1 2	Title: Lead exposure in brown bears is linked to environmental levels and the distribution of moose kills
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35 Abstract

Lead (Pb) is heterogeneously distributed in the environment and multiple sources like Pb 36 37 ammunition and fossil fuel combustion can increase the risk of exposure in wildlife. Brown bears 38 (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 39 40 the contribution of two potential sources of Pb exposure in female brown bears (n = 34individuals; n = 61 samples). We used multiple linear regressions to determine the contribution 41 of both environmental Pb levels estimated from plant roots and moose (Alces alces) kills to 42 blood Pb concentrations in female brown bears. We found positive relationships between blood 43 Pb concentrations in bears and both the distribution of moose kills by hunters and environmental 44 Pb levels around capture locations. Our results suggest that the consumption of slaughter remains 45 discarded by moose hunters is a likely significant pathway of Pb exposure and this exposure is 46 additive to environmental Pb exposure in female brown bears in Sweden. We suggest that 47 48 spatially explicit models, incorporating habitat selection analyses of harvest data, may prove useful in predicting Pb exposure in scavengers. 49

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Keywords: Ursus arctos, Pb, scavenger, slaughter remain, resource selection function

53 **1. Introduction**

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Lead (Pb) is a naturally occurring trace element that is heterogeneously distributed in the 55 environment and its geochemical cycle has been affected by human activities (Arnemo et al., 56 57 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, 58 1990; Strömberg et al., 2008), but unleaded gasoline and smelter emissions may still influence 59 environmental Pb levels (Chételat et al., 2022; Chrastný et al., 2018; Widory et al., 2018). Pb 60 61 also has a long residence time in soils and certain areas with high historic Pb depositions still 62 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 63 64 or plants (Berglund et al., 2009; Scheifler et al., 2006). For instance, passerines sampled in urban environments, where the soils were contaminated by vehicle emissions, have higher blood Pb 65 concentrations when compared to birds sampled in rural environments (Chatelain et al., 2021; 66 McClelland et al., 2019; Roux and Marra, 2007). 67 Several types of human activities, such as hunting with Pb-based ammunition, can 68 increase the level of Pb found in the environment. Bullets used in hunting rifles are designed to 69 70 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). 71 Carcasses and gut piles discarded during the hunting season have high numbers of embedded 72 73 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 74 not uniform in space because it is intrinsically linked to the distribution of hunters on the 75

76 landscape.

Spatially explicit models can be used to predict the risk of Pb exposure from multiple 77 sources in wildlife (Mateo-Tomás et al., 2016). These models are known for being sensitive to 78 scale (Johnson et al., 2021); yet we are currently lacking information on the fine-scale variations 79 in risk of Pb exposure from bullet fragments embedded in discarded slaughter remains. Many 80 studies have investigated the relationship between hunting and Pb exposure in scavengers 81 82 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, 83 2011; Singh et al., 2021), which may limit the identification of exposure sources, especially 84 85 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 86 distribution of moose (Alces alces) harvest in Sweden by analysing moose harvest locations with 87 habitat selection analysis, which originally has been developed to analyse data from GPS-88 collared animals (Northrup et al. 2022). Second, we determine whether variations in blood Pb 89 90 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 91 in scavengers were on birds, but mammalian scavengers are also likely at risk of increased Pb 92 93 exposure when feeding on slaughter remains (Brown et al., 2022; Chiverton et al., 2022; Kelly et al., 2021). 94

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

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their habitat use and foraging behaviours, brown bears could be exposed to multiple potential 100 sources of Pb, including fossil fuel combustion and ammunition. Brown bears in Sweden are 101 exposed to Pb from unconfirmed sources (Fuchs et al., 2021). The mean (SD) blood Pb level of 102 96.6 (35.6) μ g/L reported by Fuchs et al. (2021) is higher than the means of 55 (40) μ g/L and 103 $58.0 (34.7) \mu g/L$ reported in North American and other European brown bears, respectively 104 105 (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 106 107 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 108 reducing Pb exposure in wildlife. 109

110 The aim of this study is to build a spatially explicit model to quantify the contribution of 111 two potential sources of Pb to the blood Pb concentrations measured in Scandinavian brown 112 bears: Pb from plant roots (hereafter refer to environmental Pb level) and Pb from ammunition 113 used by moose hunters. We hypothesised that the environmental Pb levels, and the distribution of 114 moose kills influence blood Pb concentrations in brown bears. We predicted that blood Pb 115 concentrations in Scandinavian brown bears would be positively related to both environmental 116 Pb levels, and the probability of moose kill.

