J.A., 2020). Katmai NP, visited by approximately 40,000 visitors annually, is not connected to any road system and small aircraft is used to access the park (IRMA, 2022). Blood samples were collected in May and July in 2016 (Table 1). Recreational hunting is not permitted within all three Alaskan parks, but occasional subsistence hunting occurs at a very low level.

We used a human footprint index based on the 2013 data by Atkinson and Williams (2020) that indicates the cumulative anthropogenic pressure on an ecosystem to quantify the human impact in the different study areas. The index compiles survey and remotely sensed data on land use, represents the degree of wilderness, and has been linked to biodiversity and species extinction risk (Williams et al., 2020). We created a 2-km radius buffer around each sample location and extracted the highest index value within the buffer. Brown et al. (2023) found that background Pb contamination and the probability of hunter killed moose as a potential Pb source within a 2-km radius around the sampling location best correlated with the blood Pb concentration of bears in Scandinavia.

2.2. Sample collection

All bears, in all study areas, were chemically immobilized from a helicopter between mid-April and beginning of July, had their sex-determined, and were weighed. Capture and handling procedures in Alaska were approved by the US National Park Service (AKR_KATM_Hilderbrand_BrownBear_2014, AKR_LACL_Mangipane_BrownBear_2014, AKR_GAAR_Gustine_GrizzlyBear_2014) and the US Geological Survey (2014–01, 2015–04, 2015–06) Animal Care and Use Committee. In Scandinavia, captures were approved by the Swedish Ethical Committee on Animal Research, Uppsala, Sweden (C18/15) and the Swedish Environmental Protection Agency, Stockholm, Sweden (NV-00741-18). Age was determined by the cementum analysis of a vestigial first premolar (Hilderbrand et al., 1999; Mattson, 1993) or estimated by tooth wear (Hilderbrand et al., 2018). All Alaskan bears were four years or older. In Scandinavia, most bears had been captured as yearlings together with their mother, thus their ages were known. Blood for HM analysis was collected from the jugular vein in Scandinavia or the cephalic vein in Alaska in either 8 ml evacuated heparin trace element tubes or 4 ml evacuated K3EDTA tubes and frozen at −20 °C the same day. All samples were analyzed in the same laboratory (ALS Scandinavia AB, Luleå, Sweden). Shipping from the USA to Sweden followed the CITES regulations (export permit #19US15593D/9, import permit #4.10.18–12752/2019). Samples were prepared by closed vessel MicroWave-assisted acid digestion and HM concentration measured by high-resolution, inductively-coupled plasma sector field mass spectrometry. Concentrations, in μg/L, were for the total amount of each element. Lower level of quantification was 0.02 μg/L for As, 0.006 μg/L for Cd, 0.4 μg/L for Hg and 0.2 μg/L for Pb. Further details on the analytical methods can be found in the supplementary material as well as in Rodushkin et al. (2000) and Söderberg et al. (2023).

2.3. Sample selection

We analyzed a total of 72 blood samples in this study. From Alaska, we included 18 samples from Gates NP, 12 from Katmai NP, and 12 from Clark NP. In Scandinavia, over 300 blood samples from 71 individual bears have been analyzed for HM. To obtain a balanced data set, we subsampled the Scandinavian data set to contain only individuals $\geq$ four years, and with the variables age, sex, and body mass available. We then randomly selected only one sample per individual, and from this pool we again randomly selected 30 samples for statistical comparison with the Alaskan data.

2.4. Statistical analysis

We used generalized linear models (glm) with a gamma distribution and a log-link function to estimate differences in blood HM concentration of individual bears between the study areas in R 4.2.1 (R Core Team, 2022). We formulated the same set of candidate model for each element tested (Table SM 1), and selected top models using the second order Akaike Information Criterion (AICc) in the AICmodavg package (Mazerolle, 2019). Models within ΔAICc <2 were considered equivalent and estimates averaged using the *modavg* function. For all candidate models, we fitted the blood HM concentration as response variable and study area as explanatory variable. To this base model, we added either sex or age of the bear, both variables combined, as well as a model with an interaction of these variables. We did not include body mass due to the correlation with age (Spearman's rho = 0.65). Sex was included to control for behavioral and physiological differences due to reproduction between males and females (Hertel et al., 2018; Swenson et al., 2007; Tan et al., 2009), and age as indication of bioaccumulation. We included the interaction age × sex to test for different accumulation trends between males and females at different ages. We considered differences between the study areas significant if the 95% confidence intervals did not overlap. To facilitate model performance we kept values below level of quantification as provided by the mass spectrometry. Pointwise back transformation was used when interpreting summary tables and for predictions using the exp function in R.

2.5. Heavy metal (loids) and diet in Gates NP

Both the relative abundance of stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in guard hair and derived estimates of proportions for terrestrial protein, marine protein, and vegetation in the diet of individual bears have been previously analyzed and were available for Gates NP (Mangipane et al., 2020). In brief: Hair sections 4 cm from the root, representing growth from July through October, were selected for analysis to evaluate assimilated diet within that period (Mangipane et al., 2020). Isotopic values are reported as the relative difference from the sample isotopic ratios ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) to the standard isotopic ratios; i.e., $[(R_{Sample}/R_{Standard})\text{-}1]*1000$. PeeDee Belemnite limestone was standard for carbon and atmospheric N_2 for nitrogen. To estimate dietary proportions, $\delta^{13}C$ and $\delta^{15}N$ values were compared to source data using the bayesian isotopic mixing model MixSIAR (version 3.1.10), with sex as a fixed effect and bear ID and a process error term as random effects. Previously estimated $\delta^{13}C/\delta^{15}N$ for chum salmon (Johnson and Schindler, 2009), a sub-arctic plant baseline (Mowat and Heard, 2006), and terrestrial meat was used as source data (Mowat and Heard, 2006). This model was run on a data set including 80 samples from 58 individual bears. The proportional contribution of the analyzed dietary sources was estimated for each bear-year ID. We combined the model estimates and 95% credible intervals for individual bears where blood samples for HM were available. Hair and blood were collected at the same capture event, hair represents diet the previous year and blood HM concentration the combination of recent and long-term exposure. We assume limited annual variation on the individual level in the dominant diet, however, salmon abundance varies between years. We used glm's to test if the blood concentrations of As, Cd, Hg and Pb in bears with >50% salmon in the diet the previous year differed compared to the bears with >50% vegetation in their diet the previous year. The difference between the groups was considered significant if $P < 0.05$.

3. Results

Summary statistics for each element in each area are presented in Table 2. Arsenic concentrations in six blood samples from Scandinavian bears and one from the Gates NP were below the limit of quantification (<0.15 μg/L). The human footprint index within the 2-km buffer of each sample location was 0 for all samples from the Alaskan study areas; we therefore decided to not include the human footprint index in further modelling. In Scandinavia, the maximal human footprint index within the buffer around each sample location ranged from 1 to 18, with a mean of 9.2. None of the HM concentrations in the Scandinavian samples were correlated with the human footprint index (correlation factors ranged between $r = 0.01$ for Hg and $r = 0.17$ for As with all p-values >0.05 (0.29–0.94)).

3.1. Arsenic

Three models were considered equivalent in explaining As in blood samples: the top model contained sample area as the only predictor, another model included sample area and the interaction between sex and age, and third model included sample area and sex (Table SM 1). We based interpretation on model-averaged estimates of these three models (Table 3). Predicted As blood concentrations for 10-year-old (mean age all bears) female bears were lowest in Scandinavia (0.4 μg/L; 0.2–0.9 μg/L) but confidence intervals overlapped with Gates NP (1.0 μg/L; 0.5–1.9 μg/L) and Clark NP (1.1 μg/L; 0.5–2.4 μg/L) (Fig. 1a). Katmai NP had the highest predicted blood As concentrations (3.3 μg/L; 1.2–6.7 μg/L) but had overlapping confidence intervals with the other Alaskan areas (Fig. 1). We found that As blood concentration decreased with increasing age in males and increased with increasing age in females. In Gates NP, a five-year-old (25% percentile all bears) female had a predicted As blood concentration of 0.8 μg/L (0.5–1.2 μg/L) and a male had a concentration of 1.0 μg/L (0.8–1.5 μg/L). At 13-years of age (75% percentile), the model predicted 1.1 μg/L (0.4–3.2 μg/L) for females and 0.7 μg/L (0.2–2.0 μg/L) for males, respectively.

3.2. Cadmium

The best model contained sample area and age (Table SM 1). The

Table 2
Mean, standard deviation (SD), range and sample size (N) of blood concentrations for arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb) in μg/L of 72 brown bears (*Ursus arctos*) sampled between 2010 and 2021 in Scandinavia and national parks and preserves (NP) in Alaska, USA.

Element	Study Area	Mean	SD	Range	N
As	Gates NP	1.1	0.9	<0.15–3.1	18
	Katmai NP	3.6	2.9	0.7–10.6	12
	Clark NP	1.2	1.7	0.3–6.2	12
	Scandinavia	0.4	0.5	<0.15–2.7	30
Cd	Gates NP	0.4	0.3	0.1–1.0	18
	Katmai NP	0.1	0.1	0.1–0.3	12
	Clark NP	0.2	0.1	0.1–0.4	12
	Scandinavia	0.3	0.2	0.1–0.8	30
Hg	Gates NP	15.9	19.5	1.5–43.5	18
	Katmai NP	19.7	10.5	4.5–39.9	12
	Clark NP	40.1	32.6	5.6–119.2	12
	Scandinavia	1.4	0.7	0.5–4.0	30
Pb	Gates NP	38.4	32.3	4.7–138.6	18
	Katmai NP	10.0	8.9	3.4–37.1	12
	Clark NP	16.7	13.2	2.7–52.5	12
	Scandinavia	83.9	33.1	24.6–167.0	30

Table 3

Model averaged estimates (log scale) of a generalized linear model predicting blood concentrations for arsenic (As) and lead (Pb) in blood samples from brown bears *(Ursus arctos)* based on a sampling area in Scandinavia (reference level) and three national park and preserves (NP) in Alaska, sex of the bear and age at sampling between 2010 and 2021. All values are on log scale.

	Variable	Estimate	95% Confidence Interval	
			Lower	Upper
As	Scandinavia	−0.646	−1.471	0.180
	Gates NP	0.905	0.256	1.555
	Clark NP	1.014	0.237	1.791
	Katmai NP	2.111	1.389	2.833
	Males	−0.692	−1.954	0.570
	Age	−0.048	−0.130	0.035
	Males × Age	0.090	−0.005	0.184
Pb	Scandinavia	4.350	4.001	4.698
	Gates NP	−1.123	−1.612	−0.635
	Clark NP	−1.990	−2.571	−1.408
	Katmai NP	−2.430	−2.929	−1.930
	Sex Males	−0.157	−0.528	0.214
	Age	0.033	−0.005	0.070

model predicted that the Cd blood concentration of a 10-year-old bear (mean age all bears) was significantly higher in Scandinavia (0.4 µg/L; 0.3–0.5 µg/L) and Gates NP (0.3 µg/L; 0.2–0.4 µg/L) compared to Clark NP (0.2 µg/L; 0.1–0.2 µg/L) and Katmai NP (0.1 µg/L; 0.1–0.2 µg/L) (Fig. 1b). Predictions of Cd concentrations increased with increasing age (Table 4). For example, the model predicted a Cd blood concentration of 0.2 µg/L (0.2–0.3 µg/L) for a five-year-old bear and 0.3 µg/L (0.3–0.4 µg/L) for a 13-year-old bear in Gates NP. Models with sex of the bear were not selected (Table SM 1).

3.3. *Mercury*

The best model for Hg contained sample area, body mass, and sex (Table SM 1). Predicted blood Hg concentrations for 10-year-old bears were lowest in Scandinavia (1.5 µg/L; 1.1–2.0 µg/L). Within the Alaskan study areas, predictions for Clark NP where highest and confidence intervals did not overlap with any other area (47.8 µg/L; 31.2–73.5 µg/L) (Fig. 1c). Blood Hg concentration did not differ significantly between Gates NP (18.2 µg/L; 13.0–25.4 µg/L) and Katmai NP (20.5 µg/L; 13.9–30.2 µg/L). Mercury blood concentrations decreased with age (Table 4): For a five-year-old bear in Gates NP the model predicted a blood Hg concentration of 22.7 µg/L (14.7–34.9 µg/L) and for a 13-year-old 15.9 µg/L (11.6–21.8 µg/L).

3.4. *Lead*

For Pb, two models were equivalent based on AICc scores: The top model contained sample area and age and the second-best model also included sex (Table SM1). The model averaged predictions (Table 4) revealed a significantly higher Pb blood concentration of a 10-year-old female bear in Scandinavia (107.3 µg/L; 75.7–152.0 µg/L) than in the Alaskan areas. Within the Alaskan areas, Gates NP bears had significantly higher Pb concentration (34.9 µ/L; 21.4 µ/L – 56.9 µg/L) than Clark NP (14.7 µg/L; 8.2–26.2 µg/L) and Katmai NP (9.5 µg/L; 5.7–15.5 µg/L). Estimated 95% CI's for the Clark NP samples overlapped with the Katmai NP samples (Fig. 1d). Predictions of Pb concentrations increased with increasing age (Table 3) from 29.6 µg/L (23.8–35.7 µg/L) in a five-

Table 4

Model estimates (log scale) of a generalized linear model predicting blood concentrations for cadmium (Cd) and mercury (Hg) in blood samples from brown bears *(Ursus arctos)* based on sampling area in Scandinavia (reference level) and three national park and preserves (NP) in Alaska and age at sampling between 2010 and 2021.

	Variable	Estimate	Standard Error	T-value	P-value
Cd	Scandinavia	−1.449	0.113	−12.769	<0.001
	Gates NP	−0.267	0.162	−1.653	0.103
	Clark NP	−0.806	0.191	−4.212	<0.001
	Katmai NP	−1.146	0.166	−6.925	<0.001
	Age	0.050	0.012	4.055	<0.001
Hg	Scandinavia	0.833	0.169	4.926	<0.001
	Gates NP	2.511	0.241	10.415	<0.001
	Clark NP	3.479	0.285	12.194	<0.001
	Katmai NP	2.631	0.247	10.664	<0.001
	Age	−0.044	0.018	−2.426	0.018

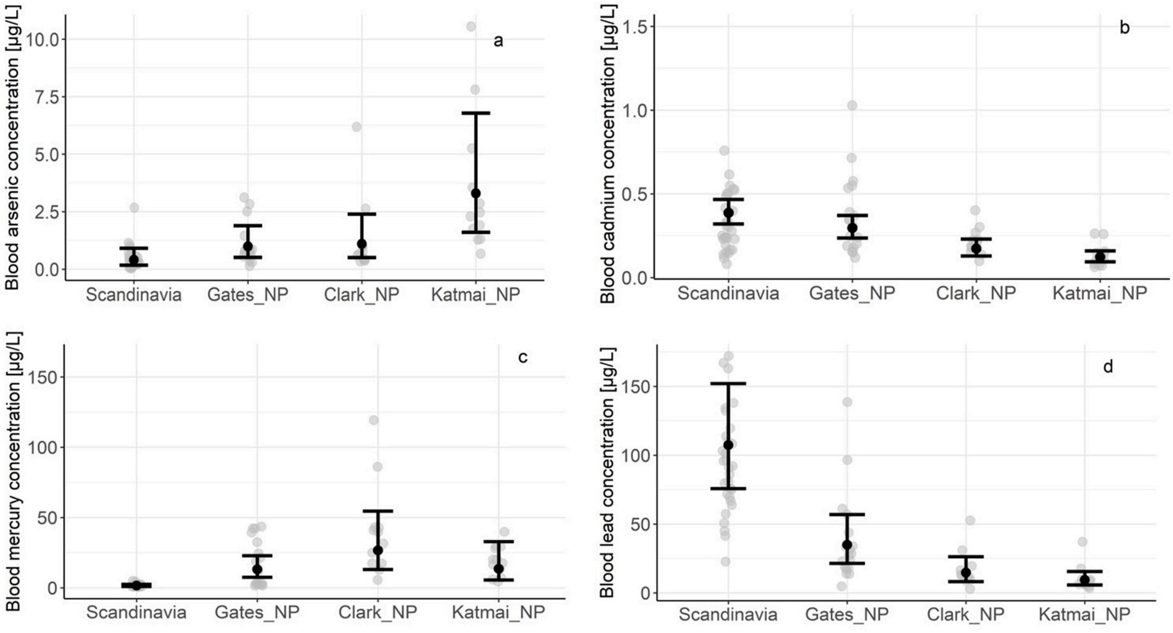

Fig. 1. Concentrations of arsenic (a), cadmium (b), mercury (c), and lead (d) in blood from brown bears *(Ursus arctos)* (µg/L, black dots) in south-central Scandinavia and three national park and preserves (NP) in Alaska, USA, predicted by generalized linear models including sample area, sex, and age of the bears as fixed factors. Error bars indicate 95% confidence intervals for the predictions. The raw data, collected between 2010 and 2021, are displayed as grey dots.

year-old to 38.5 (27.9–62.3 μg/L) in a 13-year-old female bear from Gates NP. Females had higher estimates than males; for example, in the Gates NP, a 10-year-old male had a predicted Pb blood concentration of 29.8 μg/L (20.6–43.2 μg/L), 5.1 μg/L lower than a female (34.9 μg/L; 24.1–50.6 μg/L).

