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

Aurore Aubail [a, b, *], Rune Dietz [a, *], Frank Rigét [a], Christian Sonne [a], Øystein Wiig [c], Florence Caurant [b]

[a] *National Environmental Research Institute*, Aarhus University, Frederiksborgvej 399, P.O. Box 358, DK-4000 Roskilde, Denmark

[b] *Littoral, Environnement et Sociétés (LIENSs),* UMR 6250, CNRS-Université de La Rochelle, 2 rue Olympe de Gouges, F-17042 La Rochelle cedex, France

[c] *Natural History Museum*, University of Oslo, P.O. Box 1172 Blindern, NO-0318 Oslo, Norway

* Corresponding authors:
Tel.: +33 (0)546507639; Fax.: +33 (0)546458264/ Tel.: +45 21254035; Fax.: +45 46301914
E-mail address: aaubail@yahoo.fr/rdi@dmu.dk

Abstract

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

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

Introduction

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

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

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

Since diet is the main pathway for Hg to enter marine mammals, in addition to the general anthropogenic trends a change in feeding habits would also likely result in variations in Hg exposure, and consequently, in the body concentrations of this metal. Moreover, due to the magnification of this element, a change in Hg levels of an organism may only be seen if that organism feeds on different trophic levels than it used to. The naturally occurring carbon (^{12}C and ^{13}C) and nitrogen (^{14}N and ^{15}N) stable isotopes ratios have proved to be useful tools for characterizing the primary production in marine and terrestrial ecosystems and delineating the trophic position of organisms in food webs, respectively.[26] Like trace elements, carbon and nitrogen are incorporated in the dental tissue during its growth. Combined to the analysis of Hg, the measurement of the carbon and nitrogen stable isotopes in the teeth will provide valuable information relative to potential changes in feeding habits or habitats for polar bear. The stable isotopic technique is based on the metabolic discrimination between the heavy and the light isotopes. While $^{13}C/^{12}C$ values exhibit few variations through successive trophic levels in the food chains,[27] the $^{15}N/^{14}N$ value is significantly and regularly enriched through the food chains with a value found in a consumer's tissue directly related to the one of its prey, providing thus information on one's trophic level.[28] Usually, the isotopic measurements are carried out on muscle tissues which give access to the feeding ecology from the last weeks or months, whereas performed on teeth, the isotopic data obtained will represent an average dietary estimate over the animal's lifetime or near to its lifetime,[29] erasing any potential shift of diet related to seasonal and physiological stages.

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

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

Materials and methods

Sampling procedure and preparation

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

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

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

Analytical procedures and instrumentation for Hg analysis

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

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

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

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

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

Analytical procedures and instrumentation for stable isotope measurements

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

Relative abundance of stable isotopes of nitrogen ($^{15}N/^{14}N$) and carbon ($^{13}C/^{12}C$) were determined with an Elemental Analyser connected on-line to an Isotope-Ratio Mass Spectrometer (Isoprime, Micromass, UK). Stable isotope results are expressed in delta notation (δ), defined as the part per thousand (‰) deviation from a standard material:

$$\delta\,^{15}N \text{ or } \delta\,^{13}C = [(Rsample / Rstandard) - 1] \times 10^3$$

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

Data treatment

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

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

The significance level was set to p = 0.05 and the statistical analyses were performed using the free software R, version 2.1.1.[42]

