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## Temporal trend of mercury in polar bears (*Ursus maritimus*) from Svalbard using teeth as a biomonitoring tissue

Aurore Aubail, Rune Dietz, Frank Rigét, Christian Sonne, Øystein Wiig,  
Florence Caurant

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4 **Temporal trend of mercury in polar bears (*Ursus maritimus*) from**  
5 **Svalbard using teeth as a biomonitoring tissue**  
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9 Aurore Aubail <sup>a, b, \*</sup>, Rune Dietz <sup>a, \*</sup>, Frank Rigét <sup>a</sup>, Christian Sonne <sup>a</sup>, Øystein Wiig <sup>c</sup>, Florence  
10 Caurant <sup>b</sup>  
11

12 <sup>a</sup> *National Environmental Research Institute, Aarhus University, Frederiksborgvej 399, P.O. Box*  
13 *358, DK-4000 Roskilde, Denmark*  
14

15 <sup>b</sup> *Littoral, Environnement et Sociétés (LIENSs), UMR 6250, CNRS-Université de La Rochelle, 2 rue*  
16 *Olympe de Gouges, F-17042 La Rochelle cedex, France*  
17

18 <sup>c</sup> *Natural History Museum, University of Oslo, P.O. Box 1172 Blindern, NO-0318 Oslo, Norway*  
19  
20  
21  
22

23 \* Corresponding authors:

24 Tel.: +33 (0)546507639; Fax.: +33 (0)546458264/ Tel.: +45 21254035; Fax.: +45 46301914

25 E-mail address: [aaubail@yahoo.fr](mailto:aaubail@yahoo.fr)/rdi@dmu.dk  
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28 **Abstract**

29 We examined the use of mercury (Hg) and nitrogen and carbon stable isotopes in teeth of polar bear  
30 (*Ursus maritimus*) from Svalbard as biotracers of temporal changes in Hg pollution exposure  
31 between 1964 and 2003. Teeth were regarded as a good matrix of the Hg exposure, and in total 87  
32 teeth of polar bears were analysed. Dental Hg levels ranged from 0.6 to 72.3 ng/g dry weight and  
33 increased with age the first 10 years of life. A decreasing time trend in Hg concentrations was  
34 observed over the recent four decades while no temporal changes were found in the stable isotope  
35 ratios of nitrogen ( $\delta^{15}\text{N}$ ) and carbon ( $\delta^{13}\text{C}$ ). This suggests that the decrease of Hg concentrations  
36 over time was more likely due to a lower environmental Hg exposure in this region rather than a  
37 shift in the feeding habits of Svalbard polar bears.

38

39 **Key words:** Hg, carbon, nitrogen, stable isotope, polar bear, tooth, Svalbard, temporal trend

## 40 **Introduction**

41

42 Mercury (Hg) is emitted to the atmosphere from both natural and anthropogenic sources around the  
43 world. Long-range atmospheric transport represents the major pathway of Hg to the Arctic  
44 environment.<sup>1,2</sup> However recent evidence show that ocean currents and rivers also contributes  
45 significantly to the Arctic Hg pollution.<sup>3</sup> Mercury is known to accumulate in organisms and  
46 biomagnify up the food chains,<sup>4</sup> and has therefore been detected in the body tissues of various  
47 Arctic organisms including local Inuit populations.<sup>5</sup>

48 Bioaccumulation processes in the Arctic fauna are still investigated, and various temporal trends of  
49 Hg have been provided within the Arctic Monitoring Assessment Programme.<sup>5</sup> Due to its long life  
50 span and its top position in the marine Arctic food web, the polar bear (*Ursus maritimus*)  
51 accumulates significant amounts of Hg in its tissues. Mercury in this species has been mostly  
52 investigated in soft organs such as liver or kidney,<sup>6-11</sup> but also in hair<sup>6,12-15</sup> and blood.<sup>16</sup> However,  
53 from our knowledge there is no information available concerning Hg concentrations in polar bear  
54 teeth, though this tissue could provide a good material to assess Hg bioaccumulation in this apex  
55 predator. Calcified tissues like teeth are indeed considered to be valuable archives, as they record  
56 the individual life history, environment and diet, and therefore could be used in different fields of  
57 environmental sciences.

58 Mammalian teeth comprise three anatomical defined tissues: enamel, dentine and cementum.  
59 Dentine and cementum are composed of both organic (collagen) and mineral (calcium phosphate,  
60 *i.e.* apatite) fractions that grow throughout life; in marine mammals, these two tissues deposit yearly  
61 through layers or Growth Layer Groups (GLG). Mercury, once ingested, distributes to all internal  
62 organs and tissues via blood stream,<sup>17</sup> including to the dentinal increments from blood vessels.<sup>18</sup>  
63 Contrary to soft tissues, the tooth is not remodelled or very little throughout life, and incorporated  
64 elements like Hg are thought not to be remobilised.<sup>19,20</sup> Thus, the dental tissue is a valuable material  
65 concerning life exposure investigations, and has therefore been previously investigated for Hg in  
66 rats,<sup>21</sup> marine mammals<sup>22-25</sup> and humans.<sup>19,20</sup>

67 Since diet is the main pathway for Hg to enter marine mammals, in addition to the general  
68 anthropogenic trends a change in feeding habits would also likely result in variations in Hg  
69 exposure, and consequently, in the body concentrations of this metal. Moreover, due to the  
70 magnification of this element, a change in Hg levels of an organism may only be seen if that  
71 organism feeds on different trophic levels than it used to. The naturally occurring carbon (<sup>12</sup>C and  
72 <sup>13</sup>C) and nitrogen (<sup>14</sup>N and <sup>15</sup>N) stable isotopes ratios have proved to be useful tools for