3.5. *Heavy metal (loids) and diet*

Dietary proportion estimates in combination with blood HM concentrations were available for 17 Gates NP bears. The MixSIAR outputs suggests two distinct groups: Bears using predominantly vegetation-based resources and bears focusing on salmon, represented as marine protein. Nine bears used vegetation as their main dietary resource, and the proportional dietary estimates were >75% vegetation, with 95% credible intervals entirely >50% (Fig. 2). In the salmon group, for five bears, marine protein was estimated to comprise >75% of the diet, with 95% credible intervals entirely >50%, and one bear with an estimate of 68% (44–82%) marine protein in the diet. For the remaining two bears, the estimates where close to 50%, likely combining both sources. For all bears, the model estimated the terrestrial protein source contribution to <4% with upper 95% credible intervals <25%. Bears primarily feeding on vegetation had significantly higher As blood concentrations (1.6 μg/L, SE: 0.37 μg/L, $P < 0.05$) than bears feeding on salmon (0.6 μg/L, SE: 0.17 μg/L). No significant differences in Cd blood concentration were found between bears that fed primarily on either salmon or vegetation. The estimated mean Hg blood concentration of bears that had >50% vegetation in their diet was 2.7 μg/L (SE: 0.2 μg/L) but blood Hg concentration were 11 times higher (31.7 μg/L, SE: 3.5 μg/L, $P < 0.001$) for bears with >50% salmon in their diet. In comparison, bears with >50% vegetation in their diet had triple the blood Pb concentration (56.3 μg/L, SE: 13.5 μg/L, $P = 0.016$) than bears that primarily fed on salmon (18.6 μg/L, SE: 5.4 μg/L).

4. Discussion

We report blood concentrations of four non-essential elements in four different brown bear populations in Alaska and Scandinavia. While Scandinavian bears were exposed to a larger human footprint and had higher concentrations of Cd and Pb, they had lower concentrations of As and Hg. Bears in the Gates NP, the most remote of the Alaskan areas, had the highest levels of Cd and Pb. Contrary to hypothesis *i*), it suggests that local human footprint is a poor predictor of HM exposure and intake. Bears with a marine protein-based diet had higher Hg but lower Pb concentrations compared to bears with high vegetation levels in their diet, supporting our hypothesis *ii*) connecting diet to heavy metal exposure. Bears in areas with a high level of marine proteins in the diet had higher As concentrations. However, in Gates NP, bears feeding primarily on salmon had lower As concentrations than bears feeding primarily on vegetation. No relationship between diet and Cd blood concentration was found in Gates NP. Hypothesis *iii*), suggesting increasing whole blood HM concentrations with age, was supported for Cd, Pb and As in females, whereas concentrations of As in males and Hg decreased with age.

HM are distributed into our study areas via atmospheric distribution, river run-off, and ocean currents, as well as via upstream migrating anadromous salmon. Arsenic, Cd, Hg and Pb are contaminants that cycle in the environment for long periods, before they finally are deposited in lake or ocean sediments (AMAP, 2005). For example, 60% of the annual emitted Hg are re-emissions, depositions from anthropogenic sources that are mobilized primarily due to natural processes such as wildfires (Dastoor et al., 2022). There is strong evidence that the rapid environmental changes, due to global warming, will alter contaminant pathways and cycles in unexpected and abrupt ways in the Arctic and Sub-Arctic (Macdonald et al., 2005). Increased wildfire activity, erosion, melting ice and thawing permafrost are processes that increase with global warming and have high potential for contaminant re-emission (AMAP, 2005; Chételat et al., 2022b). From a One Health perspective, we found that bears were exposed to HM likely by food

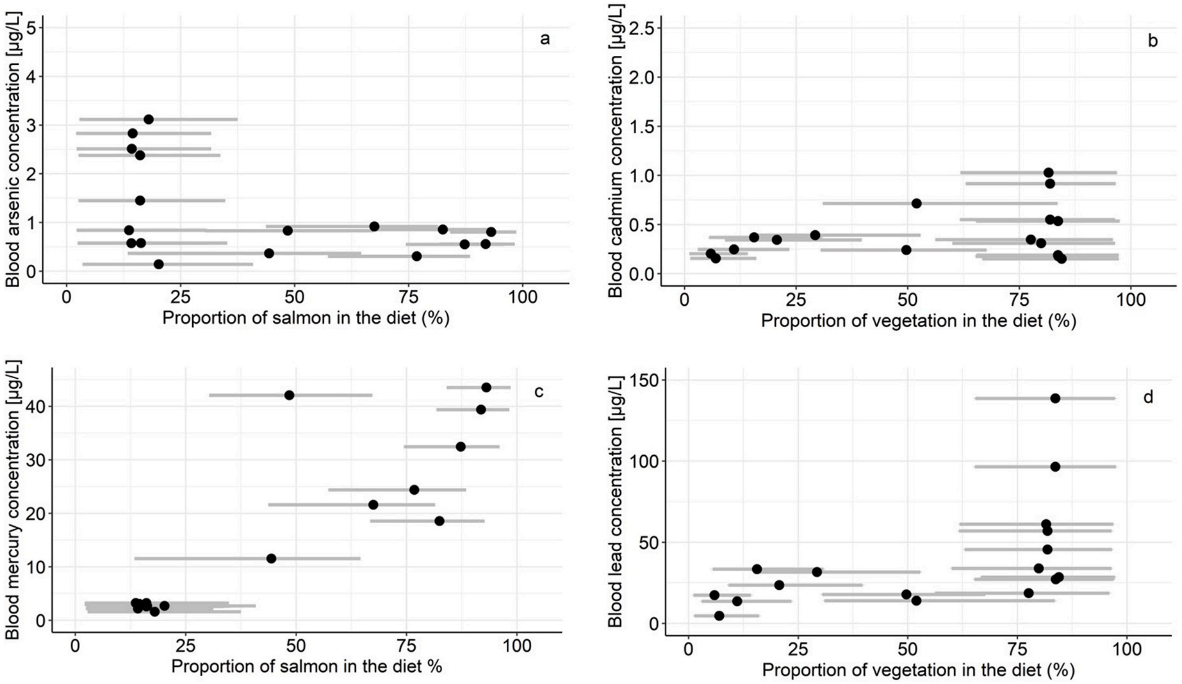

Fig. 2. Proportion of salmon (a and c) and vegetation (b and d) in the diet (black dots and grey 95% credible intervals) of 17 brown bears from the Gates of the Arctic National Park and Preserve, Alaska, USA, in relation to blood concentrations of arsenic (As), cadmium (Cd) mercury (Hg) and lead (Pb). Dietary estimates by (Mangipane et al., 2020) were based on C/N isotopes in hair; both hair and blood samples were collected in 2016. The proportion of terrestrial protein intake was estimated to be <4% for all individuals and is not shown.

items such as salmon and berries, that also are highly appreciated by humans. Protected areas, such as the national parks in Alaska, are supporting a healthy, diverse and resilient environment and although they are protected from direct human impact, they can still be vulnerable to pollution. We suggest extending contaminant monitoring in remote terrestrial ecosystems and to increase efforts to reduce both direct and re-emissions of HM.

Bears in Scandinavia feed exclusively on terrestrial resources (Stenset et al., 2016) and had the highest Pb concentrations but the lowest Hg blood concentrations. Bears in Katmai NP feed predominantly on a marine diet, consisting of migrating salmon in fresh water and flounders and clams along the seashore. Similarly, bears from Clark NP feed primarily on salmon (Rogers et al., 2020). Both Alaskan populations with a marine based diet had low Pb blood concentrations and high Hg concentrations. In comparison, bears from Gates NP either feed on spawning salmon and have high Hg and low Pb, or feed on upland terrestrial vegetation (Mangipane et al., 2020) and have low Hg with some individuals having elevated Pb blood concentrations. Besides in Gates NP, Cd concentrations were higher in Scandinavia than in Alaska, generally, reflecting the higher reported background levels there (AMAP, 2005). We suggest that HM exposure on brown bears is connected to the population typical food sources and the regional background concentrations, except for Hg, for which we suggest that background concentrations are less relevant and that migrating salmon are the dominant source of exposure.

In general, there are two ways to contextualize HM blood concentrations: The comparison to benchmark dose limits (BML) for the onset of toxicological effects or to compare individuals to the population exposure to detect deviating concentrations. Knowledge about the onset of sublethal toxicological effects of HM on bears, and for terrestrial top predators in general, is very limited (Rodríguez-Jorquera et al., 2017). We chose here to compare four populations to each other and discuss deviating results in context of each population's typical diet and human impact on the landscape level.

In bears, As concentrations were higher in Alaska than Scandinavia and highest in Katmai NP. Bears in Katmai NP also consume marine sources other than salmon. The intake of seafood has been linked to high As concentrations in human urinary samples, however in the organic form, which is less toxic and readily excreted (ATSDR, 2020). We quantified the total As blood concentration and were thus unable to distinguish between different forms of As. Only blood As concentrations in bears from Katmai NP reached a predicted mean (3.3 μg/L) which is above the lower detection limit (2.37 μg/L) of the study by Lazarus et al. (2020) on bears in Poland and Croatia, where most bears tested below that concentration. We found decreasing concentrations in females but not in males; however, effect sizes were small and must be interpreted with care. The same care should be taken when comparing between species: For example, brown rats *(Rattus norvegicus)* can bind 15–30 times more As (in the form of inorganic arsenite) to red blood cells than humans which in turn are more sensitive to As compared to other mammals (Lu et al., 2004).

Predicted Cd blood concentrations of Scandinavian bears (0.4 μg/L) were higher than in south-eastern European bears (0.29 μg/L) (Lazarus et al., 2020), which, in turn, were similar to bears from Gates NP (0.3 μg/L). This is counterintuitive, because there are higher Cd (and Pb) background concentrations in top soils in Croatia and Poland (0.10–0.38 mg/kg Cd) compared to Scandinavia (0.03–0.08 mg/kg Cd) (Salminen et al., 2005) and most likely also compared to the very remote Gates NP. This suggest that bears in Scandinavia, and to some extent Gates NP, accumulate more Cd and Pb from their environment compared to bears in Croatia and Poland. There are large differences in many aspects between these populations, but the Scandinavian and the Gates NP bears share a hyperphagic diet that is based on berries, which is different compared to bears from south-eastern Europe where hard mast dominates the fall diet (Lazarus et al., 2017; Mangipane et al., 2020; Stenset et al., 2016). Also, the predicted concentrations in samples from Clark NP and Katmai NP are lower (0.2 μg/L and 0.1 μg/L) and those bears have more marine food resources. For all four elements studied here, diet is the most likely exposure route. Cd is assumed to bioaccumulate in organisms (Järup, 2003; Zhang and Reynolds, 2019), which is supported by our result of higher Cd blood concentrations in older bears, similar to bears from Croatia where renal tissue Cd concentrations increased with age (Lazarus et al., 2017).

Mercury has biomagnification properties, increasing exposure risk in top level consumers, especially in the arctic marine system. The top 100 m of the Earth's oceans are a significant Hg reservoir and gaseous evasion of elemental Hg is restricted by arctic sea ice while advection of Hg from the oceans from the south is ongoing (AMAP, 2005). Although bears in Katmai NP also feed on other marine sources, migrating salmon are the main marine food resource for brown bears in Alaska. All five Pacific salmon species occurring in Alaska are fast growing, not top-trophic level consumers, and are considered the "best choice" for human consumption based on their low Hg concentrations (Bridges et al., 2020). However, the relationship between increased Hg blood concentrations in brown bears from the Gates NP and their $\delta^{13}C$ values indicating marine dominated diet, suggests salmon as the main source of Hg. The Hg blood concentrations we found in brown bears are also closer to polar bears than we expected. Knott et al. (2011, 2012) reported blood Hg mean concentrations of polar bears from the Bering Sea grouped by year, cohort, and sex, ranging from 39.05 μg/kg wet weight (ww) (males in 2007) to 74.62 μg/kg ww (both sexes, <4 years old in 2005). The mean Hg blood concentration from Clark NP brown bears (this study) was 40.1 μg/L (consider 1 L blood ~ 0.9 kg blood). Maximum measured concentrations in polar bears are two-fold higher than in brown bears and only in two samples from Clark NP blood Hg concentrations of >50 μg/L were measured. Our model estimated a tendency of decreasing Hg concentration with age. Visual interpretation of the measured Hg blood concentrations in relation of age of the Alaskan brown bears (not shown), indicate that the highest concentrations were measured in individuals between 7 and 13 years of age. This coincides with the age bears in these populations reach asymptotic body size (Hilderbrand et al., 2018). Such young but full-sized bears might be more successful in competing for good fishing spots, getting exposed to more Hg from salmon, while younger and older bears need to feed more on alternative source such as vegetation in addition to salmon.