Results

Mercury concentrations in relation to age and sex

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

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

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

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

Time trends

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

Discussion

Levels of Hg in the teeth

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

Biological factors

Although diet may vary substantially between genders, no difference in Hg concentrations was found between males and females, which fits the lack of difference between males and females previously reported in soft tissues of polar bears.[7-9] Contrarily, dental Hg concentrations were correlated with age (Table 4). Polar bears generally exhibit a cumulative pattern of this metal especially in their liver.[7-9,11] However, age effects on dental Hg concentrations in Arctic marine mammals are not consistent in the literature. Outridge *et al.*[22] showed that Hg concentrations in teeth of beluga whales increased with age, while Kinghorn *et al.*[53] did not find a significant age effect in the same species. Outridge *et al.*[24] observed a positive correlation between age and Hg concentrations in teeth of ringed seals, while Aubail *et al.*[25] did find a significant decrease of the dental Hg concentrations the first years of life for Greenland ringed seals; this trend was explained by early maternal transfer of Hg to pre- and postnatal individuals. Indeed, since ringed seals acquire their permanent dentition at the foetal stage,[54] the prenatal transfer of Hg may represent an important source of Hg for the dental tissues mineralised under the foetal stage, compared to the postnatal Hg incorporated from diet. In this study, a general increase of Hg concentrations in the teeth of polar bears was observed for the first 10 years of life and was followed by a more constant plateau phase (Fig. 2a). Thus, this trend could be explained by the fact that polar bears, contrarily to ringed seals, would acquire their permanent dentition at the postnatal stage. This results in the progressive accumulation of Hg throughout years with a low intake of Hg from the maternal milk, to greater Hg concentrations with an increasing efficiency of the hunting strategy and thus, amount of prey and Hg ingested. The stabilized levels of Hg observed afterwards may likely be related to the late dentinal occlusion of the tooth. Indeed, the deposition of dentine reduces the pulp area and in some species, the pulp cavity has been shown to occlude at some point of their life with the subsequent suspension of the deposition of dentine[55] and likely also elements such as Hg from blood vessels.

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

Since integration time and process of both isotopic and metallic elements are considered as relatively similar, $\delta^{15}N$ or $\delta^{13}C$ values have been used to relate Hg concentrations to trophic position or foraging location, respectively. However, Hg concentrations were not correlated with $\delta^{15}N$ or $\delta^{13}C$ values in this study and no age effect was found for $\delta^{15}N$ values, which is different from other previous investigations in which high $\delta^{15}N$ values were measured in muscle of different marine mammals in the first years of life, relative to dietary inputs from mother's milk during the period of suckling.[57-59] In those studies, the decrease in nitrogen isotope values reflects ontogenetic changes and is likely to be associated with the period from primarily feeding on milk towards primarily feeding on live prey. Likewise, no age effects were found for $\delta^{13}C$ values in our study, whereas cubs of the year were expected to present low $\delta^{13}C$ values, in response to depleted ^{13}C directly derived from maternal milk lipids.

Time trend

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

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

The atmospheric transport is the main pathway for Hg to reach the Arctic region,[5] and Svalbard area seems to be under the influence of wind flows from Eastern North America, Europe and Russia.[61] Mercury emissions have been reported as decreasing substantially from the North American, European and Russian sources in the 1990s, due to a general diminution of industrial activities and consumption of raw materials.[62-64] Although the processes between Hg emissions from a source and its accumulation by organisms are long and complex, changes in dental Hg concentrations over time in Svalbard polar bears are likely to be explained by a decrease in emissions of this metal from remote sources and subsequent transport and delivery by the winds to the Svalbard ecosystem.

Conclusion

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

Acknowledgements

We greatly acknowledge Carine Churlaud from the Centre Commun d'Analyses (La Rochelle, France), Gert Asmund, Sigga Joensen and Lene Bruun from the NERI for their valuable input on the Hg analysis, and Pierre Richard, Benoît Lebreton and Gaël Guillou from LIENSs (UMR 6250 CNRS-La Rochelle University, France) for their advice and contribution on the stable isotope analysis. We also would like to thank Thea Bechshøft from NERI for her help regarding the samples. We are grateful to Peter Outridge, Canadian Geological survey and Michael Goodsite, National Environmental Research Institute (NERI, Roskilde, Denmark) for advice and discussion underway. This study was partly financed by The Dancea Programme, KVUG (Kommissionen for Videnskabelige Undersøgelser i Grønland) and the Prince Albert II Foundation. Finally, the Poitou-Charentes region and Fund M.L. Furnestin-Faure did support financially the PhD research.