73 characterizing the primary production in marine and terrestrial ecosystems and delineating the  
74 trophic position of organisms in food webs, respectively.<sup>26</sup> Like trace elements, carbon and nitrogen  
75 are incorporated in the dental tissue during its growth. Combined to the analysis of Hg, the  
76 measurement of the carbon and nitrogen stable isotopes in the teeth will provide valuable  
77 information relative to potential changes in feeding habits or habitats for polar bear. The stable  
78 isotopic technique is based on the metabolic discrimination between the heavy and the light  
79 isotopes. While  $^{13}\text{C}/^{12}\text{C}$  values exhibit few variations through successive trophic levels in the food  
80 chains,<sup>27</sup> the  $^{15}\text{N}/^{14}\text{N}$  value is significantly and regularly enriched through the food chains with a  
81 value found in a consumer's tissue directly related to the one of its prey, providing thus information  
82 on one's trophic level.<sup>28</sup> Usually, the isotopic measurements are carried out on muscle tissues which  
83 give access to the feeding ecology from the last weeks or months, whereas performed on teeth, the  
84 isotopic data obtained will represent an average dietary estimate over the animal's lifetime or near  
85 to its lifetime,<sup>29</sup> erasing any potential shift of diet related to seasonal and physiological stages.

86 Polar bears are known to prey mainly on ringed seals (*Pusa hispida*) and to a lesser extent on  
87 bearded seals (*Erignathus barbatus*) across the Arctic.<sup>30</sup> However, there are some evidences that the  
88 ringed seal dominance in polar bear diet differs for example spatially and temporally in the  
89 Arctic.<sup>31,32</sup> Polar bears feed also on other species such as harp seals (*Phoca groenlandica*),<sup>33</sup> walrus  
90 (*Odobenus rosmarus*),<sup>34</sup> beluga whales (*Delphinapterus leucas*) and narwhal (*Monodon*  
91 *monoceros*),<sup>35</sup> and diet items like seabirds or even reindeers (*Rangifer tarandus*) have been showed  
92 to be consumed by the polar bears in Svalbard.<sup>36</sup> Sea ice is used as a platform by polar bears for seal  
93 hunt,<sup>30</sup> so that the accessibility of their main prey varies throughout the year due to sea ice extension  
94 changes. This close relation to the sea ice makes polar bears vulnerable to a warming climate and a  
95 relevant indicator of climate change effects on the ecosystem.<sup>37</sup> Global warming has resulted in  
96 significant declines in total cover and thickness of sea ice over the last decades in the Arctic.<sup>38</sup>  
97 Because of this progressive earlier break-up and later freezing of the Arctic sea ice in some areas,  
98 polar bear's access to seals is thus likely to be reduced resulting in longer periods of fasting and  
99 searching for alternative food sources. Stable isotopic measurements were therefore used  
100 complementarily with the elemental analysis in this study on polar bears to relate temporal  
101 variations of tooth Hg to potential changes in feeding habits for this species.

102 This article presents the Hg concentrations and  $^{15}\text{N}/^{14}\text{N}$  and  $^{13}\text{C}/^{12}\text{C}$  values in teeth of polar bears  
103 from Svalbard over the recent four decades. The objectives of this study are to assess the influence  
104 of the biological factors (sex and age) on Hg concentrations in teeth and to investigate the temporal  
105 pattern of Hg concentrations in Svalbard polar bears between the 1960s and the 2000s in order to  
106 evaluate whether the tooth is a good biomonitoring tissue.

107

## 108 **Materials and methods**

109

### 110 **Sampling procedure and preparation**

111 Tooth samples were obtained from 87 polar bear skulls archived at the Natural History Museum,  
112 University of Oslo (NHM), Norway. The skulls had been collected in the archipelago of Svalbard  
113 (Fig. 1), from 1964 to 2003. An overview of the samples used in statistical analysis is presented in  
114 Table 1.

115 The first premolar of the lower mandible had been used for age estimation by counting annual  
116 layers in the cementum after decalcification, thin sectioning (14 $\mu$ m), and staining in toluidine blue,  
117 as described by Dietz *et al.*<sup>39</sup> The third incisor from the lower right mandible was taken for the Hg  
118 and carbon and nitrogen stable isotopes analytical purpose. The extracted tooth was cut in three  
119 pieces using a Proxon diamond saw. The upper third incisor was used for the study of Hg content,  
120 while the middle part of the tooth was used to the determination of the stable isotopes, and the  
121 lower third incisor was kept for further potential investigations (*e.g.* additional age determination).  
122 It is assumed that contrarily to the root, the upper and middle parts of incisors have the same deposit  
123 of layers and have been similarly exposed to Hg.

124 Prior to analysis, upper and middle parts of the teeth were cleaned of external materials by abrasion,  
125 immersed subsequently in 10% nitric acid for 20s, and rinsed in several ultra-pure Milli-Q water  
126 baths. Tooth samples were dried for a minimum of 24h at room temperature, and subsequently  
127 stored in cleaned plastic flasks until analysis.