117

118 2. Material and methods

119 2.1 Study area

- 120 The study area was in Dalarna and Gävleborg counties, south-central Sweden (~61°N, 15°E).
- 121 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 122 of Scots pine (Pinus sylvestris), Norway spruce (Picea abies), and birch (Betula spp.), whereas 123 the underlayer mainly consists of berry shrubs (Vaccinium spp.), heather (Calluna vulgaris) and 124 grasses with mosses and lichens covering the ground (Elfström et al., 2008; Ordiz et al., 2013; 125 Swenson et al., 1999). The area is also characterized by a dense network of forest roads (0.7 126 km/km²) and low human density (4-7 inhabitants/km²) (Martin et al., 2010; Ordiz et al., 2013). 127 Moose hunting in Sweden during the study period was allowed from the first Monday of 128 September to the end of January and, on average, 84,000 moose are harvested annually during 129 this period. Most moose (~75%) are harvested between September and the end of October 130 (Wikenros et al., 2013). Brown bears in Sweden typically enter their den towards the end of 131 October (Evans et al., 2016; Friebe et al., 2014, 2001) and thus have access to the slaughter 132 remains discarded by hunters. Those slaughter remains likely contain Pb fragments because most 133 hunters in Scandinavia use Pb ammunition (Stokke et al., 2017). 134

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2.2 Environmental Pb concentrations and hunting variable

137 We obtained a biogeochemistry (Biogeokemi) database from the Geological Survey of Sweden

138 (© Sveriges Geologiska Undersökning) that contains the concentrations of trace elements,

including Pb, of plant roots (*Carex* spp., *Fontinalis antipyretica* or *Filipendula ulmaria*)

140 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
- 144 elements circulating in the environment (Lax and Selinus, 2005). Our study area in south-central

145	Sweden was represented by a total of 2,264 samples of plant roots in this database. We used
146	these samples to predict environmental Pb concentrations on a dry weight basis across our study
147	area by using ordinary kriging. We fitted a Matern variogram with Stein's parameterization
148	(nugget = 0.224, psill = 0.366, kappa = 0.3, range = $0 - 23,594$ m) with the <i>fit.variogram</i>
149	function [gstat package (Gräler et al., 2016)] to predict Pb concentrations across our study area
150	with a resolution of 500 m. The interpolated surface was generated with the krige function [gstat
151	package, (Gräler et al., 2016)]. We log-transformed Pb concentrations and added a constant (C =
152	2.5) to all observations to obtain normally distributed data (W = 0.99, $p = 0.06$). We then back-
153	transformed the predicted values to the original scale to obtain a map with predicted Pb
154	concentrations across our study area. We used five-fold cross validation [krige.cv function; gstat
155	package, (Gräler et al., 2016)] to assess the predictive power of the variogram.
156	The distribution of moose kills in south-central Sweden was estimated following the
157	approach in Brown et al. (in review) by applying resource selection functions (RSF) to moose
158	harvest locations during 2017-2019. Briefly, the RSF was estimated by using a logistic
159	regression that contrasted the landscape characteristics at used (i.e., moose harvest locations
160	provided by hunters) and available (i.e., random) locations (Fieberg et al., 2021; Manly et al.,
161	2002). Hunters typically use areas that are located closer too roads and that have good lateral
162	visibility (e.g., clearcuts), but avoid rugged terrain with poor visibility. The RSF included
163	landcover types (i.e., forest composition, clearcut and bogs), distance to closest road and other
164	variables such as elevation, and terrain ruggedness index, which may impede the movement of
165	hunters across the landscape. The values produced by an RSF are proportional to the probability
166	of selection (Johnson et al., 2006; Manly et al., 2002) or, in this specific example, they are
167	proportional to the probability of hunters killing a moose at any location within our study area.

168 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. (in
review).

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172 **2.3** Capture, sample collection and chemical analyses

173 We carried out a total of 61 captures of adult female brown bears (n = 34 individuals), during 174 2017-2020. The captures were carried out as part of the Scandinavian Brown Bear Research 175 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 176 177 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, 178 Denmark) during the spring (April to June). See Arnemo and Evans (2017) for more details on 179 the capture protocol. The capture location was recorded with a hand-held GPS. At each capture, 180 4 or 6 mL of blood were collected from the jugular vein with evacuated K3EDTA tubes (n = 26) 181 (Vacuette, Greiner Bio-One International GmbH, Kremsmünster, Austria) or evacuated heparin 182 trace element tubes (n = 35) (Vacuette), respectively. The samples were first stored in a cooler in 183 the field and then frozen at -20°C until they were processed in the lab. The Pb concentrations 184 was measured by inductively coupled plasma sector field mass spectrometry (ICP-SFMS, 185 186 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 187 determining whether blood Pb concentrations were correlated to life history traits. The Pb 188 concentrations in digestion blanks were low ($< 2\mu g/L$) compared with sample results and the 189 difference between measured and expected Pb concentration in the certified reference material 190