References

1 S. E. Lindberg, S. Brooks, C. J. Lin, K Scott, T. Meyers, L. Chambers, M. R. Landis and R. Stevens, *Water Air Soil Poll*., 2001, 295-302.

2 P. A. Ariya, A. P. Dastoor, M. Amyot, W. H. Schroeder, L. Barrie, K. Anlauf, F. Raofie, A. Ryzhkov, D. Davignon, J. Lalonde and A. Steffen, *Tellus Series B-Chemical and Physical Meteorology*, 2004, **56**, 397-403.

3 G. Stern, L. Loseto, R. Macdonald, F. Wang, C. Zdanowicz, P. Outridge, A. Cole, J. Chételat, H. Hintelmann and A. Steffen, in *AMAP Assessment 2011: Mercury in the Arctic*. Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway, 2011, pp. 67-83.

4 L. Atwell, K. A. Hobson and H. E. Welch, *Can. J. Fish and Aquat. Sci*., 1998, **55**, 1114-1121.

5 AMAP, in *AMAP Assessment 2002: Heavy metals in the Arctic*, Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway, 2005.

6 E. W. Born, A. Renzoni and R. Dietz, *Polar Res*., 1991, **9**, 113-130.

7 B. M. Braune, R. J. Norstrom, M. P. Wong, B. T. Collins and J. Lee, *Sci. Total Environ*., 1991, **100**, 283-299.

8 R. Dietz, E. W. Born, C. T. Agger and C. O. Nielsen, *Polar Biol*., 1995, **15**, 175-185.

9 R. Dietz, F. Riget and E. W. Born, *Sci. Total Environ*., 2000, **245**, 25-47.

10 G. Norheim, J. U. Skaare and O. Wiig, *Environ. Pollut*., 1992, **77**, 51-57.

11 S. A. Rush, K. Borga, R. Dietz, E. W. Born, C. Sonne, T. Evans, D. C. G. Muir, R. J. Letcher, R. J. Norstrom and A. T. Fisk, *Environ. Pollut*., 2008, **153**, 618-626.

12 R. Dietz, F. Riget, E. W. Born, C. Sonne, P. Grandjean, M. Kirkegaard, M. T. Olsen, G. Asmund, A. Renzoni, H. Baagoe and C. Andreasen, *Environ. Sci. Technol*., 2006, **40**, 1120-1125.

13 R. Dietz, E. W. Born, F. Rigét, A. Aubail, C. Sonne, R. Drimmie and N. Basu, *Environ. Sci. Technol*., 2011, **45**, 1458-1465.

14 R. D. P. Eaton and J. P. Farant, *Arctic*, 1982, **35**, 422-425.

15 T. W. Horton, J. D. Blum, Z. Xie, M. Hren and C. P. Chamberlain, *Polar Res*., 2009, **28**, 443-454.

16 T. Cardona-Marek, K. K. Knott, B. E. Meyer and T. M. O'Hara, *Environ. Toxicol. Chem*., 2009, **28**, 1416-1424.

17 WHO, *IPCS/International Programme on Chemical Safety*, WHO (World Health Organization), Environmental Health Criteria 101, Geneva, 1990.

18 N. Miyazaki, in *Encyclopedia of marine mammals*, ed. W. F. Perrin, B. Würsig and J. G. M. Thewissen, Academic Press, 2002, pp. 1227-1232.