128

### 129 **Analytical procedures and instrumentation for Hg analysis**

130 The Hg measurements were performed at the laboratory of the National Environmental Research  
131 Institute in Roskilde (NERI), Denmark, using a solid sample atomic absorption spectrometer AMA-  
132 254 (Advanced Mercury Analyser-254 from LECO, Sweden). The use of this instrument does not  
133 require a chemical pre-treatment, which reduces considerably contamination risks and loss of Hg.  
134 The analytical process consists of a drying period at 120°C for 50 seconds, prior to a combustion  
135 phase at 750°C for 250 seconds, which leads to the desorption of Hg from the samples.  
136 Subsequently, the Hg vapour produced is carried by an oxygen flow to a gold amalgamator, and  
137 trapped on its surface. Thereafter, the collected Hg is released from the amalgamator by a short  
138 heat-up to 900°C, and carried in a pulse through a spectrophotometer, where it is measured by UV  
139 absorption. The instrument is described in detail by Hall and Pelchat.<sup>40</sup>

140

141 As there is no commercial reference material with a tooth or bone matrix and certified for Hg, a  
142 reference material was made from two commercial Standard Reference Materials (SRMs). These  
143 were the NIST 1400 Bone ash (National Institute of Standards and Technology, USA) and the  
144 DOLT-3 (Dogfish Liver Tissue from the National Research Council of Canada). The Bone ash  
145 SRM does not contain any Hg because it has been calcined at high temperatures, but it represents a  
146 calcified tissues matrix, while the DOLT-3 contributes with the organic matrix and the certified  
147 level of Hg.

148 In order to validate the use of this customised reference material, an intercalibration between two  
149 different techniques (AMA-254 and cold-vapour Atomic Absorption Spectrophotometry on a  
150 Perkin Elmer FIMS 100) was carried out in NERI, and moreover, an inter laboratory comparison  
151 was carried out between NERI and CCA (Centre Commun d'Analyses, University of La Rochelle,  
152 France) for the AMA-254 analysis. The results showed a good accuracy (*i.e.* the recovery measured  
153 value/theoretical value) of 102.3% and a relative standard deviation of 6.3% (Table 2).

154 The analytical quality of the Hg measurements by the AMA-254 was ensured by including the  
155 customised reference material at the beginning and at the end of the analytical cycle, and by running  
156 it every 10 samples. Results of these measurements ( $n = 13$ ) showed a good precision with a  
157 relative standard deviation of 1.6%, and an accuracy of 106.6% of the assigned concentration.

158 NERI participates in the international inter-laboratory comparison exercises conducted by the EEC  
159 (QUASIMEME), and the latest 2007 analyses by AMA-254 showed satisfactory results ( $0 < z <$   
160  $0.5$ ). All data are presented on a dry weight basis (dw) and the detection limit is 0.01 ng.

161

### 162 **Analytical procedures and instrumentation for stable isotope measurements**

163 Prior to analysis, tooth samples were crushed into small pieces before being ground into  
164 homogenous powder using a ball mill (Retsch MM2000) for 2min at the amplitude of 90. Powder  
165 was then stored in small glass flasks. The carbonates of the teeth were removed by digesting the  
166 teeth with approximately 1 mL of a 4M-hydrochloric acid solution at 45°C for 48h. Subsequently,  
167 the digested contents were taken up in milli-Q ultrapur quality water, and homogenised before  
168 freezing to -20°C. Thus, the samples were frozen at -80°C for a short time before freeze drying.  
169 Finally, an aliquot of approximately 1.45 mg was taken of each obtained homogenised dried  
170 sample, weighted and loaded into tin capsules.

171 Relative abundance of stable isotopes of nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ) and carbon ( $^{13}\text{C}/^{12}\text{C}$ ) were determined  
172 with an Elemental Analyser connected on-line to an Isotope-Ratio Mass Spectrometer (Isoprime,

173 Micromass, UK). Stable isotope results are expressed in delta notation ( $\delta$ ), defined as the part per  
174 thousand (‰) deviation from a standard material:

175

$$176 \quad \delta^{15}\text{N} \text{ or } \delta^{13}\text{C} = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 10^3$$

177

178 where  $R_{\text{sample}}$  and  $R_{\text{standard}}$  are the fractions of heavy to light isotopes in the sample and  
179 standard, respectively. The international standards are the atmospheric nitrogen for  $\delta^{15}\text{N}$  and the Pee  
180 Dee Belemnite (PDB) marine fossil limestone formation from South Carolina for  $\delta^{13}\text{C}$ , and  
181 acetanilide was used as the internal laboratory reference material.

182

### 183 **Data treatment**

184 Prior to the statistical analyses, the Hg data were log-transformed (base e) to reduce skewness and  
185 fit parametric requirements of normal distribution and homogeneity of variances. Shapiro–Wilk test  
186 of normality and Bartlett test of homogeneity of variances were applied to test the assumptions of  
187 analysis of variance (ANOVA) and linear regression analysis. The assumptions were not fulfilled in  
188 few cases due to some high Hg values but ANOVA tests are robust to small deviations of the data  
189 from the normal distribution,<sup>41</sup> so that the analyses were conducted anyway.

190 Analysis of variance and multiple linear regression analyses were performed to test the influence of  
191 sex, age, and year on log-transformed Hg concentrations and stable isotopic values. Only  
192 individuals (>4 years old) were used to test the factor sex (n = 49), while only individuals collected  
193 from 1964 to 1966 (n = 57) were selected when testing the age effect. The non-parametric test  
194 Spearman rank correlation was used to investigate relationships between log-transformed Hg  
195 concentrations,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values.

196 The significance level was set to  $p = 0.05$  and the statistical analyses were performed using the free  
197 software R, version 2.1.1.<sup>42</sup>

198



## 199 **Results**

200

### 201 **Mercury concentrations in relation to age and sex**

202 Dental Hg levels were generally low, exhibiting a mean value  $\pm$  standard deviation (sd) of  $6 \pm 8.3$   
203 ng/g dw (Table 3). The highest concentration was found in a female cub (0 year old) from 1964. It  
204 was considered as an outlier due to its very high Hg concentration (72.3 ng/g dw), and was  
205 consequently excluded from graphs and statistical calculations.