Previous studies on Pb exposure in brown and American black bears proposed that Pb in their diet either come from a mixture of environmental concentrations and Pb-based ammunition in ungulate carcasses discarded by hunters (Brown et al., 2022; Fuchs et al., 2021; Lazarus et al., 2018; Rogers et al., 2012). In the Scandinavian study area, bears are likely exposed to both sources (Brown et al., 2023), but recreational hunting is prohibited and subsistence hunting occurs only at very low levels in the Alaska national parks. This suggests that environmental Pb is likely the primary source in the Alaskan study areas. The minimum blood Pb concentrations found in bears from national parks in Alaska (<5 μg/L) are below current published values for the species. Brown bears in the greater Yellowstone area have blood Pb concentrations of ≥11 μg/L (Rogers et al., 2012). In mammals, Pb bioaccumulates in bones with a half-life of 10–30 years (Andreani et al., 2019; Hryhorczuk et al., 1985; Rabinowitz et al., 1976). Lead stored in bones becomes an endogenous source during periods of nutritional stress and increased bone turnover (Bellinger et al., 2013), for example, during hibernation, pregnancy and lactation. In previous work in Scandinavia, we found all bears to have increased blood Pb concentrations and had concerns that hibernation alters Pb kinetics in bears such that the comparison of blood Pb concentrations to other mammals might not be valid (Boesen et al., 2019; Fuchs et al., 2021). However, based on the Pb blood concentrations found in the Alaskan samples, especially the high variation within Gates NP, suggests that blood is an adequate tissue to quantify Pb in brown bears. The bears with a plant-based diet in Gates NP had blood Pb concentrations >50 μg/L, which is consistent with the higher Pb concentrations reported in bears from Scandinavia where most bears also rely on vegetation during the hyperphagic period (Stenset et al., 2016). We found females to have higher Pb blood concentrations compared to males which is in line with previous work, where blood Pb concentrations significantly increased with lactation, but not age (Fuchs et al., 2021). Sex differences were also found in American black bears where Pb concentration in teeth was slightly higher in females and increased with age in both sexes (Brown et al., 2022). This highlights that blood Pb concentrations need to be interpreted with care; lower blood Pb in males is in part a result of a different physiology and not necessarily based on a different exposure.

Scandinavia has been exposed to extensive aerial Pb depositions emitted from Pb-enriched gasoline, (von Storch et al., 2003). At peak use of Pb in gasoline at the end of the 1970's, background Pb concentrations in Scandinavia were increased by an order of magnitude (Renberg et al., 2001). Since then, both deposition and concentrations of Pb in humans have decreased by > 90% (Pacyna et al., 2009; Skerfving et al., 2015). The atmospheric deposition of Cd in Scandinavia follows a similar trend. Aerial transport distance of Cd might be shorter compared to Pb, thus deposition is closer to sources resulting in a decreasing south to north exposure gradient of Cd in Scandinavia (AMAP, 2005). The Scandinavian Peninsula is closer to large combustions than Alaska and the weather conditions facilitate the aerial transport from central Europe to the north, unlike in Alaska (AMAP, 2005; von Storch et al., 2003). Nevertheless, a recent study from western Canada found that organisms, including plants, fish and mammals. Accumulate Pb emitted by industrial activities (Chételat et al., 2022a).

4.1. Conclusion

The human footprint was a poor predictor for HM exposure. The human influence on the boreal forest and tundra is considered low or very low in more than 50% of these biomes' global extent, however, indices of the human footprint are calculated based on local human infrastructure and land use change but do not consider pollution from distant sources (Keys et al., 2021; Riggio et al., 2020). Sub-arctic regions are susceptible to HM pollution from aerial depositions from entire Europe and marine currents in the Pacific, but also ongoing natural processes such as erosion by glaciers and rivers mobilize HM (Chételat et al., 2022a, 2022b). Natural processes releasing HM are altered by human activities, for example, draining of wetland mobilizes Hg in Scandinavia (AMAP, 2005). Global climate warming increases glacier run off, erosion and thawing permafrost potentially increasing HM mobilization whereas declining sea ice might allow increased Hg escape from the arctic marine system (AMAP, 2005; Macdonald et al., 2005). Establishing baseline HM exposure and continuous monitoring of HM exposure are needed, even in wilderness areas.

Author contributions

Boris Fuchs: Conceptualization, Methodology, Formal analysis, Investigation, Data Curation, Writing - Original Draft. **Kyle Joly:** Investigation, Resources, Data Curation, Writing - Review & Editing. **Grant V. Hilderbrand:** Investigation, Resources, Writing - Review & Editing, Supervision. **Alina L. Evans:** Conceptualization, Investigation, Writing - Review & Editing. **Ilia Rhoduskin:** Investigation, Resources, Writing - Review & Editing. **Lindsey S. Mangipane** Methodology, Formal analysis, Investigation, Resources, Data Curation. **Buck A. Mangipane:** Methodology, Formal analysis, Investigation, Resources, Data Curation. **David D. Gustine:** Investigation, Resources, Data Curation**. Andreas Zedrosser:** Writing - Review & Editing, Supervision. **Ludovick Brown:** Validation, Writing - Review & Editing. **Jon M. Arnemo:** Conceptualization, Investigation, Resources, Writing - Review & Editing, Supervision, Funding acquisition.

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Ethics and animal welfare

Capture and handling procedures in Alaska were approved by the US National Park Service (AKR_KATM_Hilderbrand_BrownBear_2014, AKR_LACL_Mangipane_BrownBear_2014, AKR_GAAR_Gustine_GrizzlyBear_2014) and the US Geological Survey (2014–01, 2015–04, 2015–06) Animal Care and Use Committee. In Scandinavia, captures were approved by the Swedish Ethical Committee on Animal Research, Uppsala, Sweden (C18/15) and the Swedish Environmental Protection Agency, Stockholm, Sweden (NV-00741-18). Shipping of whole blood samples from brown bears *(Ursus arctos)* from the USA to Sweden followed the CITES regulations (export permit #19US15593D/9, import permit #4.10.18–12752/2019).

Declaration of competing interest

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2023.115952.

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Contamination from leaded gasoline combustion and hunting ammunition exposes brown bears *Ursus arctos* and other wildlife to lead (Pb) in Scandinavia

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Abstract

Lead (Pb) contamination poses a threat to wildlife, primarily through exposure to anthropogenic sources. Legacy Pb from leaded gasoline has substantially increased natural background concentrations globally. Scavenging birds and mammals are at high risk, adding Pb exposure when ingesting fragments of Pb-based hunting ammunition in offal and carcasses. Brown bears, as opportunistic scavengers, exhibit elevated blood and tissue Pb concentrations in North America, southeastern Europe, and Scandinavia. This study aimed to identify the sources of blood Pb in Scandinavian brown bears.

In a first step, we compared blood Pb concentrations in bears and sympatric scavengers and non-scavengers. We then analyzed Pb isotopes in bears and food items such as ants (*Formica* ssp.), berries (*Vaccinium* ssp. and *Empretum* ssp.), ant nest material, as well as Pb-based hunting ammunition. Previous published data on leaded gasoline and natural background Pb was included. Regression models were employed to assess the isotopic mix in bear blood relative to spatial origin and annual variation in berry abundance.

Scavenging species exhibited median blood Pb concentrations of 6.5 µg/L, 83.8 µg/L and 171.8 µg/L in wolverines (*Gulo gulo*), bears and ravens (*Corvus corax*) respectively, compared to 2.1 µg/L in wolves (*Canis lupus*) and 6.2 µg/L in moose (*Alces alces*). Isotopic ratios in brown bears (^{207}Pb/^{206}Pb, mean [SD] = 0.85 [0.012]) and other species aligned with an industrial Pb mix including ammunition, with spatial variations indicating natural background Pb involvement. Ant nest material, potentially ingested by bears, showed the highest exposure potential but with concentrations (5 144 µg/kg [4 078 µg/kg]) lower than agricultural soil in Sweden. The isotopic ratio in ant nest material (0.84 [0.035]) was similar to that in ammunition (0.85 [0.021]) and we were not able to quantify relative source contribution using isotopic information. Neither isotopic ratios nor the Pb concentration in bear blood were correlated with berry abundance. We concluded that anthropogenic Pb is the major source of Pb in all studied species. Scavenging and soil ingestion contributed to elevated Pb concentrations, with wolverines being an exception.

Introduction

Heavy metals contamination of the environment originates from a mix of multiple sources, including natural processes and anthropogenic activities. Identifying heavy metal sources in a contamination mix is important to effectively trace and mitigate the contaminant release.

Lead (Pb) is a highly toxic heavy metal with no known biological function in any organism and consists of four stable isotopes. Three of them, ^{206}Pb, ^{207}Pb and ^{208}Pb are radiogenic, i.e., the end-members of radioactive decay chains starting with two uranium isotopes (^{238}U and ^{235}U) and thorium (^{232}Th) (Hansmann and Köppel, 2000). Isotope ^{204}Pb is an entirely primordial isotope i.e. older than the solar system. Due to the radioactive decay, the atomic ratio between the primordial ^{204}Pb and the radiogenic proportion of ^{206}Pb, ^{207}Pb and ^{208}Pb decreases linearly with time, whereas the ratios between ^{206}Pb, ^{207}Pb and ^{208}Pb depend on the U/Pb and Th/Pb concentration ratios of the mother material (Gulson, 2008; Hansmann and Köppel, 2000). Pb ores, where most of the industrial Pb initially has been extracted from, are geochemical anomalies with relatively low but ore-specific U/Pb and Th/Pb concentration ratios, resulting in a smaller proportion of radiogenic Pb isotopes. Pb from weathered bedrock, is more radiogenic because of a higher ratios of U/Pb and Th/Pb, resulting in higher abundance of radiogenic Pb isotopes (Hansmann and Köppel, 2000). Since most Pb ores have a specific U/Pb and Th/Pb ratio and in addition have been formed at different times, they also have a specific isotopic composition, and isotopic ratios can be used to trace ore-derived Pb back to its origin.

Pb is naturally present at very low concentrations in all terrestrial environments. In Scandinavia, precipitation of Pb transported in aerosols originating from the combustion of leaded gasoline is the major contributor of Pb pollution in the top soil (Renberg et al., 2001; von Storch et al., 2003). For example, the Pb concentration in the Oh-horizon (top layer) in southern Norwegian forest soils is 5 to 25 times higher than the Pb concentration at the C-horizon (bedrock), and 75% to 99% of that top layer Pb has anthropogenic origin (Steinnes et al., 2005). Sediment analysis in Sweden suggests that industrial pollution has increased the natural Pb background up to 1 000 times (Renberg et al., 2001). The isotopic composition of the Pb mix used as an antiknock additive in leaded gasoline in Europe is characterized by

^{207}Pb/^{206}Pb ratios that are distinctively different and much higher compared to natural background Pb concentrations in Scandinavia (Komárek et al., 2008; Renberg et al., 2001).

Leaded gasoline has been phased out in Europe and consequently Pb exposure by aerial contamination has dropped dramatically. For example, in Sweden Pb concentrations in mosses, used as bioindicators for air pollution, have decreased by 96% in the period from 1975 to 2015 (Danielsson and Karlsson, 2015).

An ongoing source of Pb exposure is accidental ingestion of fragments from spent Pb based hunting ammunition (Arnemo et al., 2016; Green and Pain, 2019). European hunters discharge over 140 000 tons of lead-based ammunition each year during hunting (ECHA, 2018). The isotopic mix in ammunition is influenced by globally traded Pb, including recycled Pb used to manufacture bullets (Koons and Grant, 2002). Sjåstad et al. (2016) have measured isotopic compositions of bullets (0.22 LR) originating from across the world and report ^{207}Pb/^{206}Pb ratios ranging from 0.81 to 0.96 and ^{208}Pb/^{207}Pb ranging from 2.32 to 2.48. These values overlap with most ore deposits used for industrial Pb production (Ellam, 2010), leaded gasoline in Europe (Komárek et al., 2008) and soil down to 60 cm in Scandinavia (Steinnes et al., 2005). Only C-horizon samples in Scandinavia have an isotopic composition outside of the range for ammunition (Pallavicini et al., 2018; Steinnes et al., 2005). Despite this variability, the isotopic composition of Pb-based bullets is highly valuable in forensic investigations, for example to link Pb poisoning of individual scavengers to a specific carcass shot with Pb-based ammunition (Finkelstein et al., 2010). At the landscape level, the isotopic composition of spent hunting ammunition is unknown, and significant uncertainty persists in attributing the isotopic composition of the Pb mix, for example in a scavenger, to its source (van den Heever et al., 2023). Recently, Bayesian mixing models have gained attention in tracing and quantifying Pb sources in environmental studies (Arrondo et al., 2020; Dietrich et al., 2021; Ray and Das, 2023). However, these models assume independence of the sources, a nonlinear mixing geometry, and the inclusion of all sources (Longman et al., 2018; Stock et al., 2018; Ward et al., 2011). Meeting these assumptions with Pb isotope data from non-specific industrial sources, such as ammunition, is challenging due to the natural collinearity of the radiogenic isotopes but also due to the rising prevalence of recycled Pb and the global proliferation of ore Pb (Ellam, 2010; Kamenov et al., 2023; van den Heever et al., 2023). In environmental Pb studies, isotope data needs to be combined with other information, especially with mix and source Pb concentrations or spatio-temporal information (Arrondo et al., 2020; Cartró-Sabaté et al., 2019; van den Heever et al., 2023).

The half-life of Pb in blood is four to five weeks in humans (Rabinowitz et al., 1976), and is assumed to be similar in bears (Arnemo et al., 2022). However, accumulated Pb in bones and internal organs is remobilized into the blood stream and concentrations eventually reach an equilibrium between bones, internal organs and blood and the kinetics is thought to follow a three-compartment model (Mattisson et al., 2010; Rabinowitz et al., 1976; Yaginuma-Sakurai et al., 2012). Blood Pb concentrations thus reflect a combination of recent uptake and total body burden. Different physiological states, such as lactation, hibernation, hydration, malnutrition, or death, might change the concentrations at which these elements are at equilibrium between the different compartments (Fuchs et al., 2021; Silbergeld, 1991; Söderberg et al., 2023).

Scavengers are at risk for Pb exposure from hunting ammunition. This has been demonstrated, either via temporal overlap of the hunting season or based on Pb blood concentrations in common ravens (*Corvus corax*) (Craighead and Bedrosian, 2008; Legagneux et al., 2014), golden *eagles (Aquila chrysaetos*) (Ecke et al., 2017) or using Pb isotopes in griffon vultures (*Gyps fulvus*), white-backed vultures (*Gyps africanus*) and white tailed eagle (*Haliaeetus albicilla*) (Arrondo et al., 2020; Helander et al., 2009; van den Heever et al., 2023).

Brown bears (*Ursus arctos*) are prone to environmental Pb exposure, both in Scandinavia, south-central Europe and North America (Fuchs et al., 2023, 2021; Lazarus et al., 2020; Rogers et al., 2012). Possible routes of exposure in bears are ingestion of Pb via food items as fragments from ammunition when scavenging on hunting remains; soil and ant nesting material, milk (in dependent off-spring), as well as exposure of fetuses during pregnancy (Brown et al., 2023; Fuchs et al., 2021; Lazarus et al., 2020). The predominant sources of Pb exposure in brown bears, however, remain unclear in all these studies. Brown bears in Scandinavia hibernate for up to six months between October and April (Evans et al., 2016). During hibernation bears do not feed, drink, defecate or urinate, i.e. all major pathways for both uptake and excretion of Pb are shut down (Nelson et al., 1983). During the active phase, bears feed on berries (*Vaccinium* spp*., Empetrum nigrum*), ants (mainly ants of the genera *Formica* and *Camponotus*) and vertebrates, predominantly moose (*Alces alces*) (De Cuyper et al., 2023; Hertel et al., 2018; Rauset et al., 2012; Stenset et al., 2016; Swenson et al., 1999).

Fecal analysis estimates a debris content dominated by ant hill material of about 16% in spring and summer, and 6% during the berry season in the fall (Stenset et al., 2016). Ingestion of Pb from foods thus includes the background Pb of berries, ants, ant hill material and moose. Bears in Scandinavia predate on neonate moose calves in early summer (Ordiz et al., 2020; Rauset et al., 2012; Swenson et al., 2007). Throughout the remaining active season, scavenging is the predominant pathway for meat consumption (Ordiz et al., 2020).