19 R. Eide, G. B. Wesenberg and G. Fosse, *Scand. J. Dent. Res*., 1993, **101**, 1-4.

20 H. M. Tvinnereim, R. Eide and T. Riise, *Sci. Total Environ*., 2000, **255**, 21-27.

21 R. Eide and G. R. Wesenberg, *Environ. Res.*, 1993, **61**, 212-222.

22 P. M. Outridge, R. Wagemann and R. McNeely, *Environ. Toxicol. Chem.*, 2000, **19**, 1517-1522.

23 P. M. Outridge, K. A. Hobson, R. McNeely and A. Dyke, *Arctic*, 2002, **55**, 123-132.

24 P. M. Outridge, K. A. Hobson and J. Savelle, *Sci. Total Environ.*, 2009, **407**, 6044-6051.

25 A. Aubail, R. Dietz, F. Rigét, B. Simon-Bouhet and F. Caurant, *Sci. Total Environ.*, 2010, **408**, 5137-5146.

26 B. J. Peterson and B. Fry, *Annu. Rev. Ecol. Syst.*, 1987, **18**, 293-320.

27 B. S. Chisholm, D. E. Nelson and H. P. Schwarcz, *Science*, 1982, **216**, 1131-1132.

28 M. Minagawa and E. Wada, *Geochim. Cosmochim. Ac.*, 1984, **48**, 1135-1140.

29 P. M. Outridge, in *Mercury: Sources, Measurements, Cycles and Effects*, Mineralogical Association of Canada, Ottawa, 2005, ch. 11, pp. 217-234.

30 I. Stirling, in *Encyclopedia of marine mammals*, ed. W. F. Perrin, B. Würsig and J. G. M. Thewissen, Academic Press, 2002, pp. 945-948.

31 S. J. Iverson, S. Stirling and S. L. Lang, in *Top Predators in Marine Ecosystems*, ed. I. L. Boyd, S. Wanless and C. J. Camphuysen, Cambridge University Press, 2006, pp. 98-117.

32 M. A. McKinney, R. J. Letcher, J. Aars, E. W. Born, M. Branigan, R. Dietz, T. J. Evans, G. W. Gabrielsen, D. C. G. Muir, E. Peacock and C. Sonne, *Environ. Sci. Technol.*, 2011, **45** (3), 896-902.

33 A. E. Derocher, O. Wiig and M. Andersen, *Polar Biol.*, 2002, **25**, 448-452.

34 W. Calvert and I. Stirling, *Bears - Their Biology and Management*, 1990, **8**, 351-356.

35 L. F. Lowry, J. J. Burns and R. R. Nelson, *Can. Field-Nat.*, 1987, **101**, 141-146.

36 A. E. Derocher, O. Wiig and G. Bangjord, *Polar Biol.*, 2000, **23**, 675-678.

37 K. L. Laidre, I. Stirling, L. F. Lowry, O. Wiig, M. P. Heide-Jorgensen and S. H. Ferguson, *Ecol. Appl*, 2008, **18**, 97-125.

38 K. M. Kovacs, C. Lydersen, J. E. Overland and S. E. Moore, *Mar. Biodiv.*, 2011, **41**(1), 181-194.

39 R. Dietz, M. P. Heide-Jørgensen, T. Harkonen, J. Teilmann and N. Valentin, *Sarsia*, 1991, **76**, 17-21.

40 G. E. M. Hall and P. Pelchat, *Analyst*, 1997, **122** (9), 921-924.

41 J. H. Zar, in *Biostatistical analysis: International edition*. Pearson Education, 2009.

42 R Development Core Team, in *R: A language and environment for statistical computing. Foundation for Statistical Computing*, Vienna, Austria, 2008, ISBN 3-900051-07-0, URL http://www.R-project.org.

43 M. A. Ramsay and I. Stirling, J Zool, Lond, 1988, **214**, 601-634.

44 A. Aguilar, A. Borrell and T. Pastor, *J. Cetacean Res. Manag. Special Issue*, 1999, **1**, 83-116.

45 T. J. Brookens, J. T. Harvey and T. M. O’Hara, *Sci. Total Environ.*, 2007, **372**, 676–692.

46 T. J. Brookens, T. M. O’Hara, R. J. Taylor, G. R. Bratton and J. T. Harvey, *Mar. Poll. Bull.*, 2008, **56**, 27-41.

47 R. Dietz, N. Basu, B. Braune, T. O’Hara, T. Scheuhammer and C. Sonne, in *AMAP Assessment 2011: Mercury in the Arctic*. Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway, 2011, pp.113-137.