206 Log-transformed Hg concentrations in polar bear teeth were not influenced by gender (one-way  
207 ANOVA,  $F = 3.00$ ,  $p = 0.09$ ), so data were pooled across sexes in the further data analyses. In  
208 contrary, log-transformed Hg concentrations increased significantly with age (Spearman's  
209 correlation,  $\rho = 0.50$ ,  $p < 0.001$ ). A general increase of Hg concentrations in the teeth of polar bears  
210 was observed for the first 10 years of life and followed by a plateau phase (Fig. 2a).

211

### 212 **Stable isotopic values in relation to sex, age and Hg concentrations**

213 The  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values ranged from 17.7‰ to 21.8‰ and -17.4‰ to -14.8‰, respectively (Table  
214 3). Neither  $\delta^{15}\text{N}$  nor  $\delta^{13}\text{C}$  values in polar bear teeth were influenced by gender ( $F = 0.01$ ,  $p = 0.92$ ;  
215  $F = 0.17$ ,  $p = 0.68$ ), or correlated with age ( $\rho = -0.02$ ,  $p = 0.90$ , Fig. 2b and  $\rho = -0.11$ ,  $p = 0.42$ , Fig.  
216 2c, respectively); and no relationship was found between log-transformed Hg concentrations and  
217  $\delta^{15}\text{N}$  or  $\delta^{13}\text{C}$  values ( $\rho = 0.11$ ,  $p = 0.29$  and  $\rho = 0.01$ ,  $p = 0.91$ , respectively) (Table 4).

218

### 219 **Time trends**

220 Sub-adult and adult individuals with age between 3-10 years ( $n = 37$ ) were selected for the time  
221 trend analyses in order to limit the overlap of the periods of life of the animals and in the same time  
222 to cover the entire 40 years period. In addition, since polar bear cubs are nursed until their third year  
223 of life,<sup>43</sup> selecting individuals older than 2 years excludes the animals which have been feeding  
224 exclusively on maternal milk. The yearly average age of the sampled individuals (3-10 years) did  
225 not follow any time trend (linear regression,  $F = 0.54$ ,  $p = 0.47$ ), hence no age normalisation was  
226 conducted. However, a significant decreasing trend in Hg concentrations of 2.1% per year was  
227 found over the 4 decades period between 1964 and 2003 ( $F = 9.66$ ,  $p = 0.004$ , Fig. 3a), while no  
228 significant temporal trend was found for  $\delta^{15}\text{N}$  or  $\delta^{13}\text{C}$  values for the same period ( $F < 0.01$ ,  $p =$   
229  $0.94$ , Fig. 3b and  $F = 0.42$ ,  $p = 0.52$ , Fig. 3c, respectively).

230

## 231 **Discussion**

232

### 233 **Levels of Hg in the teeth**

234 Diet is the main pathway for Hg in to the body of marine mammals.<sup>44</sup> Thus, the feeding habits  
235 mainly determine the Hg primary load of a species. However, another important factor is the  
236 excretion routes where hair has been shown to play an important role for pinnipeds or polar bears  
237 similar to the one of feathers for birds.<sup>45-47</sup> Polar bears, as top predators of the Arctic marine food  
238 web, have been shown to accumulate high Hg concentrations (several ppm) in soft tissues<sup>7,8,9,10</sup> and  
239 fur<sup>6,12,13</sup> while Hg concentrations in teeth in our study are about 1000 times lower. These levels are  
240 consistent with the levels reported in teeth of other Arctic marine mammals. Thus, Outridge *et al.*<sup>23</sup>  
241 reported dental Hg concentrations under 2 ng/g dw for modern samples of walrus. A geometric  
242 mean ( $\pm 2$  standard errors) of 4.4 ( $\pm 1.7$ ) to 8.6 ( $\pm 3.7$ ) ng/g was found in ringed seals of 5-25 years  
243 old from Amundsen Gulf in Canada.<sup>24</sup> Aubail *et al.*<sup>25</sup> reported dental Hg concentrations ( $\pm$  sd) of  
244 2.94 ( $\pm 1.99$ ) ng/g in ringed seals from West Greenland and of 5.75 ( $\pm 6.20$ ) ng/g in ringed seals  
245 from East Greenland. However, dental Hg concentrations measured in polar bears are lower than  
246 those found in beluga whales by Outridge *et al.*<sup>23</sup>, who reported a mean Hg concentration ( $\pm$  sd) of  
247 98.4 ( $\pm 109$ ) ng/g dw for animals 6-26 years old and Hg concentrations ranging from 6.4 ( $\pm 13.3$ ) to  
248 292.3 ( $\pm 36$ ) ng/g in individuals 10-60 years old from the Beaufort Sea.<sup>24</sup> A geographical difference  
249 in general environmental Hg exposure could likely contribute to this difference, since polar bears  
250 from Svalbard and their primary food the ringed seal have been shown to generally exhibit lower  
251 Hg concentrations than Canadian or Greenlandic individuals.<sup>6,9,48-50</sup> Moreover, it has been shown  
252 that during periods of reliable food access, polar bears mainly consume seal blubber,<sup>51</sup> which is not  
253 exhibiting high Hg contents<sup>52</sup> and could therefore result in lower dental Hg levels in polar bears  
254 compared to beluga whales. However, again the excretion also plays a major role as toothed whales  
255 do not have the hair excretion route as the polar bears which results in relatively higher  
256 concentrations in *e.g.* meat and brain.<sup>47</sup>