Blood Pb concentrations of Scandinavian brown bears correlate with both the environmental background concentration and ungulate harvest distribution (Brown et al., 2023). 98% of Swedish moose hunters used Pb based rifle ammunition (Stokke et al., 2017). Gut piles of harvested moose are commonly left in the forest, and if the meat is kept for private consumption, hide, head, trimmings, and bones are either dumped in the forest, used as dog food or as a bait to attract scavengers.

Goals and hypotheses

The main objective of this study was to assess the contribution of different sources to Pb concentrations in the blood of a large omnivore, the Scandinavian brown bear. As potential exposure sources, we considered natural background Pb, industrial background Pb primarily from leaded gasoline emissions, and industrial Pb from hunting ammunition. Our analyses involved quantifying Pb concentrations and isotopic compositions in samples of items consumed by bears, including ants, ant nesting material, berries, and hunting ammunition. To enhance our source data, we incorporated Pb isotopic composition from the existing literature on preindustrial background Pb, historical aerosol data in Sweden, and Pb added to gasoline.

We tested several hypotheses, each either supporting or challenging specific sources of Pb contamination. To provide a context, we compared the Pb concentration in the blood of omnivorous brown bears alongside with blood Pb concentrations of other Scandinavian wildlife species on a carnivore – omnivore – herbivore continuum, i.e. wolves (*Canis lupus*) – wolverines (*Gulo gulo*) – common ravens – moose. Further we compared Pb concentrations in berries, ants and ant nest material as an indication of exposure potential of these items.

We hypothesized 1) a food source-related variation in Pb concentrations among the species investigated and predicted that 1.1) strictly carnivorous wolves would have the lowest Pb concentrations because of their reluctance towards scavenging on hunting remains compared to omnivorous bears, ravens and wolverines, which have been shown to feed on hunting remains (Gomo et al., 2017; Ordiz et al., 2020; Wikenros et al., 2023, 2013). In this regard, Pb concentrations and isotopic mix from moose, a strict herbivore, provides background Pb concentration unaffected by ammunition and ants/ant nest. We correlated Pb isotopic abundances and concentrations in ants with their nest material hypothesizing that the primary Pb exposure of ants would be through soil and nesting material and thus both the ant's Pb concentration and isotopic ratios would correlate with that of the nesting material.

We continued in an analytical approach, relating Pb blood concentrations and isotopic ratios in bears to spatial and temporal variation. We hypothesized 2) a spatial variation in Pb isotopic composition in bear samples and predict to detect spatial variation in natural background Pb but not industrial Pb. We furthermore hypothesized 3) an annual variation in the isotopic composition of bears blood Pb and predict that this variation is correlated with berry abundance, anticipating that in poor berry years, bears might supplement with alternative food sources such as gut piles or ants. Finally, recent exposure to ammunition fragments, increase Pb concentration and may alter Pb isotopic composition, we correlate blood Pb concentration and Pb isotopic composition in bear blood and predict 4) a less radiogenic isotopic composition in samples with a higher blood Pb concentration.

Methods

Study area

All samples in this study are from south-central Scandinavia, either from within or close to the project area of the Scandinavian Brown Bear Research Project (61°N, 14°E) (Scandinavian Brown Bear Research Project, 2020) (Figure 1, Figure S1). The study area is predominantly covered by intensively managed coniferous forest (*Pinus sylvestris* and *Picea abies*) interspersed with wetlands, and an alpine area along the Swedish-Norwegian border. Forest stands are typically clear-cut at 80 to 100 years of age followed by soil scarification and planting (Martin et al., 2010). Human density is <9/km^2 distributed in small settlements throughout the entire study area. Roads, used for forestry and recreation occur at a density of 0.3 km/km^2 and 0.7 km/km^2 for paved and gravel roads, respectively. Between 2010 and 2020, the annual moose harvest rate was 0.23/km^2 (Länsstyrelserna, 2020); 98 % of the moose were shot with Pb-based ammunition and their digestive tracts, organs, and slaughter remains were mostly left in the forest (Stokke et al., 2017). In 2023, brown bear harvest density was 0.008/km^2 in Gävleborg County, and 0.003/km^2 in Dalarna County (Länsstyrelserna, 2023).

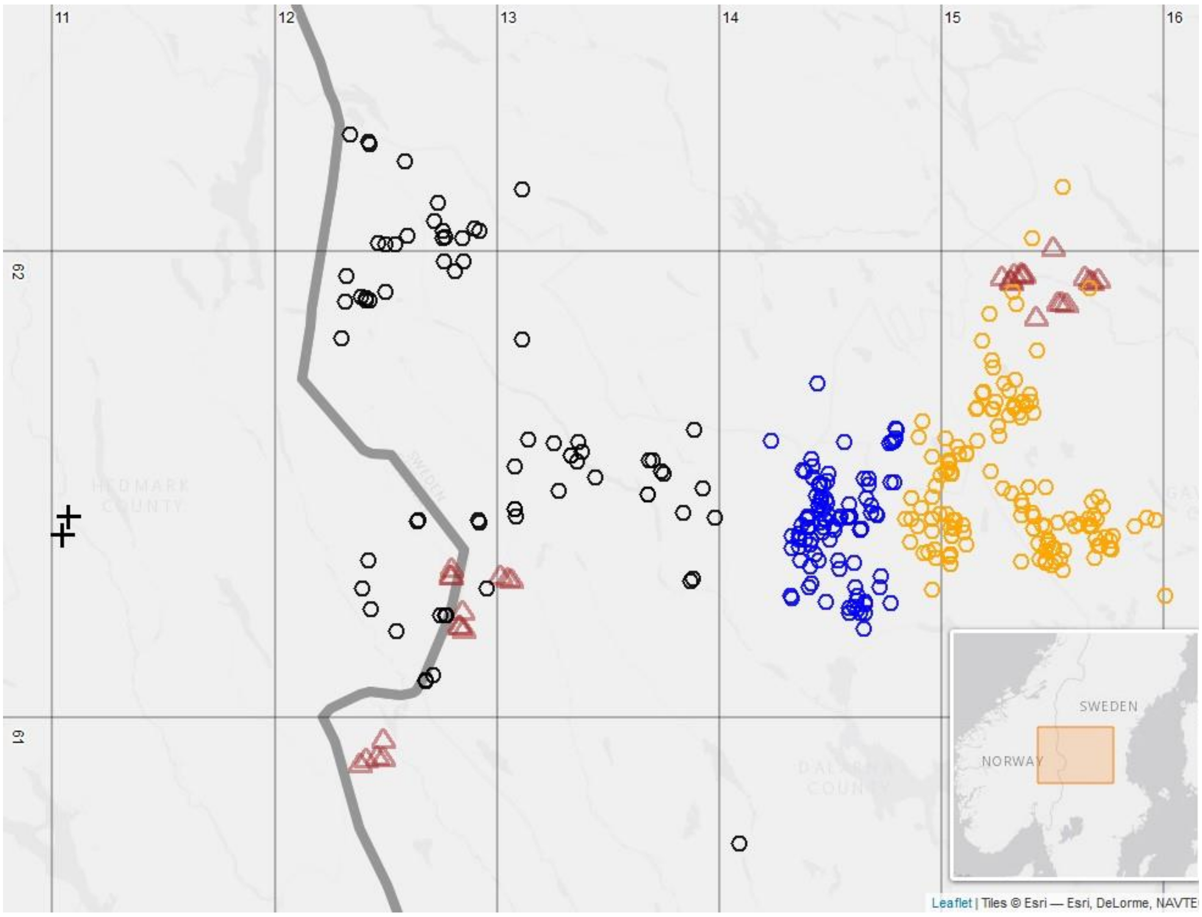

Figure 1: Blood sampling locations of brown bears (*Ursus arctos*) (open circles), moose (*Alces alces*) (open triangles), and common ravens (*Corvus corax*) (cross), sampled between 2018 and 2022. Bears are grouped in a western (black) a central (blue) and an eastern (yellow) cluster. To enhance clarity, locations for other sampled species are detailed in the supplementary materials (Figure S1).

Blood sample collection

Bears, wolves, moose and wolverines were chemically immobilized for GPS collaring in several wildlife research projects (Table S1), for details on capture see Arnemo and Evans (2017). Captures were approved by the Swedish Ethical Committee on Animal Research, Uppsala Sweden, the Swedish Environmental Protection Agency, the Norwegian Food Safety Authority and the Norwegian Environment Agency (Table S1). The mammary glands of bears and wolverines were palpated and considered lactating when milk was present.

Blood was collected from the jugular vein in bears, moose, and wolverine and from the cephalic vein in wolves. Either 4 mL evacuated K_3EDTA tubes (EDTA) (Vacuette, Greiner Bio-One International GmbH, Kremsmünster, Austria) or 6 mL evacuated heparin trace element tubes (TE) (Vacuette) were used for blood sampling. At least two blood samples (same sample tube type) were taken. Blood samples were frozen the same day and kept at −20 °C during storage and shipment to the laboratory (ALS Scandinavia AB, Luleå, Sweden). Samples were shipped and analyzed in one to two batches each year.

Ravens were trapped in two live-traps in eastern Norway during their hunting season by local landowners attempting to reduce small game predation (Figure S1). Ravens were euthanized at the trap site with a powerful hit to the head after dark on the same day they had entered the trap. Blood was sampled into evacuated EDTA or TE tubes from the heart immediately after death. Raven traps were baited with hunting remains (internal organs) from moose predominantly shot with Pb-based rifle bullets.

Other biological samples

We located the ant nest (mound) for sample collection that was closest to the intersections of a 12x12 km grid that covered the range where we collected blood samples from bears (Figure S1). No ant species selection was performed at this stage. The exact location of the nest was recorded and approximately 25 ants were collected and stored in 5 mL polypropylene (pp) tubes suitable for element analysis (Screw Cap, Sarstedt, Nürnbrecht, Germany). From each nest, 4 cc^3 material was collected from 5 cm below the surface, at approximately two thirds up the mound and stored in cryovials. Five ants from each nest were later submitted to an entomologist for species identification. All ant samples were collected in September 2022.

We collected bilberries (*Vaccinium myrtillus*), lingonberries (*Vaccinium vitis vide*a) and crowberries (*Empetrum nigrum*). Berries were collected on randomly assigned 1m^2 sample plots in the western part of the study area in August (Figure S1) for a study assessing berry density in relation to forest status (Hertel et al., 2018). Berries were stored in pp bags and kept frozen at -20° during storage and shipment to the laboratory.

Rifle ammunition

We bought eight batches of Pb-based rifle ammunition used for big game hunting in Scandinavia in late 2019. Based on findings of Stokke et al. (2017) reporting frequently used bullets for moose hunting in Sweden, we selected bullets produced by Lapua (Mega, cal. 30.06) and Norma (Alaska cal. 30.06, Oryx cal. 6.5x55, cal. 30.06 and cal. 9.3x62, Vulkan cal. 30.06). From each batch we scraped approximately 50 mg Pb from the tip of one bullet using ceramic scalpels. Bonded bullets were cut open using a metal saw. A first scraping of Pb from the bullet core was discarded to minimize contamination. Samples were stored in 2 mL pp tubes (Cryovial, Avantor, Radnor, USA). We extended the data set by adding data from another eight batches purchased in Spain, reported by Arrondo et al. (2020).

Element concentration

Concentrations of 72 elements and Pb isotopic composition were analyzed for all samples except for two batches of blood samples (n=90) from bears, collected in 2017 and 2018, where a reduced suite of analyses was performed (with no Pb isotope ratio measurements).

Blood, ant, ant nest material and berries were prepared for analysis by closed vessel microWave-assisted acid digestion and element concentrations were measured by high-resolution inductively-coupled plasma sector field mass spectrometry (ICP-SFMS, ELEMENT XR, ThermoScientific, Bremen, Germany). For a detailed description and validation of analytical method for whole blood, see Fuchs et al. (2023), Rodushkin et al. (2000), and Söderberg et al. (2023), for berries, see Rodushkin et al. (1999), and for ant and ant nest material see Pallavicini et al. (2018). Ammunition fragments were cleaned by 1 minute soaking in 7.9 M HNO_3, followed by dissolution of the fragments in the same matrix. Digests remaining after determination of element concentrations were diluted to target Pb concentration of 2 µg/L and Pb isotopic ratios were measured by ICP-SFMS equipped with stable introduction system using bracketing standards (NIST SRM 981, National Institute of Standards and Technology, NIST, Gaithersburg, MD, USA) matching sample solutions in acid strength and analyte concentration for instrumental mass bias correction (Olofsson et al., 2000).

Pb isotopic ratios can be measured with higher precision using multiple collector ICP-MS (MC-ICP-MS), however, the total amount of Pb in volume of blood samples available for analysis was often insufficient for this technique. Moreover, separation of analyte from matrix, required by MC-ICP-MS, would make analyses significantly more time consuming and labor intensive and this option was not used in this study. Mean in-run repeatability was 2.6% RSD for Pb concentrations, 0.24% RSD for $^{208}Pb/^{206}Pb$ and 0.18% RSD for $^{207}Pb/^{206}Pb$. Long term reproducibility, i.e. the deviation of obtained values from paired bear blood sampled analyzed in independent sessions, was 5.4% RSD for Pb concentrations, 0.44% RSD for $^{208}Pb/^{206}Pb$ and 0.39% RSD for $^{207}Pb/^{206}Pb$. For more information on quality control of ICP-SFMS measurements see supplementary information.

Pb isotope in leaded gasoline

To obtain isotopic ratios (^{206}Pb, ^{207}Pb, ^{208}Pb) for leaded gasoline in Europe we used literature cited by Komárek et al. (2008). We added aerosol data measured in Sweden in 1988 (Hopper et al., 1991) to the gasoline mix in order to include Pb isotopic mixtures for atmospheric depositions in Scandinavia.

Pb isotope in soil

Data on soil background Pb is available from the geochemical atlas of Europe (GEMAS) (O'Connor, 2014), downloaded on 2023-07-26 from geoportal.de ("GEMAS," 2021). We extracted all 18 point-measurements of ^{206}Pb/^{207}Pb and ^{207}Pb/^{208}Pb from the GEMAS agricultural soils that laid within a convex polygon based on the bears sampling locations, and in a surrounding 40 km buffer.

Bilberry production index

Berry abundance was measured in the southern range of the bear study area in the Siljansfors Experimental Forest in Dalarna, using the same method as in Hertel et al. (2018). In brief: All berries are counted on 54 circular 0.25 m^2 sized permanent sample plots in late summer every year. We fitted a linear model with the annual average production over all sample plots between 2013 and 2022 as response and the calendar year as predictor. From this model we calculated the residual distance to each datapoint and scaled this index between 0 and 1. We then tested for correlation between the berry index and the bear blood Pb concentration measured the subsequent spring.

Descriptive interpretation

For hypothesis 1, we describe median values, standard deviations (SD), and ranges of blood Pb concentrations across the different species. Isotopic compositions between bears, other wildlife, biological samples and industrial Pb isotopes were visually assessed and discussed based on three isotope plots.

Spatial distribution

In a prior study (Fuchs et al., 2021), we detected spatial disparities in Pb exposure within the brown bear population in Scandinavia. Therefore, bears were stratified into spatial clusters according to the west to east coordinates of their sampling locations. We simplified the clusters used in Fuchs et al. (2021) into a western, a central and an eastern cluster, to obtain a higher proportion of individual bears sampled multiple times in each cluster enhancing model convergence (Figure 1). We delinated breaks at longitude < 14.2, 14.2 – 14.8 and > 14.8.