48 R. Dietz, P. Paludan-Müller, C. T. Agger and C. O. Nielsen, *NAMMCO Scientific contributions*, 1998, **1**, 242-273.

49 R. J. Norstrom, R. E. Sweinsberg and B. T. Collins, *Sci. Total Environ.*, 1986, **48**, 195-212.

50 F. Riget, D. Muir, M. Kwan, T. Savinova, M. Nyman, V. Woshner and T. O'Hara, *Sci. Total Environ.*, 2005, **351**, 312-322.

51 I. Stirling and E. H. McEwan, *Can. J. Zool.*, 1975, **53**, 1021-1027.

52 P. Johansen, D. Muir, G. Asmund and F. Riget, *Sci. Total Environ.*, 2004, **331**, 189-206.

53 A. Kinghorn, M. M. Humphries, P. Outridge and H. M. Chan, *Sci. Total Environ.*, 2008, **402**, 43-50.

54 B. E. Stewart, S. Innes and R. E. A. Stewart, *Mar. Mamm. Sci.*, 1998, **14**, 221-231.

55 A. A. Hohn, in *Encyclopedia of marine mammals*, ed. W. F. Perrin, B. Würsig and J. G. M. Thewissen, Academic Press, 2002, pp. 6-13.

56 D. E. Kenny and C. Bickel, *International Zoo Yearbook*, 2005, **39**, 205-214.

57 R. Dietz, F. Riget, K. A. Hobson, M. P. Heide-Jorgensen, P. Moller, M. Cleeman, J. de Boer and M. Glasius, *Sci. Total Environ.*, 2004, **331**, 83-105.

58 K. A. Hobson and J. L. Sease, *Mar. Mamm. Sci.*, 1998, **14**, 116-129.

59 S. C. Polischuk, K. A. Hobson and M. A. Ramsay, *Can. J. Zool.*, 2001, **79**, 499-511.

60 H. M. Tvinnereim, R. Eide and G. R. Fosse, International Conference on Human Health Effects of Mercury Exposure, Faroe Islands, 1997.

61 R. W. Macdonald, T. Harner, J. Fyfe, H. Loeng and T. Weingartner, in *AMAP, Assessment 2002: The Influence of Global Change on Contaminant Pathways to, within, and from the Arctic*. Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway, 2003.

62 AMAP/UNEP, in *Technical Background Report to the Global Atmospheric Mercury Assessment*, Arctic Monitoring and Assessment Programme/UNEP Chemicals Branch, 2008 (http://www.chem.unep.ch/mercury/Atmospheric_Emissions/Technical_background_report.pdf).

63 J. Munthe, M. Goodsite, T. Berg, J. Chételat, A. Cole, A. Dastoor, T. Douglas, D. Durnford, R. Macdonald, D. Muir, P. Outridge, J. Pacyna, A. Ryzhkov, H. Skov, A. Steffen, K. Sundseth, O. Travnikov, I. Wängberg and S. Wilson, in *AMAP Assessment 2011: Mercury in the Arctic*. Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway, 2011, pp. 9-44.