257

### 258 **Biological factors**

259 Although diet may vary substantially between genders, no difference in Hg concentrations was  
260 found between males and females, which fits the lack of difference between males and females  
261 previously reported in soft tissues of polar bears.<sup>7-9</sup> Contrarily, dental Hg concentrations were  
262 correlated with age (Table 4). Polar bears generally exhibit a cumulative pattern of this metal  
263 especially in their liver.<sup>7-9,11</sup> However, age effects on dental Hg concentrations in Arctic marine

264 mammals are not consistent in the literature. Outridge *et al.*<sup>22</sup> showed that Hg concentrations in  
265 teeth of beluga whales increased with age, while Kinghorn *et al.*<sup>53</sup> did not find a significant age  
266 effect in the same species. Outridge *et al.*<sup>24</sup> observed a positive correlation between age and Hg  
267 concentrations in teeth of ringed seals, while Aubail *et al.*<sup>25</sup> did find a significant decrease of the  
268 dental Hg concentrations the first years of life for Greenland ringed seals; this trend was explained  
269 by early maternal transfer of Hg to pre- and postnatal individuals. Indeed, since ringed seals acquire  
270 their permanent dentition at the foetal stage,<sup>54</sup> the prenatal transfer of Hg may represent an  
271 important source of Hg for the dental tissues mineralised under the foetal stage, compared to the  
272 postnatal Hg incorporated from diet. In this study, a general increase of Hg concentrations in the  
273 teeth of polar bears was observed for the first 10 years of life and was followed by a more constant  
274 plateau phase (Fig. 2a). Thus, this trend could be explained by the fact that polar bears, contrarily to  
275 ringed seals, would acquire their permanent dentition at the postnatal stage. This results in the  
276 progressive accumulation of Hg throughout years with a low intake of Hg from the maternal milk,  
277 to greater Hg concentrations with an increasing efficiency of the hunting strategy and thus, amount  
278 of prey and Hg ingested. The stabilized levels of Hg observed afterwards may likely be related to  
279 the late dentinal occlusion of the tooth. Indeed, the deposition of dentine reduces the pulp area and  
280 in some species, the pulp cavity has been shown to occlude at some point of their life with the  
281 subsequent suspension of the deposition of dentine<sup>55</sup> and likely also elements such as Hg from  
282 blood vessels.

283 The highest dental Hg content in our study (72.3 ng/g dw) was displayed by one of the five cubs of  
284 the year, whereas the four others exhibited low dental Hg concentrations (from 0.9 to 3.4 ng/g dw).  
285 Kenny and Bickel<sup>56</sup> reported that a 6 months old polar bear cub still had some deciduous incisors at  
286 its age, thus, the hypothesis that the deciduous incisor may actually have been sampled and  
287 analysed instead of the permanent one for that yearling cannot be eliminated. The high Hg value  
288 displayed by that particular individual could likely correspond to the Hg maternal input during  
289 foetal stage, an input which has been shown to be of important contribution for the dental tissues of  
290 ringed seals.<sup>25</sup>

291 Since integration time and process of both isotopic and metallic elements are considered as  
292 relatively similar,  $\delta^{15}\text{N}$  or  $\delta^{13}\text{C}$  values have been used to relate Hg concentrations to trophic position  
293 or foraging location, respectively. However, Hg concentrations were not correlated with  $\delta^{15}\text{N}$  or  
294  $\delta^{13}\text{C}$  values in this study and no age effect was found for  $\delta^{15}\text{N}$  values, which is different from other  
295 previous investigations in which high  $\delta^{15}\text{N}$  values were measured in muscle of different marine  
296 mammals in the first years of life, relative to dietary inputs from mother's milk during the period of

297 suckling.<sup>57-59</sup> In those studies, the decrease in nitrogen isotope values reflects ontogenetic changes  
298 and is likely to be associated with the period from primarily feeding on milk towards primarily  
299 feeding on live prey. Likewise, no age effects were found for  $\delta^{13}\text{C}$  values in our study, whereas  
300 cubs of the year were expected to present low  $\delta^{13}\text{C}$  values, in response to depleted  $^{13}\text{C}$  directly  
301 derived from maternal milk lipids.

302

### 303 **Time trend**

304 Contrary to soft tissues, which are generally degraded over time, dental tissues are well preserved  
305 from degradation. In addition, mammal teeth are stored and preserved within jaws in museum  
306 collections for decades, and their easy access allow thus to provide long-term time series of data. As  
307 biological archives, teeth are thought to reflect the Hg exposure through diet at the time of  
308 formation since dental Hg matches blood Hg at the time of formation and mineralisation of the  
309 tooth. Therefore, Hg concentrations in teeth reflect an average exposure of the period of life during  
310 which the dental tissues have been mineralised.<sup>29</sup> Thus, it is worth noticing that the sampling year  
311 (in this case equalling death of the animal) does not represent the year of Hg exposure or  
312 accumulation for this animal, but terminates a lifetime period of exposure.