Statistical analysis

Data preparation and analysis were performed with R version 4.2.3 (R Core Team, 2023). We fitted linear mixed models (lmm) for the $^{207}Pb/^{206}Pb$ ratio using the *lmer* function (Bates et al., 2015). We chose the $^{207}Pb/^{206}Pb$ ratio as response variable because it is reported for most other environmental Pb studies in Scandinavia. For the Pb blood concentrations, we fitted generalized linear mixed models (glmm) using the *glmer* function with a gamma distribution to avoid predictions below zero and a logarithmic link function (Bates et al., 2015). For both responses, we used the Akaike Information Criterion (AIC) to compare candidate models fitted via maximum likelihood, representing different a priori formulated hypotheses (Mazerolle, 2019). We used delta AIC = 2 as a cut-off value to differentiate between models.

Spatial differences in bear blood Pb isotopes

To test our hypothesis 2), whether spatial variation indicates more influence of natural background Pb compared to industrial Pb we fitted a lmm with the $^{207}Pb/^{206}Pb$ ratio as response and the affiliation to the spatial cluster as a fixed effect. We compared this model to a null model (i.e. no spatial effect) and to a model with an additive fixed effect indicating whether the bear was lactating at sampling. We included lactation because lactating bears likely remobilize Pb from their bones increasing Pb blood concentration and potentially changing isotopic composition (Fuchs et al., 2021). Bear id was added as random intercept to all models.

Annual variation in Pb concentration and Pb isotopic composition

To test for interannual variation of Pb blood concentration and the $^{207}Pb/^{206}Pb$ isotopic ratio, we selected bears with samples from at least two different years. We were particularly interested whether annual variation in Pb was related to bilberry availability (hypothesis 3). For each response variable, we formulated three candidate models with different fixed variables a priori: 1) a null model, 2) a model with calendar year and 3) a model with bilberry index. We allowed random variation on the intercept for each Bear id as well as for each spatial cluster to account for individual and spatial variation.

Relation between Pb isotopic composition and Pb blood concentration

To assess whether the bears' blood ^{207}Pb/^{206}Pb ratio was corelated with the Pb blood concentration, we tested a set of candidate models representing different outcomes of hypothesis 4: 1) no relation between the isotopic ratio and the blood Pb concentration, 2) the isotopic ratio is inversely correlated with the Pb blood concentration. We further tested whether a bear is lactating or not would improve model fit. In all candidate models we included the calendar year, the spatial cluster and the Bear Id as random intercept components.

Ant and ant nest material

We correlated Pb concentrations from ants of a mound with their nesting material using generalized linear models with a gamma distribution and a log link function for Pb and a gaussian distribution for Pb isotopes. We compared a model with the ants' Pb concentration as the response and the nesting material Pb concentration and the spatial cluster as fixed variables, to a model without the spatial group and a null model. Similar for the relation of the ^{207}Pb/^{206}Pb ratio in ants and ant nesting material, however we also included a model with the spatial cluster as the only fixed term to test if the Pb isotopic ratio is best explained by the spatial origin. We simplified clusters to a western and an eastern spatial group due to sample size (Figure S1).

Results and Discussion

Blood Pb concentrations

Brown bears had the highest median blood Pb concentrations (83.8 µg/L) among the tested mammalian species (Table 1). The lowest measured Pb blood concentration (20.4 µg/L) among the 355 samples from 167 brown bears was still higher than any blood Pb concentration measured in wolves or moose (Table1). Median blood Pb concentrations in wolves and moose were 3% and 7%, respectively, compared to that of bears.

The highest blood Pb concentration among all species was measured in a sample from a raven (849 µg/L), all other ravens had blood Pb concentrations < 405 µg/L. All ravens were trapped between mid-October and end of November, overlapping with the moose hunting season. Large parts of the biomass from hunting remains are consumed by ravens and other corvid species (Gomo et al., 2017; Legagneux et al., 2014). Ravens have previously been documented to be exposed to ammunition Pb during hunting season with median blood Pb concentrations of 107 µg/L, substantially lower than the 171.8 µg/L presented here (Craighead and Bedrosian, 2008). In another study, blood Pb concentrations in ravens increased with the moose harvest density and Pb isotopic composition changed towards an ammunition-like isotopic composition (Legagneux et al., 2014).

The wolves' median Pb blood concentration was 2.1 µg/L. The low Pb blood concentration in wolves as well as the large difference to sympatric brown bears has been previously documented in the Yellowstone area, USA, as well as in kidneys, liver and muscle Pb concentrations in Croatia (Lazarus et al., 2017; Rogers et al., 2012). Wolves included in this study were either territorial adults (n= 21) or pups within their natal territory (n=10), both documented to be very reluctant to scavenge especially on carcass remains that are killed or placed by humans, including hunting remains (Wikenros et al., 2023, 2013). The predominant prey of these wolves are neonate to one-year-old moose calves (Sand et al., 2008).

Of the sampled 18 wolverines, 11 were lactating females with a median blood Pb concentration of 6.7 µg/L, similar to the concentrations for the remaining seven animals (6.4 µg/L). Two animals were sampled in January, the rest in March and April (2016 -2022). Scavenging was the major food source based on foraging studies conducted on these wolverines (Petter Wabakken, unpubl. data), and several individuals used bait sites with leftovers from ungulates, possibly shot with Pb based ammunition. To our knowledge, no blood Pb concentrations for wolverines have been published elsewhere. Liver concentrations in wolverines from Yukon and Nunavut in Canada indicate low Pb exposure (Chételat et al., 2022; Hoekstra et al., 2003).

Blood Pb concentrations of moose were low, with the narrowest range and the lowest maximal concentration measured (Table 1). Scandinavian wolves and moose are useful sentinels to assess background Pb exposure. Moose are selective feeders consuming a range of shrubs and herbs, but we assume Pb ingestion is likely through unintentional uptake of soil when feeding near the ground. Median concentrations < 10 µg/L found in wolves, moose and wolverines are much lower than threshold concentrations used to define Pb contamination, for example 100 µg/L in ravens (Craighead and Bedrosian, 2008; Legagneux et al., 2014) or 200 µg/L in vultures (Arrondo et al., 2020).

Table 1: Mean, median, standard deviation (SD), range and sample size for blood lead (Pb) concentrations in brown bears (*Ursus arctos*), moose (*Alces alces*), common raven (*Corvus corax*), wolf (*Canis lupus*) and wolverine (*Gulo gulo*) sampled in Scandinavia between 2018 and 2023.

Species	Mean µg/L	Median µg/L	SD µg/L	Range µg/L	n
Brown Bear	89.1	83.8	35.5	20.4 – 220.5	355
Moose	7.0	6.2	3.4	2.0 – 14.0	31
Common Raven	192.1	171.8	131.8	48.3 – 849.3	45
Wolf	3.5	2.1	4.0	0.8 – 20.0	31
Wolverine	10.1	6.5	11.8	2.7 – 54.5	18

In accordance with our hypothesis 1, the scavenging ravens and bears in comparison had high Pb concentrations, with the wolverine as the exemption. In ravens, Pb blood concentration follows a distinct seasonal pattern, with a sharp increase at the start of the hunting season (Legagneux et al., 2014) and a second peak when hunting remains reappear during the snowmelt (Craighead and Bedrosian, 2008). Hunting remains are seasonal sources available in late summer, fall and to a lesser extent during the snow melt. Little is known about the resources utilized by ravens throughout the year, aside from carrion. Such seasonal pattern could partly explain the lower blood Pb concentration in wolverines which were sampled 3 to 4 months post hunting season. Mean Pb blood concentration in Scandinavian brown bears is 60% higher during hibernation as compared to mid-summer (Hydeskov, 2023). This suggest a seasonal decrease, however Pb is bound to red blood cells, and red blood cell counts (RBC) are 30% higher during hibernation likely due to dehydration (Græsli et al., 2015), complicating the analysis of seasonal variation in bears.

Lead concentrations in environmental samples

Pb concentration in berries was similar or lower compared to reference sites in northern Sweden and Finland (Pöykiö et al., 2005; Rodushkin et al., 1999). The European commission has set maximal allowed Pb concentration in fruits to 100 µg/kg wet weight (ww), corresponding to about 11 µg/kg dry weight (dw), assuming 89% water content (Rodushkin et al., 1999). Median concentration of our samples was 8 µg/kg dw, but the highest concentration measured in crowberries of 72 µg/kg dw exceeded this level by factor >6 (Table 2). While bilberries fall to the ground when ambient temperatures fall substantially below 0° C, lingonberries and crowberries partly remain on the bushes and are consumed by bears the following spring (Stenset et al., 2016). Nevertheless, Pb exposure at these concentrations are unlikely to explain the blood Pb concentration in bears. For context, in experimental studies, laboratory rats (*Ratus norwegicus*) that were exposed to drinking water with highly bioavailable Pb-acetat at a concentration of 50 000 µg/L (reported as 50 ppm) had a blood Pb concentration of 110 µg/L after three months of exposure (Virgolini et al., 2008).

Table 2: Dry weight lead (Pb) concentration in ants and ant nesting material (*Formica* ssp.) as well as from forest berries (bilberries (*Vaccinium myrtillus*), lingonberries (*Vaccinium vitis vide*a) and crowberries *Empetrum nigrum*)), sampled in Scandinavia between 2018 and 2022. Presented are mean, median, standard deviation (SD), range and sample size (n).

Matrix	Mean µg/kg	Median µg/kg	SD µg/kg	Range µg/kg	n
Ant	592	254	693	79 – 2 418	29
Ant nest Material	5 144	3 995	4 078	294 – 17 652	27
Berries	12	8	13	3 – 73	33

Median Pb concentration in ants (Table 2) were within the range reported in northern Sweden comparing wet weight concentrations of ants at a Pb mine to a reference site (Berglund et al., 2010). Using a dry weight to wet weight ratio of 3.6 as suggested by Berglund et al. (2010) ant Pb concentrations in our study, overlapped with both the reference site and the mining site, suggesting significant exposure in some colonies.

Pb in soil and dust is considered to be 60 times more bioavailable than Pb in food or water (ATSDR, 2020). The median concentration in ant nest material was 3 995 µg/kg dw with the highest concentration at 17 600 µg/kg dw (Table 2). In comparison, the median in agricultural soil in Sweden is 17 000 µg/kg dw and the 5th percentile is 8 000 µg/kg dw (Eriksson et al., 2010). Ant nesting material consisted of organic matter, mainly spruce and pine needles, gum resin and sand. Median Pb concentrations for needles in northern Sweden was 120 µg/kg dw (Pallavicini et al., 2018), suggesting that the majority of Pb is associated with the inorganic part of the nest material.

Stokke et al. (2017) reported a mean loss of 2.8 g Pb from each bullet in moose shot with Pb-based expanding rifle ammunition. The loss is due to fragmentation of the bullet in the shot animal, fragments which are accidentally ingested by scavengers. The bioavailability of Pb is likely higher the smaller the fragments are due to the increased surface to volume ratio, Kollander et al. (2017) found 27 to 50 million Pb nanoparticles (40 -750 nm) per 1 g of meat around the wound channel in a roe deer (*Capreolus capreolus*) and a wild boar shot with Pb-based bullets. Absorption of Pb from the gastro-intestinal tract varies with age, health and physiological status, e.g. children can absorb 40-50% of water-soluble Pb, whereas adults only absorb 3-10% (ATSDR, 2020). A bear consuming one lung from a Pb shot moose can potentially ingest 1 g of Pb. In comparison, to ingest 1 g of Pb a bear would need to consume > 250 kg of ant nest material (at median concentration).

Pb isotopic ratios

The summary of the Pb isotopic ratios for ammunition, ants, ant nesting material, whole blood from bears, wolves and wolverines is presented in Table 3. We joined the data from bilberries, lingonberries and crowberries because there was complete overlap in all values. We segregated the moose into a western and an eastern cluster because the Pb isotopes based on whole blood differed between the clusters with small variation within the group (Table 3).

The ^{207}Pb/^{206}Pb and ^{208}Pb/^{207}Pb ratios in soil in the study area overlapped with expected values from bedrock, representing background values with little contamination from industrial Pb (Table 3, Figure 2-4). Steinnes et al. (2005) reported ^{207}Pb/^{206}Pb ratios < 0.78 for C-horizon samples in the very west of our study area. We used the soil data as reference for natural background Pb.

The ^{207}Pb/^{206}Pb and ^{208}Pb/^{207}Pb ratios of leaded gasoline is distinctly different from the natural background in the study area (Table 3, Figure 3-4). The distribution of Pb isotopes originating from gasoline is relatively narrow, even when aerosol measurements are included, because only Pb from a few deposits have been used to produce Pb additives for European gasoline (Komárek et al., 2008).

Mean $^{207}Pb/^{206}Pb$ and $^{208}Pb/^{207}Pb$ ratios in whole blood from ravens, bears and wolverines were similar to those of ammunition (Table 3). Consequently, these scavenging species exhibit a close overlap with ammunition on the three-isotope plot (Figure 2). However, the isotopic composition of ant nesting material, shares a similar isotopic mix preventing a quantitative estimation of the contribution of individual sources to the mix in bear blood (Table 3, Figures 2-4). Similar isotopic compositions as for ant nesting material have been reported for spruce and pine needles in northern Sweden (Pallavicini et al., 2018) and top soil in south-eastern Norway (Steinnes et al., 2005). Soil and ammunition were also reported to be a major contributors to the blood Pb in vultures in Spain and South Africa (Arrondo et al., 2020; van den Heever et al., 2023).

Further, the isotopic composition in blood samples from moose displays similar spatial variations as found in bears (Figure 3). The isotopic ratios for eastern moose group overlaps well with those for the berries, sampled in the eastern part of the study area (Table 3, Figure 3). Bilberry brushwood is an important feeding plant for moose (Wam and Hjeljord, 2010). The spatial segregation is also visible in the bear samples, where the eastern cluster differs, consistent with berries and the eastern moose group (Figure 4).

Mixing models are one way to statistically quantify source contribution in an isotopic mix. Binary mixing models assume that, two known end members (sources) contribute to the mix (Longman et al., 2018). These models might be useful to assess Pb contribution in environmental samples such as berries or ant nest material, assuming a binary mix between natural background Pb and gasoline. For mixes with more than two sources, partly pre-parameterized Bayesian mixing models such as MixSiar (Stock et al., 2018) or Simmr (Govan and Parnell, 2023), available within the R environment, have been used to quantify source contribution in Pb isotopic mix (Arrondo et al., 2020; Dietrich et al., 2021; Longman et al., 2018; Ray and Das, 2023). In case of the bears, the mixing geometry (Figure 2- 5) violates basic assumptions of these models, namely that the convex hull of the source distribution should overlap with the mix distribution (Stock et al., 2018; Ward et al., 2011). Thus, we decided not to apply such models on the blood sample data.

Table 3: Mean and standard deviation of the mean (SD), range, and sample size (N) for lead (Pb) isotope in matrices presented in this study.