64 E. G. Pacyna and J. M. Pacyna, *Water Air Soil Pollut.*, 2002, **137**, 149-165.

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

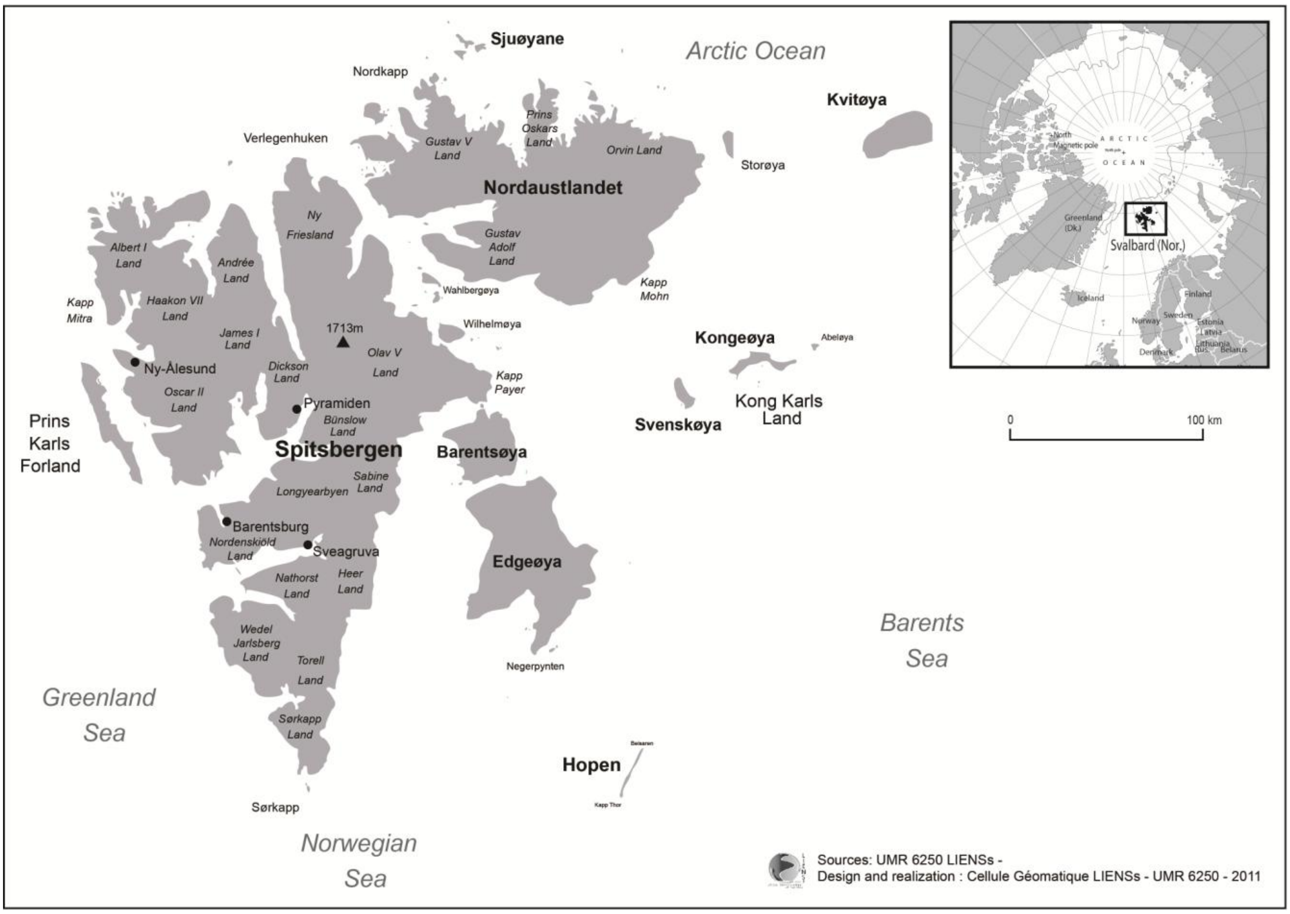

Fig. 2 Age (years) *vs* Hg concentrations (in ng/g dw) (a), *vs* $\delta^{15}N$ values (in ‰) (b) and *vs* $\delta^{13}C$ values (in ‰) (c) in teeth of polar bears from Svalbard, 1964-1966. The smoothing line (robust, locally weighted scatterplot smoothing system based on the LOWESS algorithm) represents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