313 A decrease in Hg levels over time was found in the teeth of polar bears from Svalbard. The absence  
314 of temporal trends in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values over the four studied decades did not support the  
315 hypothesis of a temporal variation in feeding or foraging habits, but did mostly point towards a  
316 reduction in environmental Hg exposure. A temporal decrease in Hg concentrations has been  
317 observed previously in polar bear hair from East Greenland between 1973 and 2001. This was  
318 explained by reduced general environmental Hg levels attributed to a reduction in Hg emissions  
319 from European and North American sources.<sup>12</sup> In addition, our results are also in agreement with  
320 previous investigations on Hg content in human deciduous teeth from Norway, which described a  
321 decreasing time trend of the dental Hg levels from the 1970s to the 1990s likely reflecting a  
322 decrease in environmental Hg burden in Norway.<sup>60</sup>

323 The atmospheric transport is the main pathway for Hg to reach the Arctic region,<sup>5</sup> and Svalbard area  
324 seems to be under the influence of wind flows from Eastern North America, Europe and Russia.<sup>61</sup>  
325 Mercury emissions have been reported as decreasing substantially from the North American,  
326 European and Russian sources in the 1990s, due to a general diminution of industrial activities and  
327 consumption of raw materials.<sup>62-64</sup> Although the processes between Hg emissions from a source and  
328 its accumulation by organisms are long and complex, changes in dental Hg concentrations over time