Matrix	Mean (SD) Range		N	References
	$^{207}Pb/^{206}Pb$	$^{208}Pb/^{207}Pb$		
Ammunition	0.85 (0.021) 0.81 - 0.88	2.44 (0.018) 2.41 - 2.47	16	Arrondo et al., 2020 (N=8); This study (N=8)
Ant	0.86 (0.023) 0.75 - 0.87	2.40 (0.017) 2.36 - 2.44	29	This study
Ant Nesting Material	0.84 (0.035) 0.71 - 0.87	2.44 (0.028) 2.39 - 2.52	27	This study
Bear Blood	0.85 (0.012) 0.82 - 0.88	2.44 (0.014) 2.39 - 2.49	242	This study
Berries	0.85 (0.018) 0.82 - 0.89	2.42 (0.018) 2.36 - 2.45	33	This study
Gasoline / Aerosoles	0.88 (0.018) 0.84 - 0.94	2.41 (0.024) 2.34 - 2.45	132	Hansmann and Köppel, 2000; Hopper et al., 1991; Monna et al., 1997; Resongles et al., 2021
Moose Blood East	0.85 (0.005) 0.84 - 0.86	2.42 (0.006) 2.41 - 2.44	16	This study
Moose Blood West	0.86 (0.003) 0.86 - 0.87	2.45 (0.004) 2.44 - 2.46	15	This study
Raven Blood	0.85 (0.011) 0.82 - 0.89	2.44 (0.014) 2.39 - 2.49	45	This study
Soil	0.78 (0.054) 0.71 - 0.85	2.48 (0.022) 2.46 - 2.51	18	GEMAS, 2021
Wolf Blood	0.87 (0.020) 0.83 - 0.92	2.43 (0.014) 2.40 - 2.47	31	This study
Wolverine Blood	0.85 (0.019) 0.80 - 0.88	2.45 (0.019) 2.42 - 2.50	18	This study

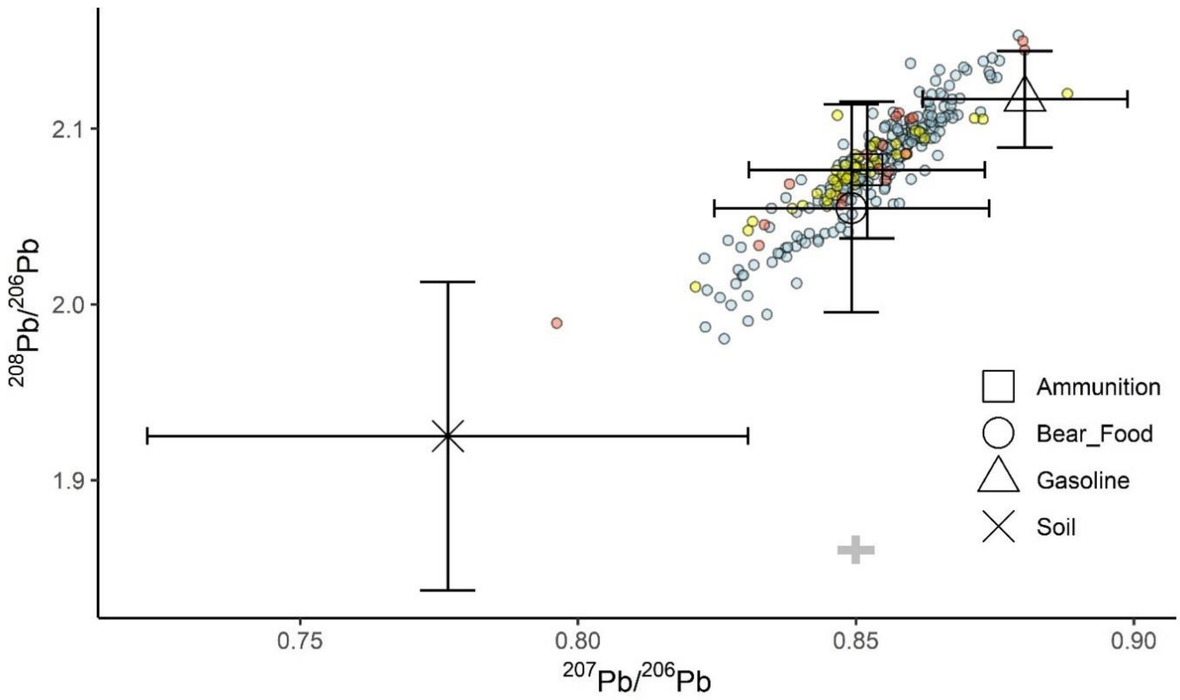

Figure 2. Three isotope plot ($^{208}Pb/^{206}Pb$ vs $^{207}Pb/^{206}Pb$) in whole blood samples from brown bear (*Ursus arctos*) (blue dots), common ravens (*Corvus corax*) (yellow dots) and wolverines (*Gulo gulo*) (red dots) sampled in Scandinavia between 2019 and 2022, as well as the mean and standard deviation of agricultural soils in the study area (cross), Pb-based rifle ammunition used for hunting (square) and European leaded gasoline (triangle). Ants (*Formica* ssp.), ant nest material and berries (*Vaccinium* ssp., *Empetrum nigrum*.) representing bear food are grouped together for better visibility (circle). Isotope data from soil is from the geochemical atlas Europe ("GEMAS," 2021), ammunition is joined data from this study and Arrondo et al. (2020). Isotopic composition of European leaded gasoline is reported by Hansmannn and Köppel (2000), Hopper et al. (1991), Monna et al. (1997), and Resongles et al. (2021). The grey cross indicates measurement uncertainty of the Pb isotopic composition in blood.

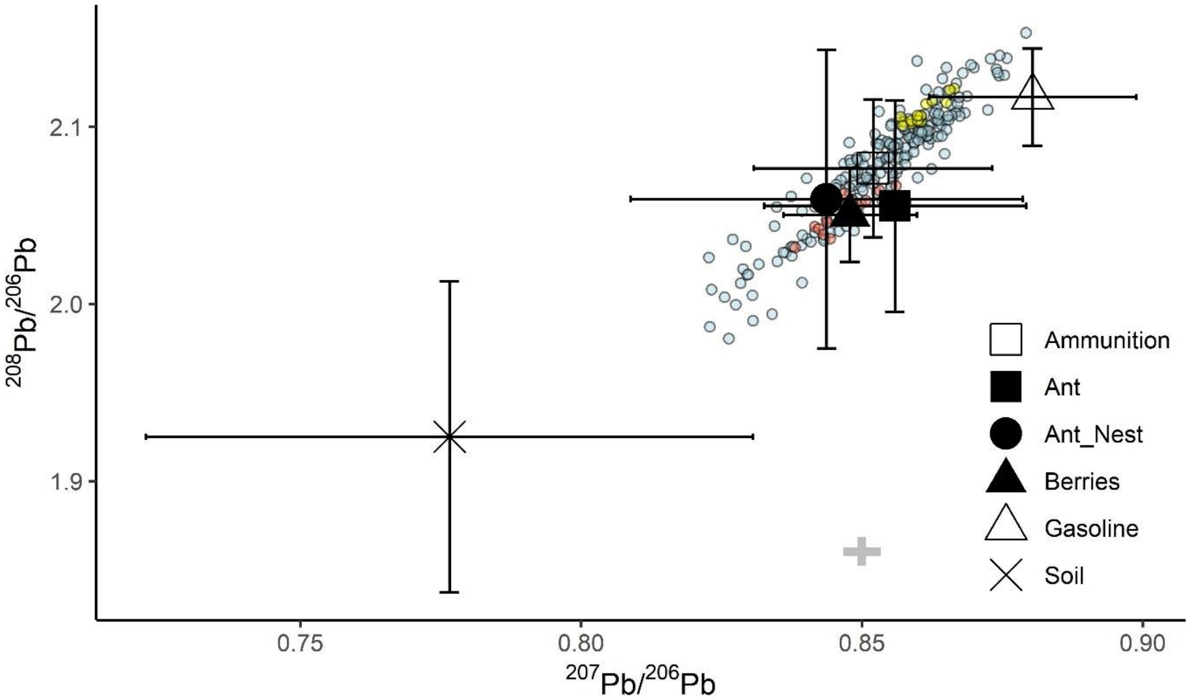

Figure 3. Three isotope plot ($^{208}Pb/^{206}Pb$ vs $^{207}Pb/^{206}Pb$) in whole blood samples from brown bear (*Ursus arctos*) (blue dots) and moose (*Alces alces*) divided in a eastern (yellow dots) and a western (red dots) group sampled in Scandinavia between 2019 and 2022. As well as the mean and standard deviation of agricultural soils in the study area (cross), Pb-based rifle ammunition used for hunting (open square) and European leaded gasoline (open triangle). Ants (*Formica* ssp) (filled square), ant nest material (filled circle) and berries (*Vaccinium* ssp., *Empetrum nigrum*) (filled triangle). Isotope data from soil is from the geochemical atlas Europe ("GEMAS," 2021), ammunition is joined data from this study and Arrondo et al. (2020). Isotopic composition of European leaded gasoline is reported by Hansmannn and Köppel (2000), Hopper et al. (1991), Monna et al. (1997), and Resongles et al. (2021). The grey cross indicates measurement uncertainty of the Pb isotopic composition in blood.

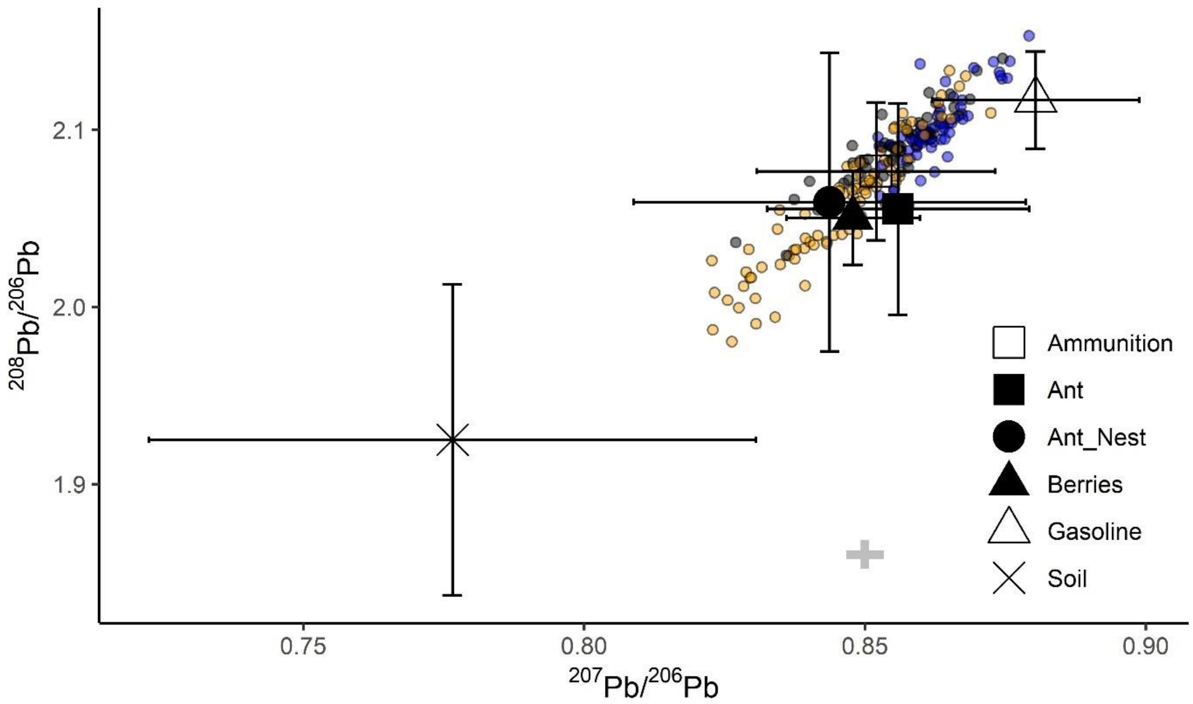

Figure 4. Three isotope plot ($^{208}Pb/^{206}Pb$ vs $^{207}Pb/^{206}Pb$) in whole blood samples from brown bear (*Ursus arctos*) divided in a eastern (yellow dots) and a central (blue dots) and a western spatial cluster (black dots) sampled in Scandinavia between 2019 and 2022. As well as the mean and standard deviation of agricultural soils in the study area (cross), Pb-based rifle ammunition used for hunting (open square) and European leaded gasoline (open triangle). Ants (*Formica ssp.*) (filled square), ant nest material (filled circle) and berries (*Vaccinium* ssp., *Empetrum nigrum*) (filled triangle). Isotope data from soil is from the geochemical atlas Europe ("GEMAS," 2021), ammunition is joined data from this study and Arrondo et al. (2020). Isotopic composition of European leaded gasoline is reported by Hansmann and Köppel (2000), Hopper et al. (1991), Monna et al. (1997), and Resongles et al. (2021). The grey cross indicates measurement uncertainty of the Pb isotopic composition in blood.

Spatial differences in bear blood Pb isotopes

In this model, 242 samples (62 lactating) from 120 individual bears were analyzed. The model relating the $^{207}Pb/^{206}Pb$ in the bear blood to the spatial cluster affiliation and the lactational status of the bears had the lowest AIC value, predicting a more radiogenic $^{207}Pb/^{206}Pb$ ratio in bear blood from the eastern part of the study area (0.848, 95% CI: 0.846 – 0.850) compared to the central part (0.861, 95% CI: 0.859 – 0.865) (Figure 5, Table 4, Table S3). Predictions overlapped for the central and the western part (0.854, 95% CI 0.851 – 0.857). Lactating bears had slightly higher ratios. We hypothesized (2.1) that spatial variation in the isotopic composition would indicate natural background Pb as a potential source. We previously found that Pb blood concentrations in female bears from the western parts of the study area where 30 to 60 µg/L higher compared to the central and eastern areas (Fuchs et al., 2021). The isotopic ratios in the bear blood samples from areas with higher Pb concentrations were within the typical distribution of industrial Pb but less radiogenic and thus closer to the expected natural background. Possibly bears in the western part have similar exposure to industrial Pb as in the eastern part of the study area but higher relative exposure to natural Pb sources. The most likely contributor to Pb with isotopic composition typical for natural background is soil ingestion when consuming ants.

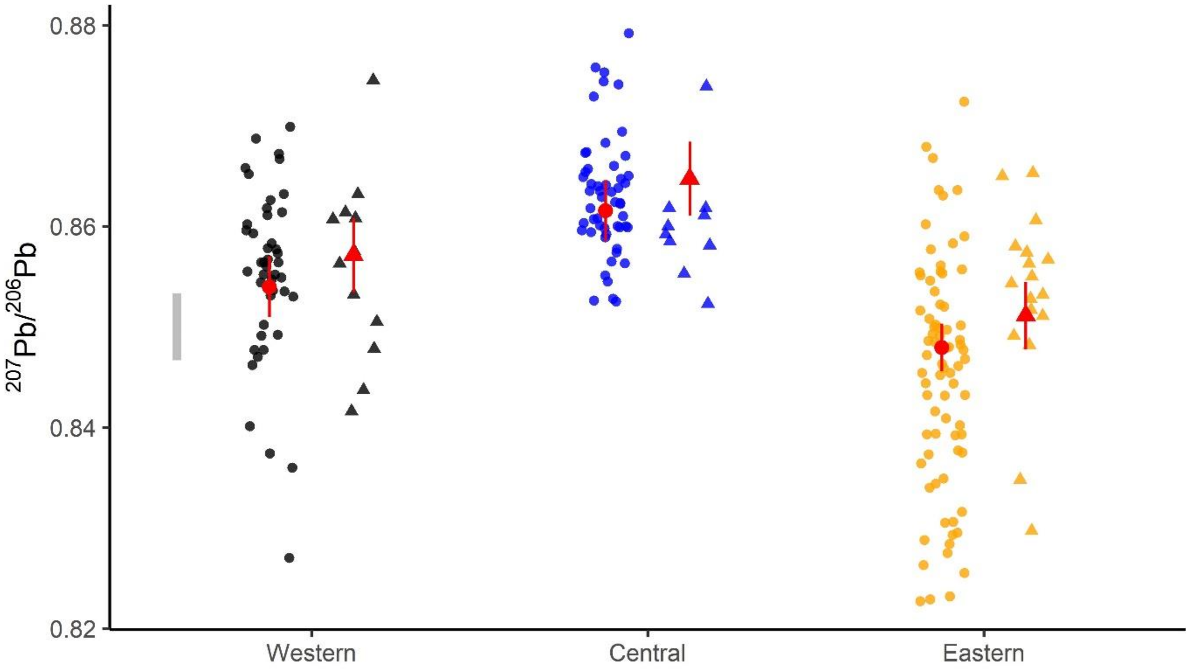

Figure 5: Ratios of lead (Pb) isotopes $^{207}Pb/^{206}Pb$ in 304 brown bears (*Ursus arctos*) whole blood samples from Scandinavia sampled between 2019 and 2022, depending on spatial cluster and their lactational status (circles: nonlactating males and females; triangles: lactating females). As well as the prediction and 95 % confidence intervals (red) of a linear mixed model with the spatial cluster and lactational status as fixed and individual id as random intercept predictor. The grey vertical bar indicates measurement uncertainty. Spread on the x axis within cluster and lactational status is random to increase visibility.