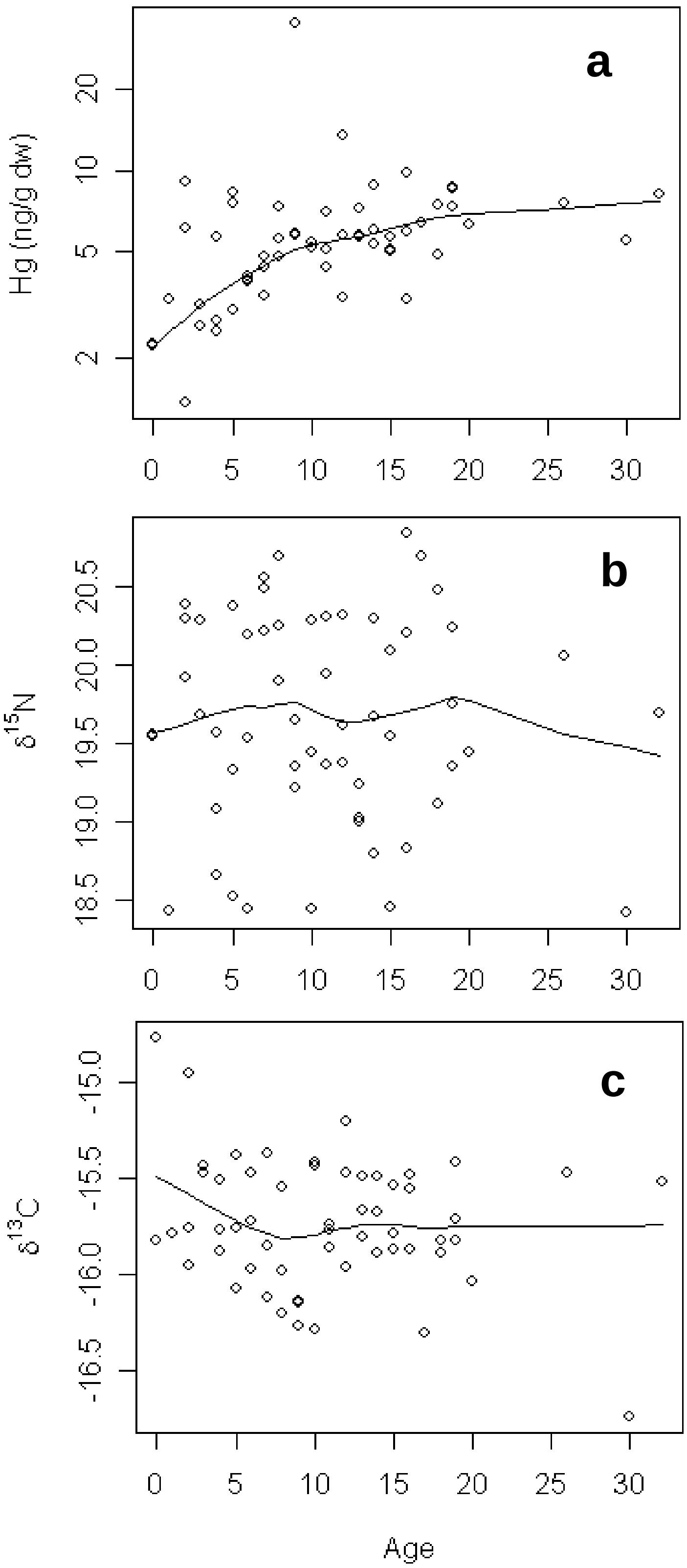

Fig. 3 Year *vs* dental Hg concentrations (ng/g dw) (a), *vs* $\delta^{15}N$ values (b) and *vs* $\delta^{13}C$ values (c) in polar bears from Svalbard, aged from 3 to 10 years old. Smoothing lines (robust, locally weighted scatterplot smoothing system based on the LOWESS algorithm) represent the fitted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