329 in Svalbard polar bears are likely to be explained by a decrease in emissions of this metal from  
330 remote sources and subsequent transport and delivery by the winds to the Svalbard ecosystem.

331

### 332 **Conclusion**

333 Teeth can provide long term elemental and isotopic composition and important knowledge on  
334 lifetime dietary and contaminant exposure of the studied animals. However, biological factors like  
335 age are important to investigate, when using dental tissues to assess temporal trends of Hg. Indeed,  
336 age seems to have a great influence on Hg concentrations and in addition, it seems necessary to  
337 determine if the studied species develops its permanent dentition at the foetal or postnatal stage for  
338 a better understanding of the relation age-Hg concentrations. In our study, the combined use of the  
339 elemental and isotopic measurements allowed to eliminate feeding behavior as a factor of influence  
340 on Hg temporal patterns. Thus, the temporal trend observed in the teeth of polar bears from  
341 Svalbard over the recent four decades seemed to reflect the decrease in the deposition and  
342 subsequent bioaccumulation of Hg in Svalbard ecosystem, following the decrease in Hg emissions  
343 to the atmosphere from Europe and North America during the late 20<sup>th</sup> century.

344

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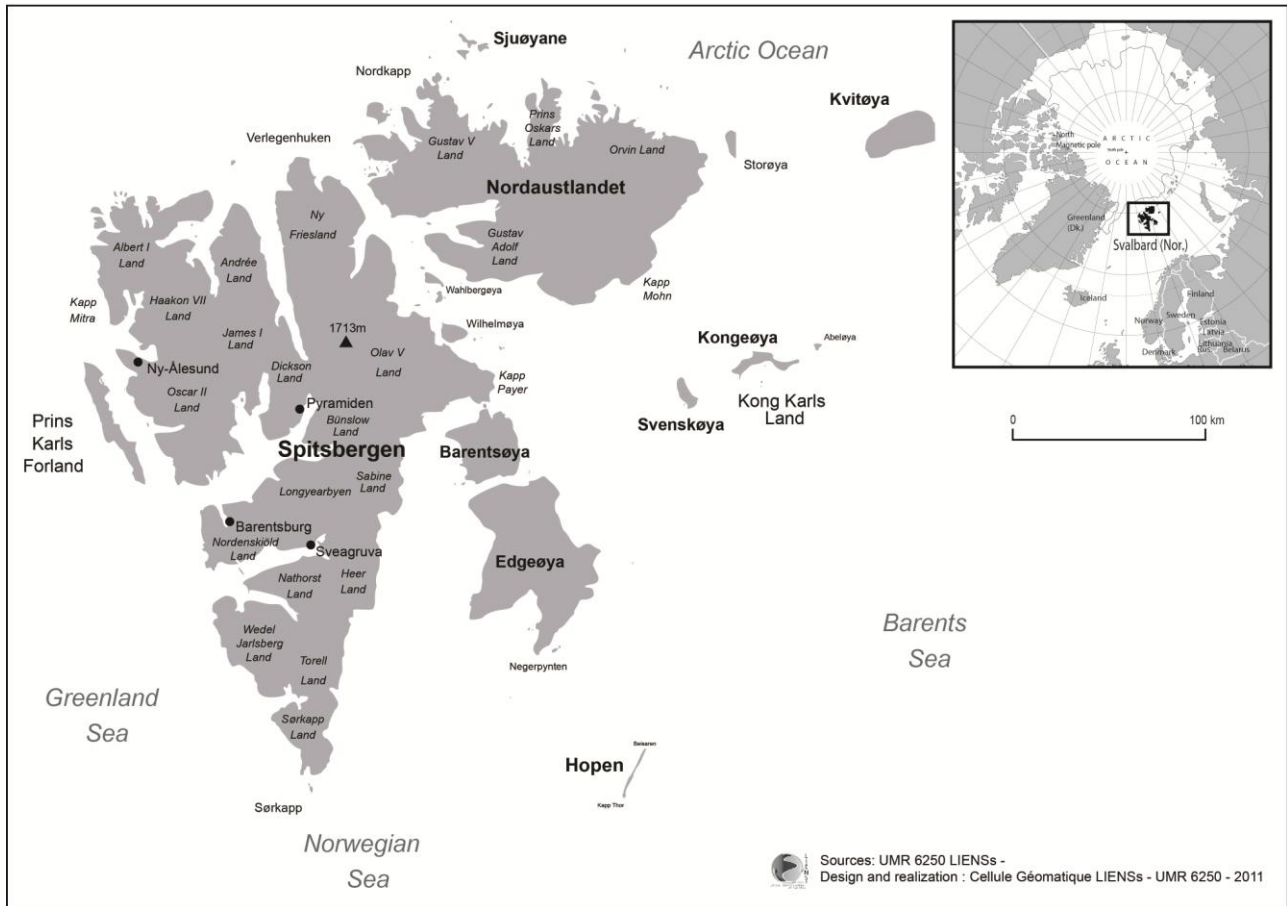
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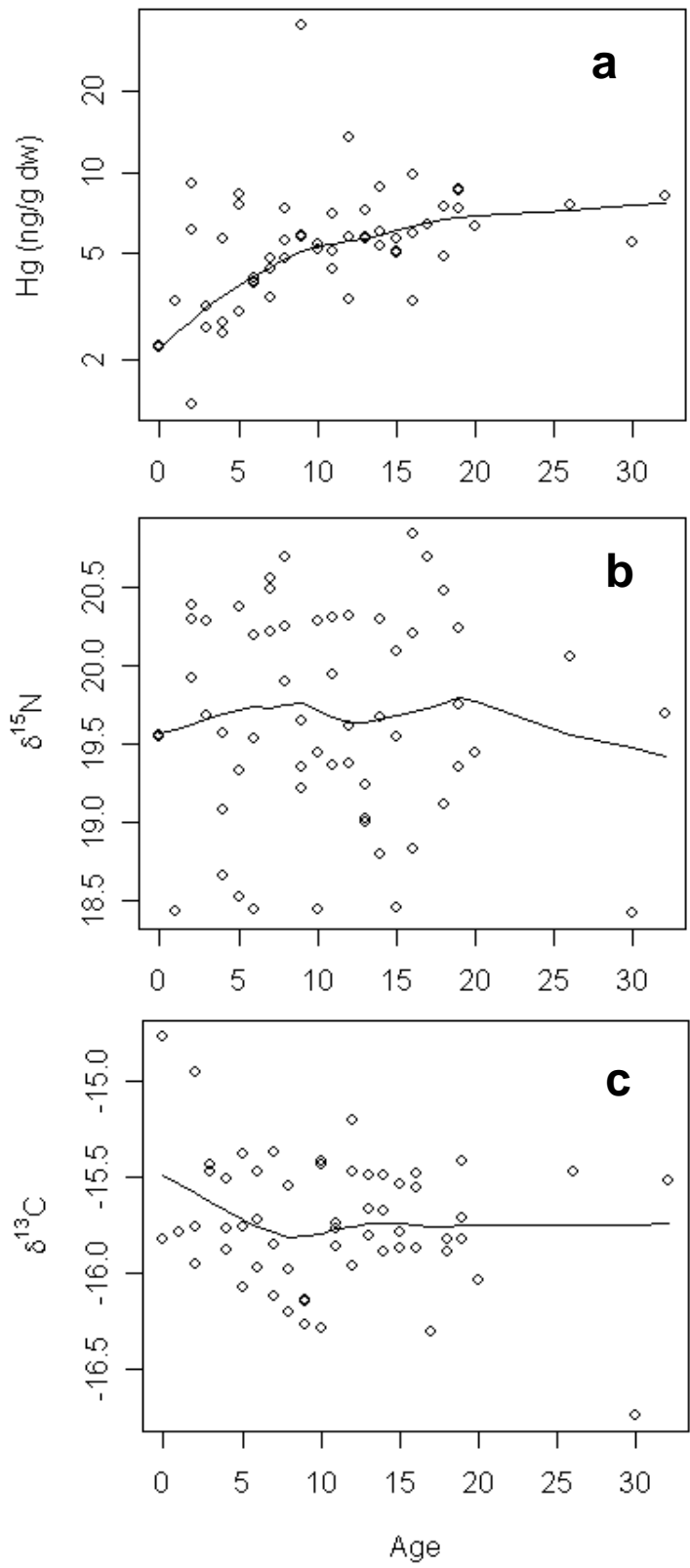
462 **Fig. 1** Map of Svalbard (Norway), where polar bear samples have been collected