Table 4: Model selection based on Akaike information criterion (AIC) for five groups of mixed candidate models relating the lead (Pb) isotopic ratio (^{206}Pb/^{207}Pb), and the Pb blood concentration (Pb conc.) from Scandinavian brown bears (*Ursus arctos*), sampled between 2017 and 2022 to the sample location (Cluster), the lactational status of the bear (Lactation), the day of the year (YDay) and the calendar year (Year) the bear was sampled, and an index for bilberry (*Vaccinium myrtillus*) abundance. The Id of the bear (Bear Id), Cluster and Year were also used as random intercepts. Models with the lowest AIC, and a difference of ΔAIC > 2 were selected for interpretation. K indicates the number of fitted parameters, the AICw the weight of each candidate model within the selection and the LL the log likelihood. Interpreted models are in bold.

Hypothesis	Response	Fixed model terms	Random intercepts	K	AIC	ΔAIC	AICw	LL
Spatial Variation (2.1)	**^{206}Pb/^{207}Pb**	**Cluster + Lactation**	**Bear Id**	**6**	**-1293.29**	**0**	**0.82**	**652.85**
		Cluster	Bear Id	5	-1290.19	3	0.09	650.24
		Null	Bear Id	3	-1247.77	46	0.00	626.94
Annual Variation (3.1)	**^{206}Pb/^{207}Pb**	**Year**	**Bear Id** **Cluster**	**7**	**-1182.61**	**0**	**1.00**	**598.67**
		Bilberry Index	Bear Id Cluster	5	-1109.19	73	0.00	559.79
		Null	Bear Id Cluster	4	-1097.37	85	0.00	552.81
	Pb conc.	**Year**	**Bear Id** **Cluster**	**7**	**2087.60**	**0**	**1.00**	**-1034.39**
		Bilberry Index	Bear Id Cluster	5	2105.62	18	0.00	-1047.68
		Null	Bear Id Cluster	4	2128.53	41	0.00	-1060.18
^{206}Pb/^{207}Pb in relation to Pb conc. (4)	^{206}Pb/^{207}Pb	Pb conc.	Bear Id Cluster Year	6	-1365.78	0	0.46	689.09
		Null	Bear Id Cluster Year	5	-1325.35	0	0.37	687.82
		Pb Conc. + Lactation	Bear Id Cluster Year	7	-1363.72	2	0.17	689.13

Annual variation of blood Pb concentration and $^{207}Pb/^{206}Pb$ in bears

For the Pb blood concentration, 231 samples from 80 individual bears sampled two to six times over six years were available. Pb isotopes were available for 145 of these blood samples measured on 54 individual bears, sampled two to four times over two to four years. The model with calendar year as predictor outperformed the null model and the model with bilberry abundance index as predictor for both the Pb blood concentration and the associated $^{207}Pb/^{206}Pb$ ratio (Table 4). However, annual variation was limited: predicted Pb concentrations were lowest in 2020 (64.5 µg/L) but 95% confidence intervals (50.3 – 82.7 µg/L) overlapped with all other assessed years (Figure 6, Table S4). Similarly, the model with calendar year outperformed the model with the bilberry index for the isotopic ratio of $^{207}Pb/^{206}Pb$. The year 2022 (0.864) differed most, however with confidence intervals that overlapped all other predicted years (0.856-0.871) (Table S3). We hypothesized (3.1) that annual variation in Pb concentration and isotopic composition could indicate the relative importance of a particular source, reasoning that in poor berry years, bears might spend more time searching for hunting remains or ants. We found some annual variation that was not connected to the berry production and the variation was weak and not synchronous between blood Pb concentration and the isotopic composition.

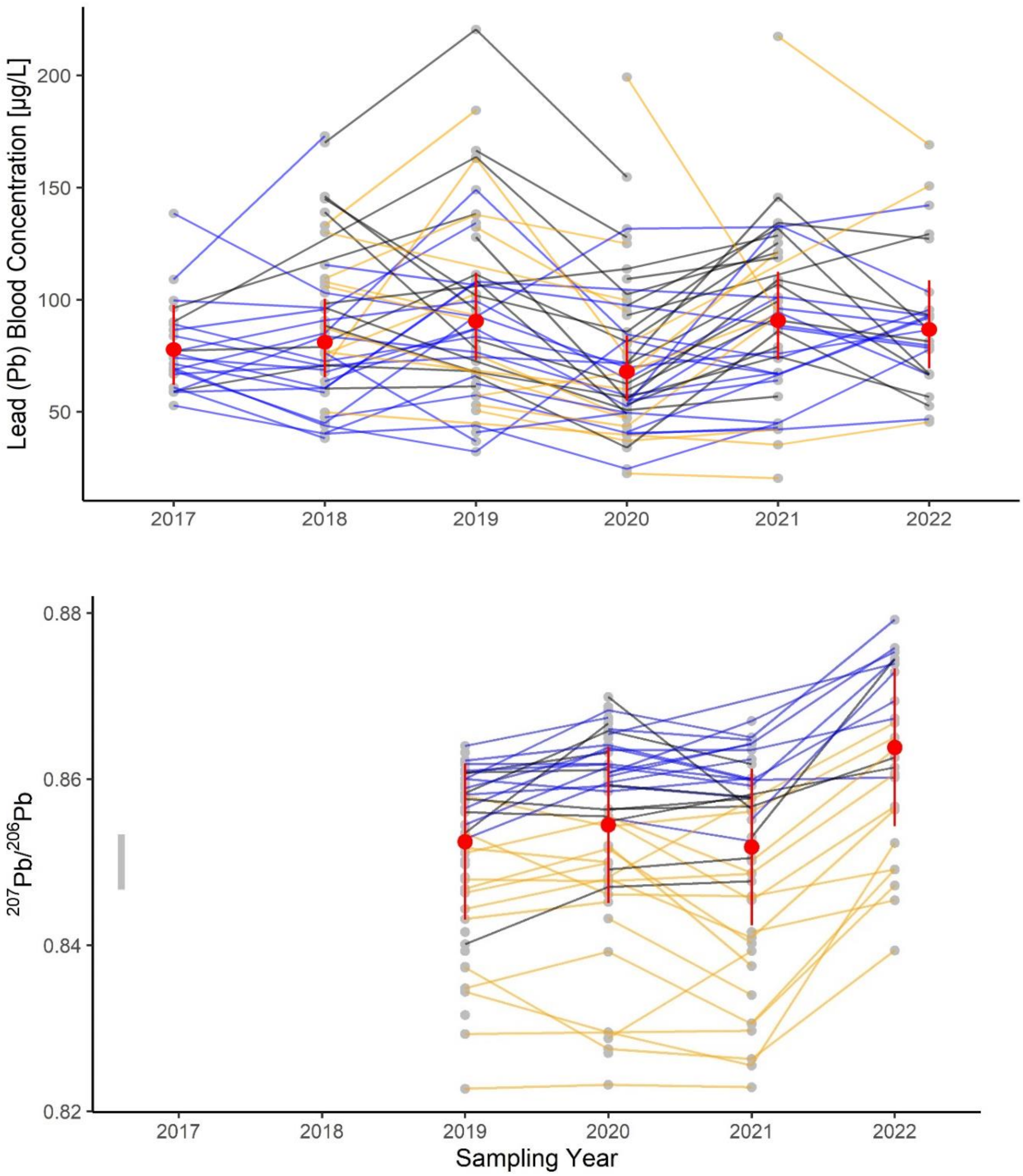

Figure 6. Annual variation in lead (Pb) blood concentration (a) and the associated ^{207}Pb/^{206}Pb ratio (b) in brown bears (*Ursus arctos*) sampled between 2017 and 2022 in Scandinavia. Only bears with repeated measurements are included (grey dots) and repeated observations are connected with solid lines. Line colors indicate the spatial cluster individuals were sampled, black: western, blue: central, yellow: eastern. Red circles and vertical lines indicate model predictions and 95% confidence intervals of a linear mixed model predicting Pb blood concentration (a) and the associated ^{207}Pb/^{206}Pb ratio (b) with year as fixed variable and random intercepts for individual bears and spatial cluster. The grey vertical bar indicates measurement uncertainty.

Relation between Pb and Pb isotopes

The ^{207}Pb/^{206}Pb ratio did not correlates with the blood Pb concentration in the brown bears, indicated by the lowest AIC level attributed to the null model (Table 4). Our interpretation is that there is no isotopically distinct single source that could explain variation in the blood Pb concentration of brown bears in Scandinavia.

Ant and ant nesting material

We obtained Pb concentrations and Pb isotopic ratios of 27 paired ant and ant nest samples, of which 23 were *Formica*, species (*F. lugubirs*, *F. aquilonia*, *F. polyctena*, *F. pratensis* including several probable hybridisations) and four were C*optoformica*, species (*F. exsecta*). The selected model explaining the Pb concentration in the ants contained the Pb concentration of the nest and the spatial group as an additive variable (Table S5). At an average nest material concentration of 5144 µg/kg, the model predicted a significantly lower ant Pb concentration in the western group (191 µg/kg, 95% CI: 130 – 281 µg/kg) as compared to the eastern group (785 µg/kg, 95% CI: 571 – 1079 µg/kg). The Pb concentration in the ants increased with increasing Pb concentration of the nesting material (Table S6), and the predicted increase was stronger in the western than in the eastern group, however, with high variation (Figure 8a). For the ^{207}Pb/^{206}Pb ratio, the selected model contained the Pb isotopic ratio of the nesting material only (Table S5). The ^{207}Pb/^{206}Pb ratio of the ants increased with the ^{207}Pb/^{206}Pb ratio of the nest (Figure 8b, Table S6). However, with an increasing ^{207}Pb/^{206}Pb in the nest material, this relation increasingly diverged from a one-to-one correlation (Figure 8b).

We expected ants to be more contaminated by their nest material, although the ants' Pb concentration correlated significantly with the Pb concentration in the nesting material the relation was far from perfect. We found ant nests with ^{207}Pb/^{206}Pb ratios < 0.8, similar to ratios for some soil C-horizon considered to represent natural Pb in this area, independent of the Pb concentration or the spatial group. Possibly, ants have moved soil particles from deeper less contaminated soil to the mount. The ants, however, are exposed to other sources representing an industrial Pb mix. Brown bears in the study area consume ants during the entire active season. In spring, snow is often melting faster on ant nest than in the surrounding area and makes them easily available to bears. We have sampled bears in late spring and early summer in a period when ants consist about 20% of the total nutrition intake (Stenset et al., 2016).

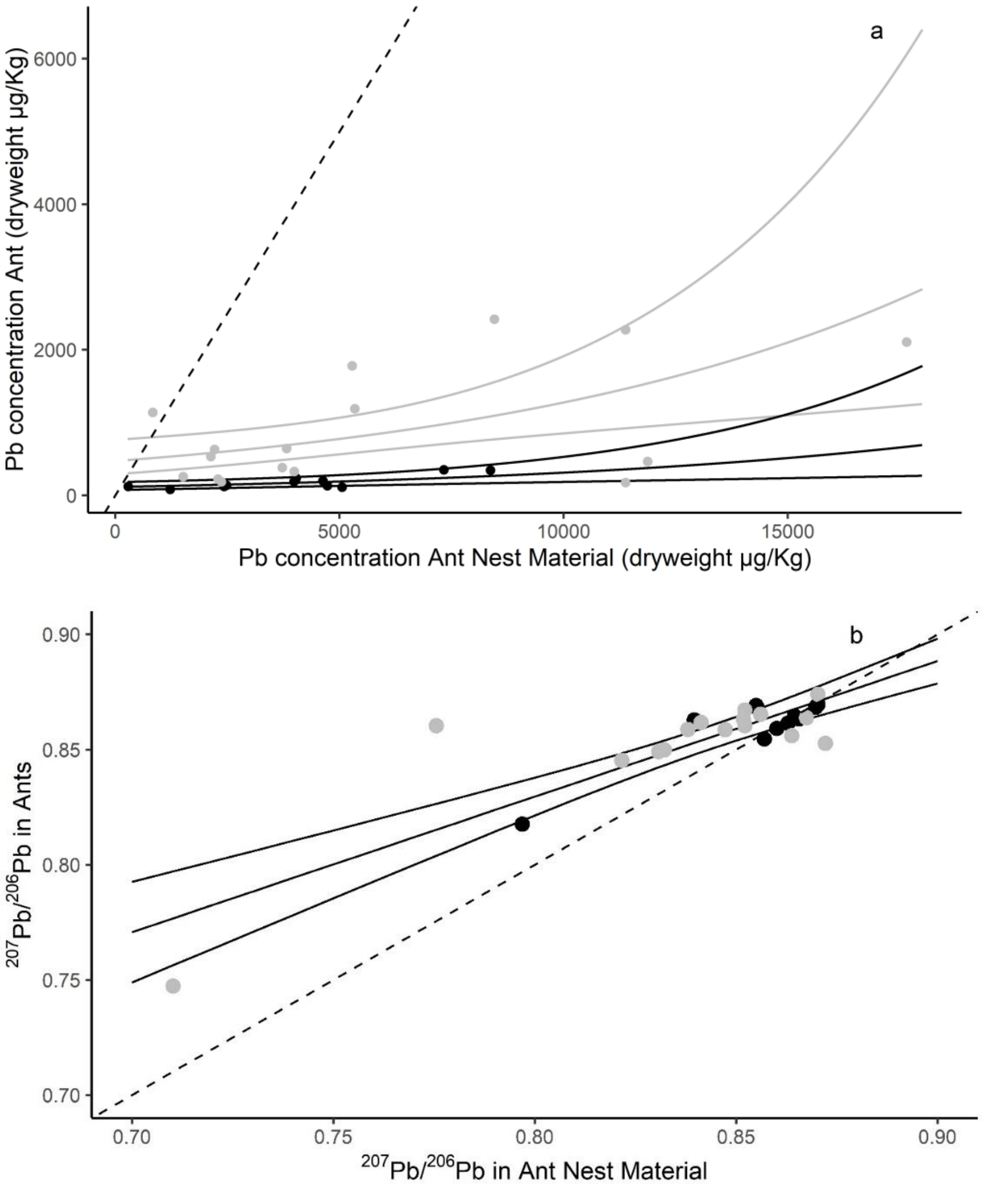

Figure 8: Predictions and 95 % confidence intervals of the Pb dry weight concentration in ants (*Formica* and *Coptoformica* ssp.) sampled 2022 in Scandinavia in relation to the Pb dry weight concentration of their nesting material, estimated by a generalized linear model (a) as well as for ^{207}Pb/^{206}Pb ratio (b) in the same samples. Grey dots and lines are from an eastern spatial cluster, black from a western cluster. The spatial cluster was not selected for the isotopic ratio model. The dashed line indicates a one-to-one relation. Measurement uncertainty of ^{207}Pb/^{206}Pb measurements was smaller than the size of the data points.