191 (Seronorm Trace Elements Whole Blood Levels 1 and 2 from SERO AS, Norway) was < 6%

192 (Fuchs et al. 2021). See Fuchs et al. (2021) for further details about sample collections and

193 Rodushkin et al. (2000) for further details about chemical analyses.

194

195 **2.4 Statistical analyses**

196 We calculated the mean environmental Pb concentrations, and the mean relative probability of 197 moose kill (fall $_{t-1}$) within circular buffers with a radius of 2, 4 and 6 km around the capture 198 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 199 200 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 201 concentrations only changed according to sampling year, and another model in which blood Pb 202 concentration in female brown bears was affected by their lactation status (Table 1). Other 203 models within that set contained either the relative probability of moose kill, or the 204 environmental Pb concentrations. The last model contained a combination of all the variables 205 206 (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 207 because it was previously shown that this variable was not related to blood Pb concentrations in 208 209 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

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Akaike's Information Criterion corrected for small sample size (AICc) by using the *aicmodavg* 213 package (Mazerolle, 2020) in R. We considered models with a $\triangle AICc < 2$ to be equivalent. We 214 first conducted the model selection for each scale (2, 4 and 6 km) separately and then carried out 215 a second AICc with the top-ranked model of each scale to determine the best performing model. 216 We used diagnostic plots to ensure that model assumptions were fulfilled. We log-transformed 217 218 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 219 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 = -0.02220 221 0.87). All statistical analyses were conducted in R version 4.1.0 (R Core Team, 2021).

222

223 **3. Results**

Female brown bears had a mean (SD) blood concentration of 91 (36) µg/L. Lactating females (n 224 = 34) had a mean blood Pb concentration of 104 (36) μ g/L (range: 56-221 μ g/L), whereas this 225 value was 73 (36) μ g/L (range: 25-155 μ g/L) in non-lactating females (n = 27). Our kriging 226 model predicted environmental Pb concentrations ranging from 4.6 to 95 mg/kg (dry weigth) on 227 228 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 229 procedure revealed that observed and predicted values of environmental Pb concentrations were 230 231 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 232 = 0.45). The mean of residuals was < 0.01, which also indicates that the prediction errors were 233 small relative to predicted values. 234

235	The model that performed best at explaining blood Pb concentrations in bears was the
236	Full model (Akaike weight = 0.86) that included effects of the lactation status, the sampling year,
237	the environmental Pb concentration and the relative probability the moose kill (fall $_{t-1}$) extracted
238	within 2 km buffers around the capture locations (Table 2). The second-best performing model
239	was the Environmental Pb models (Akaike weight = 0.14), which had a \triangle AICc of 3.69 with the
240	best model (Table 2). The other models received considerably less support as their Δ AICc were
241	> 19 with the top-ranked model (Table 2). The results were similar across scales (Table A1), but
242	the 2 km-scale performed best (Table A2). Thus, we only discuss the results with variables
243	extracted at 2 km and present the other results as supporting information (Table A3).
244	The following effect sizes and confidence intervals (95% CI) are expressed in percent of
245	change, and they were calculated by back-transforming the coefficients (multiplied by the
246	standard deviation for continuous variables), subtracting 1 and multiplying the results by 100.
247	Our models indicated that blood Pb concentration in female brown bears was positively related
248	to the environmental Pb concentration around the capture location (Figure 2; Table 3). For every
249	unit-increase of 16.02 mg/kg in environmental Pb concentrations (1 unit of standard deviation),
250	the blood Pb concentrations in female brown bears increased by 18.5 % (Lower: 10.5%, Upper:
251	27.1%). Similarly, our model predicted higher blood Pb concentrations in brown bears captured
252	in areas where hunters were more likely to kill moose during the previous fall (Figure 2; Table
253	3). Blood Pb concentrations in brown bears increased by 9.1% (Lower: 1.5%, Upper: 17.2%) for
254	every increase of 0.17 (1 unit of standard deviation) in the relative probability of moose kill.
255	Lactating females also had blood Pb concentrations that were 37.7% higher (Lower: 17.8%,
256	Upper: 61.0%) when compared to non-lactating females.