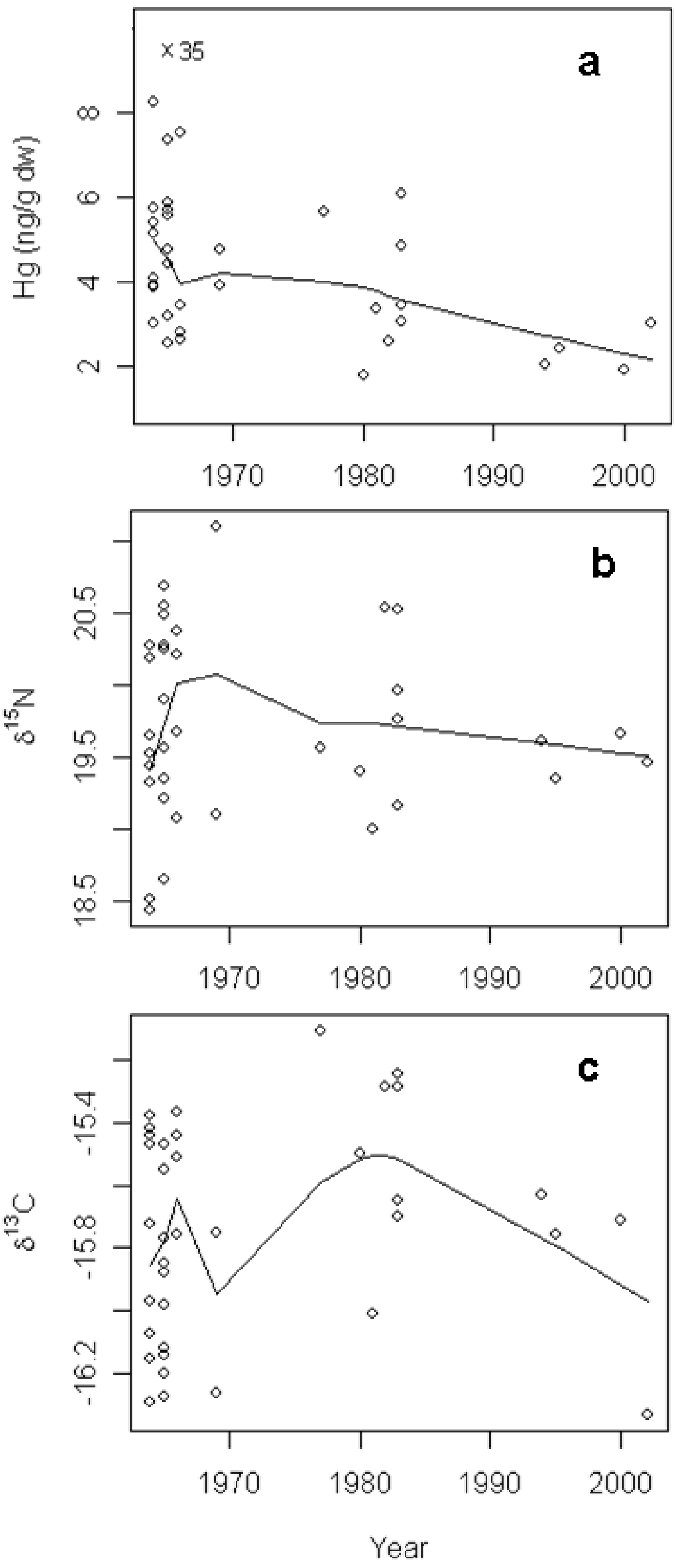

Table 1 Sex, age and numbers of Svalbard polar bears for both sub-groups (individuals collected from 1964 to 1966, and individuals aged from 3 to 10 years old) used to test the age effect and to investigate the temporal trend, respectively, and for all individuals

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

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

		Hg concentration		
	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.

Table 3 Mercury concentrations (units in ng/g dw) and isotopic ratio of carbon and nitrogen (‰) range, median and mean ± standard deviation in teeth of polar bears from Svalbard (n = 87)

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

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

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