Conclusions

We found no isotopically distinct single source that could explain variation in the blood Pb concentration of brown bears in Scandinavia. Isotopic compositions of ammunition used for hunting, and environmental background Pb in ant nest material were too similar for an analytical distinction, but both were consistent with those in bear blood. Variations in isotopic compositions among spatially grouped bears and moose were similar and hinted towards environmental background Pb as source. We found ant nest materials to have much higher Pb concentrations than any other tested food item, but at levels similar or below that of agricultural soils in Sweden. At these concentrations and considering the limited ingestion, ant nest materials alone are very unlikely to explain the elevated blood Pb concentrations in bears. We found blood concentrations in non-scavenging wolves and herbivore moose to be less than 10% as compared to bears and ravens known to feed on hunting remains. Ingestion of Pb fragment from spent hunting ammunition is very likely a significant source for Pb exposure for bears and other scavengers in Scandinavia. The Pb exposure in these wildlife species could be easily prevented using non-lead hunting ammunition. We found a basic isotopic model with aerial depositions from leaded gasoline combustion as the non-radiogenic extreme and natural Pb in deep soil as the radiogenic extreme, all biological samples, as well as ant nest materials fall in between and represent a mixture of the two.

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Supplementary materials

Quality control lead (Pb) concentration and Pb isotopes

At least 2 preparation blanks and set of matrix-matched certified reference materials (CRMs: BCR 634, BCR 635, BCR 636 Human Blood from Institute for Reference Materials and Measurements, Geel, Belgium for whole blood, NIST 1547 Peach leaves from NIST for plants and berries, NIST SRM 982 from NIST for ammunition) was prepared and analyzed with each preparation batch (maximum 35 samples). Duplicate preparation/analyses of at least 10% of samples were performed in each analytical session. Method limit of detection (assessed as 3 times the standard deviation (SD) for preparation blanks) is 0.08 µg/L for blood and 2 µg/kg for plant matrices. Mean in-run repeatability (assessed using relative SD (RSD) for results for parallel samples (n=52) analyzed within single analytical session was 2.6% RSD for Pb concentrations, 0.24% RSD for ^{208}Pb/^{206}Pb and 0.18% RSD for ^{207}Pb/^{206}Pb. As samples for this study have been analyzed in a number of analytical sessions spanning several years, we assessed long term reproducibility. We submitted 23 randomly selected blood samples from bears, previously analyzed in four different batches as blind samples for new analyses. Long term reproducibility was 5.4% RSD for Pb concentrations, 0.44% RSD for ^{208}Pb/^{206}Pb and 0.39% RSD for ^{207}Pb/^{206}Pb, thus roughly two times higher than in-run reproducibility.

Accuracy of Pb concentration measurements (assessed as % recoveries for CRMs) was in 94-103% range (Table S2). Lead isotopic ratios obtained for NIST SRM 982 agrees with tabulated ratios within measurement uncertainties. To the best of our knowledge, there are no whole blood or plant material CRMs with tabulated isotopic ratios commercially available. For whole blood, accuracy of the method was tested by participation in ICP-MS comparison program organized by the Centre de Toxicologie du Québec (CTQ, blood sample ICP02B-08), which is part of the Institut National de Santé Publique du Québec (INSPQ) (Table S2). For plant materials, Pb isotopic ratios for NIST SRM 1457 is in good agreement with previously published MC-ICP-MS ratios (Rodushkin et al., 2016).

Supplementary Figure

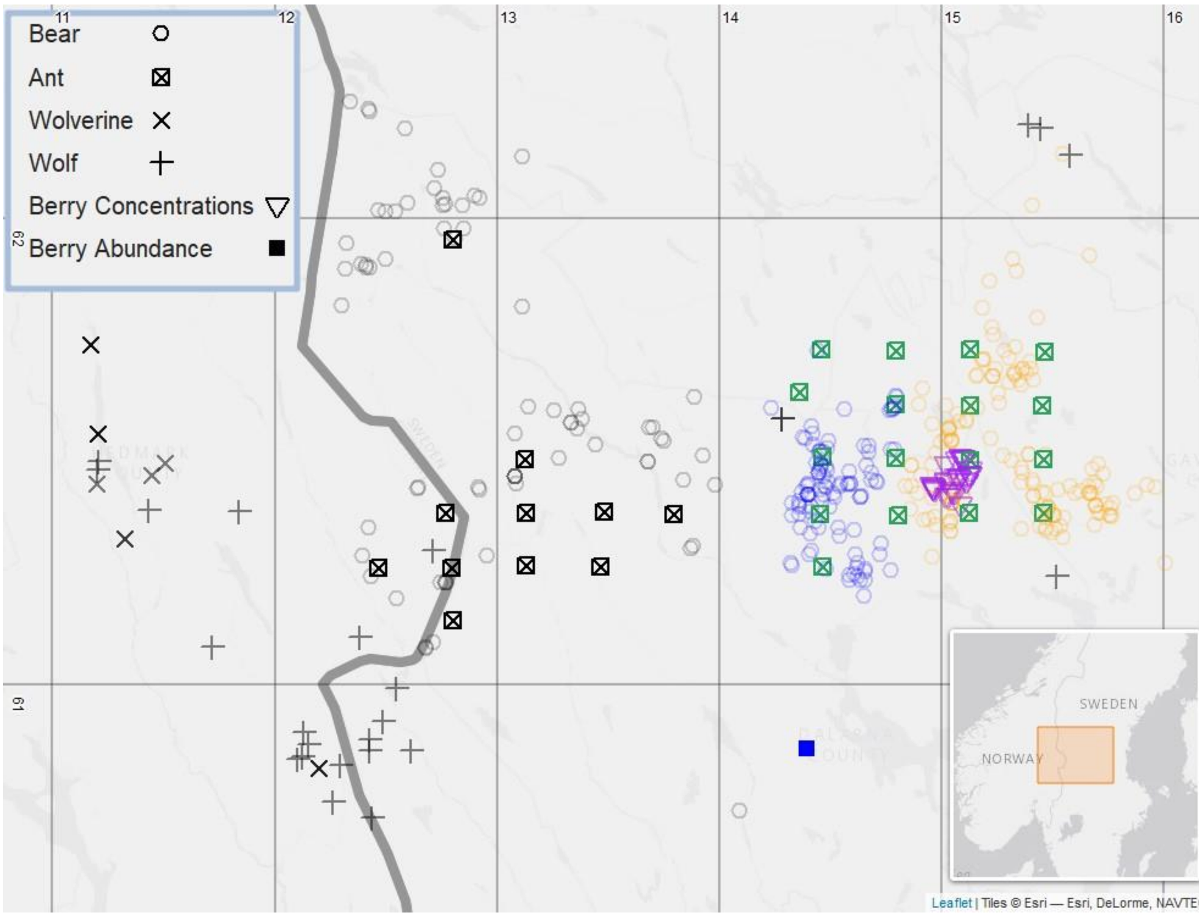

Figure S1: Sample locations for brown bears (*Ursus arctos*), ants (*Formica* ssp.) and ant nest material (Ant), wolverines (*Gulo gulo*), wolves (*Canis lupus*), forest berries (*Vaccinium* ssp. and *Empetrum n*.) for lead (Pb) concentration and Pb isotopic composition analysis. Annual bilberry (*Vaccinium myrtillus*) abundance was obtained from the Siljan experimental forest. Bears are grouped in a western (black) a central (blue) and an eastern (yellow) cluster. Ant and ant nesting material are grouped in a western (black) and eastern (grey) cluster. One sample location of a wolf is further south and not shown on this map.

Supplementary Tables

Table S1: Ethical approval document identifiers in relation to the various included research projects and host organizations.

Project name	Species	Country	Hosting institution	Ethical approval document id
Scandinavian brown bear research project (SBBRP)	Brown bear (*Ursus arctos*)	Norway	Norwegian Institute for Nature Research (NINA)	FOTS ID, 19368
		Sweden	Swedish University of Agricultural Sciences (SLU)	Dnr 5.8.18–03376/2020
Scandinavian wolf research project SKANDULV	Wolf (*Canis lupus*)	Norway	Inland Norway University of Applied Sciences (INN)	FOTS id 15370
Scandinavian forest wolverine project, Skogsjerv	Wolverine (*Gulo gulo*)	Norway	INN	FOTS id 19625
Grensevilt	Moose (*Alces alces*)	Norway	INN	FOTS id 15170
Brannfeltet	Moose (*Alces alces*)	Sweden	SLU	Dnr A3

Table S2. Lead (Pb) concentrations and isotopic ratios obtained for certified reference materials (CRM) and sample from interlaboratory performance evaluation exercise (CTQ)

Reference material	Pb conc. measured (SD, n>3), µg/L or µg/Kg	Pb conc. certified (SD), µg/L or µg/Kg	^{207}Pb/^{206}Pb measured (SD, n>3)	^{208}Pb/^{206}Pb certified/ published (SD)	^{208}Pb/^{207}Pb measured (SD, n>3)	^{208}Pb/^{207}Pb certified/ published (SD)
BCR-634	43.4 (1.7)	46 (5)	0.8424 (0.0014)	NA	2.048 (0.004)	NA
BCR-635	205 (11)	210 (24)	0.8639 (0.0011)	NA	2.119 (0.003)	NA
BCR-636	533 (30)	520 (50)	0.8690 (0.0010)	NA	2.134 (0.003)	NA
CTQ ICP02B-08	10.3 (0.2)	10.3 (0.6)	0.7663 (0.0006)	0.7669 (0.0028)	1.914 (0.001)	1.918 (0.004)
NIST SRM 1547	824 (47)	870 (30)	0.8249 (0.0030)	0.8244 (0.0004)	2.044 (0.010)	2.046 (0.007)
NIST SRM 982	matrix	matrix	0.4675 (0.0006)	0.4671 (0.0002)	1.000 (0.003)	1.0016 (0.0004)

Table S3: Estimates, standard error (SE) of the estimates and corresponding T-values of linear mixed models predicting the lead (Pb) isotopic ratio (^{206}Pb/^{207}Pb), depending on a sample location (cluster), calendar year (Year) and lactational status of the bear (Lactation), in blood samples from brown bears sampled in Scandinavia between 2019 and 2022.

Model	Fixed effects	Estimates	SE	T-value
^{206}Pb/^{207}Pb ~ Cluster + Lactation	Cluster West	0.854	0.002	563.4
	Cluster Center	0.008	0.002	3.6
	Cluster East	- 0.006	0.002	-3.2
	Lactation	0.003	0.001	2.3
^{206}Pb/^{207}Pb ~ Year	Year 2019	0.853	0.005	179.1
	Year 2020	0.002	0.001	2.3
	Year 2021	-0.001	0.001	-0.7
	Year 2022	0.011	0.001	9.8

Table S4: Estimates, standard error of the estimates (SE) and corresponding T and P – values of a generalized linear mixed model predicting the lead (Pb) concentration depending in calendar year (Year) in blood samples from brown bears (Ursus arctos) in Scandinavia, sampled between 2017 and 2022. Estimates and SE are in µg/L on the log scale.

Model	Fixed effects	Estimates	SE	T-value	P-value
Pb ~ Year	Year 2017	4.35	0.12	37.75	< 0.001
	Year 2018	0.04	0.06	0.70	0.487
	Year 2019	0.15	0.06	2.50	0.013
	Year 2020	-0.14	0.06	- 2.18	0.029
	Year 2021	0.15	0.07	2.36	0.018
	Year 2022	0.11	0.07	1.52	0.128

Table S5: Model selection based on Akaike information criterion (AIC) for two sets of generalized linear candidate models relating the dry weight lead (Pb) concentration and isotopic ratio (^{206}Pb/^{207}Pb), of ants (*Formica* ssp.), sampled in 2022 in Scandinavia to dry weight Pb concentration and isotopic ratio in the corresponding nesting material and the sample location (Cluster). Models with the lowest AIC, and a difference of ΔAIC > 2 were selected for interpretation. K indicates the number of fitted parameters, the AICw the weight of each candidate model within the selection and the LL the log likelihood. Interpreted models are in bold.

Response	Fixed model terms	K	AIC	ΔAIC	AICw	LL
Pb conc. ant	**Pb conc. nest + Cluster**	**4**	**380.87**	**0**	**1.00**	**-185.52**
	Pb conc. nest	3	399.35	18	0.00	-196.16
	Null	2	405.85	25	0.00	-200.68
^{206}Pb/^{207}Pb ant	**^{206}Pb/^{207}Pb nest**	**3**	**-153.83**	**0**	**0.77**	**80.44**
	^{206}Pb/^{207}Pb + Cluster	4	-151.39	2.43	0.23	80.61
	Null	2	-121.12	32.71	0.00	62.81
	Cluster	3	-119.29	34.54	0.00	63.17

Table S6: Estimates, standard error of the estimates (SE) and corresponding T and P – values of a generalized linear models predicting the dry weight lead (Pb) concentration and the isotopic ratio ^{206}Pb/^{207}Pb of ants (*Formica* ssp.) sampled in Scandinavia in 2022, depending on the dry weight Pb concentration and the isotopic ratio in their nesting material as well as the sample locations grouped in a eastern (East) and a western cluster. Estimates and SE are in µg/Kg on the log scale.

Response	Fixed model terms	Estimate	SE	T-value	P-value
Pb concentration	Intercept	4.74	0.22	21.5	< 0.001
	Pb Nest Material	9.97 e^{-5}	3.01 e^{-5}	3.3	0.003
	Cluster East	1.41	0.25	5.8	< 0.001
^{206}Pb/^{207}Pb ant	Intercept	0.359	0.06	5.9	< 0.001
	^{206}Pb/^{207}Pb nest	0.589	0.07	8.2	< 0.001

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Lead (Pb) is a toxic heavy metal and there is no level of exposure that is known to be without harmful effects. Lead has been removed from products such as gasoline and paint but is still used in hunting ammunition. Exposure levels to Pb in humans and the general environment have dramatically declined during the past 40 years. Brown bears in Scandinavia, however, have blood Pb concentrations that contradict this general development. In this PhD thesis, blood Pb concentrations of brown bears and other wildlife species and Pb concentrations in bear food items were used to map Pb exposure levels within the population and to trace environmental sources of Pb.

Blood Pb concentration in Scandinavian bears was on average 27 times higher compared to sympatric non-scavenging wolves. Brown bears in areas with higher environmental background Pb concentrations and a denser distribution of hunter killed moose, had higher blood Pb concentrations. The Pb isotopic composition in the blood from bears overlapped with both natural food items and industrial Pb used to manufacture hunting ammunition. Ant nest material had the highest potential for Pb exposure among natural food items, concentrations in berries were very low. Bears in Scandinavia are highly exposed to Pb from industrial sources both from environmental background Pb and Pb-based hunting ammunition, on a level likely to impair individual health. Mean (range) blood Pb concentration in all 355 Scandinavian bear samples included in this study was 89 µg/L (20 – 221 µg/L). Lactating females are particularly exposed due to remobilization of Pb from bone during lactation and nursing offspring from exposure to Pb in early life through milk.