258 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 266 of Pb that circulates in the environment within their home range. Bears could be exposed to Pb 267 268 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 269 270 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 271 concentrations estimated from plant roots may not be entirely representative of Pb concentrations 272 273 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 274 incorporate Pb from atmospheric sources (Klaminder et al., 2005); berries may also be coated 275 276 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 277 estimate and compare the amount of Pb that circulates in the different regions of our study areas. 278 Our conservative approach with buffers of various radii centred on capture locations was 279

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 281 animals can be refined further by incorporating movement or space use parameters (Sorais et al., 282 2021, 2020). Adding data from GPS transmitters could have improved the performance of our 283 model. Spatially explicit models also need to account for the physiological state of an individual 284 (i.e., reproductive status) and failure to do so may introduce bias in the models, as evidenced by 285 286 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) 287 from bones into the bloodstream and lactating females are exposed to an additional endogenous 288 source of Pb (Fuchs et al., 2021). 289

Our results also indicate that high probabilities of moose kills are linked to higher blood 290 Pb levels in brown bears. Some concerns have been raised previously regarding the risk of Pb 291 exposure from bullet fragments in mammalian scavengers (Kelly et al., 2021; Legagneux et al., 292 2014; Rogers et al., 2012), but so far this source of Pb exposure had mostly been reported in 293 294 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 295 (Helander et al., 2021; Pain et al., 2019; Slabe et al., 2022). For instance, Pb from ammunition 296 297 has been linked to increased risk of mortality (Singh et al., 2021) and altered flight behaviour (Ecke et al., 2017) in avian scavengers, and has prevented the recovery of the California condor 298 (Gymnogyps californianus) (Finkelstein et al., 2012). 299

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

behaviour have been reported in golden eagles at blood Pb levels (25 μ g/L) well below toxicity 304 thresholds for birds (Ecke et al., 2017), and it has been reported that eagles with blood Pb levels 305 above 25 µg/L were more likely to die compared to individuals below this threshold (Singh et 306 al., 2021). Although the conclusions of these studies cannot be directly translated to brown bears, 307 they suggest that subclinical effects of increased Pb exposure can occur at levels well below 308 309 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 310 deleterious effects in bears. If subclinical or sublethal effects of Pb occur in brown bears, the 311 consumption of slaughter remains discarded by hunters could be considered an evolutionary trap 312 (Schlaepfer et al., 2002), because there is no reason for an opportunistic omnivore to not 313 consume easily accessible, energy-rich and easily digestible foods when encountered (DeVault et 314 al., 2003; Pritchard and Robbins, 1990). However, this 'easy meal' acquired at low costs may be 315 deleterious in the long term, because it may contain high concentrations of Pb (Gremse et al., 316 317 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 318 their behaviour in order to gain access to slaughter remains during the fall and concluded that the 319 320 scavenging behavior of bears in Sweden is mostly opportunistic (Brown et al. In review). Despite this seemingly low exposure rate, we found a positive relationship between blood Pb levels, and 321 322 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 323 find slaughter remains (Lafferty et al., 2016; Legagneux et al., 2014; Ruth et al., 2003), 324 suggesting that bears from other populations may be at greater risk of Pb exposure than 325 Scandinavian brown bears, especially in areas where the peak of the hunting season is earlier 326

during their active period. The risk of increased Pb exposure should thus be evaluated in bearsand 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 329 330 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 331 332 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 333 number of animals that were harvested with a firearm within a specific area (Helander et al., 334 2021; Kelly and Johnson, 2011); however, this approach is based on the assumption that the 335 distribution of hunter kills is uniform within the area. This assumption is inaccurate in most 336 cases. Hunter kills are neither randomly nor uniformly distributed across the landscape, but are 337 rather concentrated around specific features that provide accessibility, concealment, and/or 338 visibility, depending on the hunting style (Gaynor et al., 2022; Norum et al., 2015). Ignoring the 339 340 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 341 (DeVault et al., 2003). However, mammals do not travel as efficiently as most avian scavengers 342 343 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 344 hunter kill sites. Using an RSF-based approach on ungulate kill sites provided by hunters may be 345 useful for predicting the fine-scale distribution of hunter kills and, by extension the increased 346 risk of Pb exposure, within an area. 347

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 350 disadvantage is that, depending on the number of included variables, it may still require hundreds 351 of harvest locations, and by extension, it also requires the cooperation of many hunters, which 352 may choose to not disclose or collect information on harvest locations for research purposes. 353 Wildlife management agencies can however obtain this information relatively easily from 354 355 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, 356 but their results may be difficult to interpret properly. Fortunately, multiples tools are now 357 358 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., 359 2017). 360