463

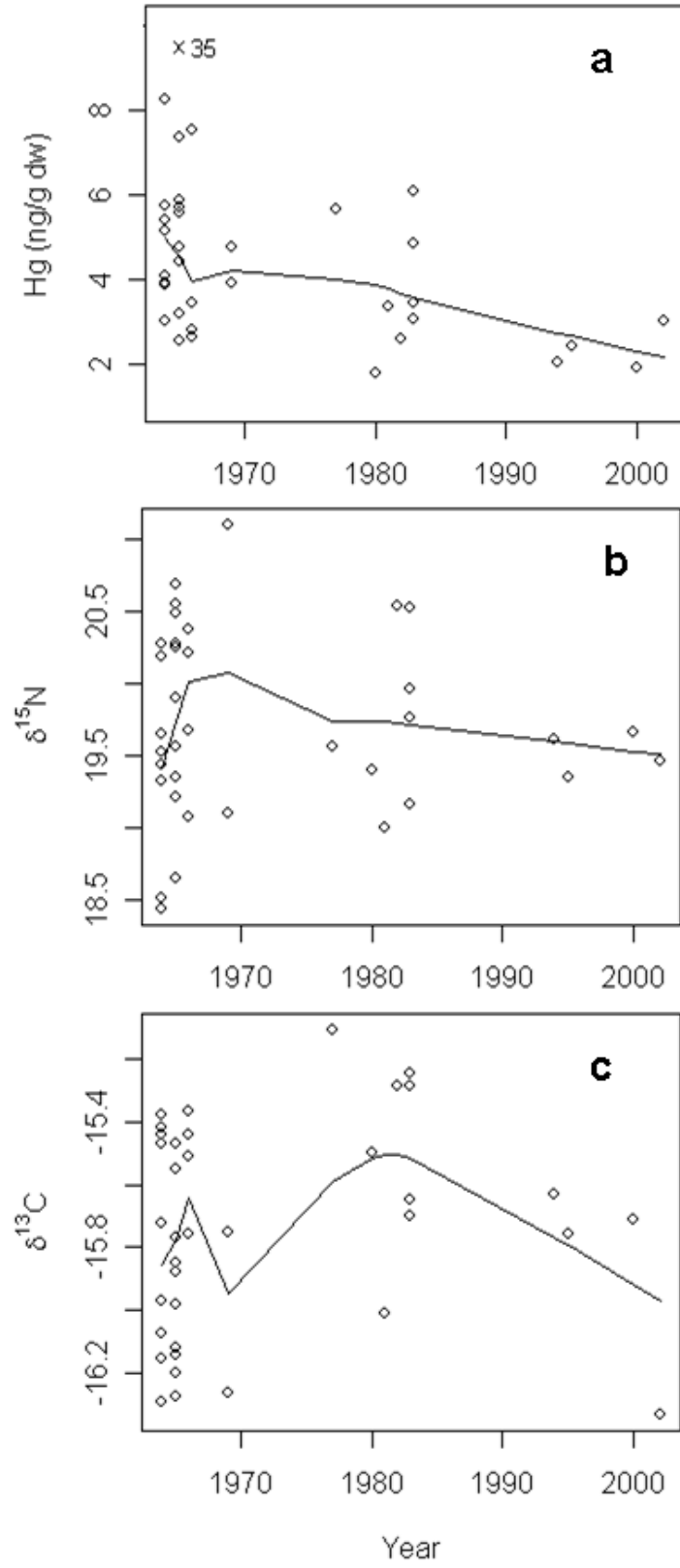


464

465 **Fig. 2** Age (years) vs Hg concentrations (in ng/g dw) (a), vs  $\delta^{15}\text{N}$  values (in ‰) (b) and vs  $\delta^{13}\text{C}$   
466 values (in ‰) (c) in teeth of polar bears from Svalbard, 1964-1966. The smoothing line (robust,  
467 locally weighted scatterplot smoothing system based on the LOWESS algorithm) represents the  
468 fitted non-linear trend of the values. Note that y axis is a logarithmic scale on Fig. 2a  
469  
470



471 **Fig. 3** Year vs dental Hg concentrations (ng/g dw) (a), vs  $\delta^{15}\text{N}$  values (b) and vs  $\delta^{13}\text{C}$  values (c) in  
472 polar bears from Svalbard, aged from 3 to 10 years old. Smoothing lines (robust, locally weighted  
473 scatterplot smoothing system based on the LOWESS algorithm) represent the fitted non-linear trend  
474 of the values  
475



476  
477

478 **Table 1** Sex, age and numbers of Svalbard polar bears for both sub-groups (individuals collected  
 479 from 1964 to 1966, and individuals aged from 3 to 10 years old) used to test the age effect and to  
 480 investigate the temporal trend, respectively, and for all individuals  
 481

Period	Male		Female		Unknown sex		All	
	n	Mean age $\pm$ sd	n	Mean age $\pm$ sd	n	Mean age $\pm$ sd	n	Mean age $\pm$ sd
1964-1966	32	11.0 $\pm$ 7.2	22	9.9 $\pm$ 5.0	4	12.5 $\pm$ 15.2	58	10.7 $\pm$ 7.1
3-10 years old	19	6.6 $\pm$ 2.0	13	6.2 $\pm$ 2.6	5	6.6 $\pm$ 1.9	37	6.5 $\pm$ 2.2
All	42	9.5 $\pm$ 7.3	32	8.4 $\pm$ 5.3	13	10.1 $\pm$ 10.0	87	9.2 $\pm$ 7.1

482  
 483  
 484 **Table 2** Analytical data for the customised reference material (units in ng/g dw), number of  
 485 analyses (n), Hg concentration mean value, its standard deviation (sd) and the relative sd  
 486

	<u>Hg concentration</u>			
	n	mean	sd	Relative sd %
AMA CCA	8	96.3	3.5	3.7
AMA NERI	10	105	6	5.7
FIMS NERI	5	106.3	3.6	3.4
Measured concentration *	23	102.3	6.4	6.3
Theoretical concentration **	1	100	4.1	4.1

\* The measured concentration is the average of the Hg concentrations determined by the three sequences of analyses.

\*\* The theoretical concentration is based on the DOLT-3 certified concentration in Hg.

487  
 488  
 489 **Table 3** Mercury concentrations (units in ng/g dw) and isotopic ratio of carbon and nitrogen (‰)  
 490 range, median and mean  $\pm$  standard deviation in teeth of polar bears from Svalbard (n = 87)  
 491

Measurements	Range	Median	Mean $\pm$ sd
Hg	0.6 - 72.3	4.9	6.0 $\pm$ 8.3
$\delta^{15}\text{N}$	17.7 - 21.8	19.6	19.6 $\pm$ 0.7
$\delta^{13}\text{C}$	-17.4 - (-)14.8	-15.8	-15.8 $\pm$ 0.4

492  
 493  
 494 **Table 4** Correlation matrix of Spearman's correlation\* coefficient between age, stable isotopes  
 495 values and Hg concentrations (using log-transformed Hg values)  
 496

Variable	Age	Log Hg
Log Hg	0.50 (p < 0.001)	-
$\delta^{15}\text{N}$	-0.02 (p = 0.90)	0.11 (p = 0.29)
$\delta^{13}\text{C}$	-0.11 (p = 0.42)	0.01 (p = 0.91)

\* Spearman's correlations were tested on all individuals excluding the outlier of 72.3 ng/g dw (n = 86). Moreover, when the correlation involved the age factor, only individuals from the 1964-1966 period were selected (n = 57).