Potential limitations of our study include the relatively small sample size, the absence of 361 males from the analyses, and the timing of blood sampling. A sample size of 34 females may be 362 363 small for many species, but considering that, in 2008, the entire population of bears in Sweden was estimated at ~3,300 individuals (Kindberg et al., 2011), our sample size can be considered 364 acceptable. Due to the absence of samples from adult males, we also could not investigate 365 366 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 367 remains; however, it is unlikely a problem in our study because avoidance of males by females 368 with dependent offspring is more common during the mating season (June-July) compared to the 369 fall when slaughter remains are available (Steyaert et al., 2013). Another potential limitation of 370 our study is that it may be difficult to relate Pb concentrations from blood samples collected 371 during spring to moose hunting activities that occurred during the previous fall. Other studies 372

have shown that the blood Pb concentrations in scavengers decrease during the winter and spring 373 (Craighead and Bedrosian, 2008; Slabe et al., 2022) and our model may have underestimated the 374 contribution of ammunition as a Pb source due to the timing of blood sampling. Nevertheless, 375 our conclusions are similar to those reported by Arrondo et al. (2020) who also found that soil 376 was an important source of Pb exposure in vultures from Spain. Additionally, it is also possible 377 378 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 379 the frequency of this behaviour. 380

An alternative explanation based on Pb kinetics is likely a better explanation for the 381 relationship between spring blood Pb levels and the distribution of moose kills during the 382 previous fall. We do not know if and how hibernation affects Pb kinetics, but blood half-life of 383 elimination may be extended because bears do not urinate nor defecate during this period 384 (Nelson et al., 1983), thereby suggesting no or minimal excretion from the body during the 385 386 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 387 typically reflect short time exposure, but this parameter also depends on the equilibrium between 388 389 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 390 during periods of nutritional stress, gestation and lactation (Arnemo et al., 2022). Bears do not 391 eat nor drink during hibernation and Pb may be mobilized from bones in all individuals; this 392 phenomenon was especially obvious in lactating females (Fuchs et al., 2021). Therefore, blood 393 Pb concentrations in bears during the spring are likely the results of a mixture of recent intakes 394

Brown, Ludovick; Fuchs, Boris; Arnemo, Jon Martin; Kindberg, Jonas; Rodushkin, Ilia; Zedrosser, Andreas; Pelletier, Fanie. Lead exposure in brown bears is linked to environmental levels and the distribution of moose kills. Science of the Total Environment 2023 ;Volum 873. s 10.1016/j.scitotenv.2023.162099. and long-term exposure from blood-organ-bone equilibrium (i.e., mobilisation of Pb frombones).

397

398 5. Conclusion

399 We found a link between the distribution of moose kills by hunters and the blood Pb 400 concentrations in bears; however, the environmental Pb level was a greater contributor to bears' 401 blood Pb concentrations. Pb from ammunition is mainly available for a few weeks during the 402 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 403 404 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 405 scavengers. We also propose that an RSF-based approach with harvest locations provided by 406 hunters should be relatively easy to implement in other systems, thereby improving our capacity 407 to better understand the risk of increased Pb exposure from bullet fragments in scavengers. This 408 study suggests that regulations on both Pb ammunition and other anthropogenic Pb emissions are 409 needed to reduce Pb exposure in bears. 410

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Figure 2. Predicted blood Pb concentrations (μ g/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).

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	$\log(Ph) \sim 1$	Ph is constant
1 (411	105(10) 1	
Year	$\log(Pb) \sim Year$	Pb is influenced by year only
Lactation	$log(Pb) \sim Lactation$	Pb is influenced by lactation only
RSF hunt	$log(Pb) \sim Probability of moose kill +$	Pb is influenced by probability of
	Lactation + Year	moose kill, lactation and year
Environmental Pb	log(Pb) ~ Environmental Pb +	Pb is influenced by
	Lactation + Year	Environmental Pb, lactation and
		year
Full	log(Pb) ~ Environmental Pb +	Pb is influenced by
	Probability of moose kill + Lactation	Environmental Pb, probability of
	+ Year	moose kill, lactation and year

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.

	Κ	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, $\Delta AICc$ = the AICc difference with the top-ranked model, w = Akaike weight within the set and LL = log-likelihood of the model.

Table 3. Parameters of the top-ranked model used to predict blood Pb concentrations [log(Pb μ g/L)] in female brown bears (n = 34 individuals; n = 61 samples) from south-central Sweden, during 2017-2020.

		S.E.	95% CI	
Variables	Estimate		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.