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Abstract
Total mercury (Hg) concentrations were measured in teeth of ringed seals from Qeqertarsuaq, central West Greenland (1982 to 2006) and Ittoqqortoormiit, central East Greenland (1986 to 2006). Stable isotopic ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) were determined as well to provide insights into diet variations between regions or through time. Mercury concentrations decreased the first years of life of the animals suggesting that Hg had been transferred from the mother to the foetus and newborn. The Hg concentrations in teeth were significantly lesser in ringed seals from central West Greenland compared to those from central East Greenland. Carbon and nitrogen stable isotopic values measured in the animals differed also significantly between the two regions. Increasing temporal trends of dental Hg concentrations between 1994 and 2006 were observed in ringed seals from both central West Greenland and central East Greenland. These increases were attributed to global changes in environmental Hg levels since no temporal trends in $\delta^{15}N$ values were found to support the hypothesis of a diet shift over time. Furthermore, a decreasing temporal trend in $\delta^{13}C$ values was observed in the teeth of seals from central East Greenland, and explained by a likely change over time towards more pelagic feeding habits; alternatively, the so-known Seuss effect was thought to be responsible for this decrease. Finally it was concluded that the tooth of ringed seal was a good monitoring tissue to assess Hg trends.

Key words: mercury, stable isotopes, carbon, nitrogen, ringed seal, *Phoca hispida,* teeth, spatial and temporal trends

1. Introduction

Mercury (Hg) is a global contaminant which is emitted to the atmosphere from both natural and anthropogenic sources all around the world (Pacyna and Pacyna, 2002). The important volatility of the elementary form of Hg allows this metal to be long range transported (Brooks et al., 2005), from its sources of emission to the most remote areas such as the Arctic regions (Pacyna and Pacyna, 2002). The recently discovered phenomenon Atmospheric Mercury Depletion Event demonstrates that the cycle of Hg in the Arctic is complex, and involves processes at the polar sunrise that lead to the production of bio-available forms of Hg (Brooks et al., 2005).

Mercury is known to accumulate in organisms and to biomagnify up the food chains (Caurant et al., 1996; Dietz et al., 1996; Rigét et al., 2007). Due to its high position in the marine Arctic food chain and its long life span, the ringed seal (*Phoca hispida*) accumulates particularly great concentrations of Hg in its tissues (Dietz et al., 1996, 1998), though with a large variability resulting from biological and ecological influences (Aguilar et al., 1999). This species has a circumpolar distribution and is thought not to be migratory (Maxwell, 1967), although some subadults have been reported to disperse over considerable distances (> 1000 km), as documented by Reeves et al. (1992) and Kapel et al. (1998). Because of this supposed high degree of site fidelity and its ubiquitous occurrence, the ringed seal is a good candidate for monitoring contaminants for both spatial and temporal trends in Arctic marine food chains, and has therefore been selected by the international AMAP, Arctic Monitoring and Assessment Programme, as an essential monitoring species (AMAP, 2003).
Within the framework of the AMAP and other programmes, samples of ringed seals such as liver, kidney, muscle, and jaws have been collected from the local Inuit subsistence hunting in different regions of Greenland through the last twenty-five years. The liver is the main organ

for Hg accumulation in marine mammals including pinnipeds (Wagemann and Muir, 1984), and most studies dealing with Hg in seals have been carried out on liver among other soft tissues (Dietz et al., 1998; Rigét et al., 2005; Wagemann, 1989).

The tooth is a hard tissue that presents the advantage, compared to soft tissues, to be long term preserved in ancient material, and to provide a good chemical record of life-history events of the individual. Indeed, Hg is incorporated with other trace elements into the crystalline apatite structure of teeth during their mineralisation (Tvinnereim et al., 2000). As the teeth are relatively stable tissues, the incorporated elements are thought not to be remobilised, or very little, throughout the life of individuals (Eide et al., 1993; Haller et al., 1993; Tvinnereim et al., 2000). Thus, because teeth appear to be excellent archives, they have been investigated for their content of trace elements in rats (Eide and Wesenberg, 1993), marine mammals (Caurant et al., 2006; Outridge et al., 2000, 2002, 2009), and humans (Eide et al., 1993; Tvinnereim et al., 2000), and are considered to be reliable indicators of Hg exposure (Eide and Wesenberg, 1993; Outridge et al., 2000; Tvinnereim et al., 2000).

Teeth have been used also to measure ratios of the naturally occurring stable isotopes of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$), which have demonstrated to provide insights into mammals feeding ecology (Hobson et al., 1997; Hobson and Welch, 1992). The stable isotopic technique is based on the metabolic discrimination between the heavy and the light isotopes. Consumers are enriched in ^{15}N relative to their food and consequently $\delta^{15}N$ measurements serve as indicators of a consumer trophic position (Tieszen et al., 1983). By contrast, $\delta^{13}C$ values vary little along the food chain and are mainly used to determine primary sources in a trophic network (DeNiro and Epstein, 1978). In the marine environment, $\delta^{13}C$ values can also indicate inshore versus offshore, or pelagic versus benthic, contribution to food intake (Hobson et al. 1994). The recent combined use of elemental and isotopic measurements appears to be a useful tool for the study of marine mammal ecology (Das et al.,

2003; Hobson et al., 2004) providing information on diet, geography, and trophic transfer of contaminants. Positive correlations between δ^{15}N values and Hg concentrations have been described within Arctic marine food webs (Atwell et al., 1998; Rigét et al., 2007), suggesting that part of the Hg variation may be linked to a biomagnification process, and several recent studies have been combining Hg and stable isotopes measurements to assess feeding variations for marine mammals (Born et al., 2003; Dietz et al., 2004; Hobson et al., 2004).

In this study, total Hg concentrations and δ^{13}C and δ^{15}N values have been determined in teeth of ringed seals from central West and central East Greenland. The aims of this work were: (i) to investigate the influence of biological factors such as gender or age, (ii) to assess potential temporal and spatial trends of Hg with support provided by the stable isotopes, and (iii) to evaluate tooth as a Hg monitoring matrix for this species.

2. Materials and methods

2.1. Sampling procedure and preparation

Tissue samples were obtained from 285 ringed seals collected from the traditional Inuit hunt in Qeqertarsuaq, Disko Bay, located at 69° 50′ N, 54° 00′ W (central West Greenland, CWG; n = 115), and in Ittoqqortoormiit, Scoresbysund, located at 70° 50′ N, 22° 00′ W (central East Greenland, CEG; n = 170). Sampling took place in May and June, in eight years within the last 25 years (1982 and 1986 for CWG and CEG respectively and 1994, 1999, 2000, 2001, 2002, 2004 and 2006 in common to both regions) (Fig. 1, Table 1). Because of a greater hunting pressure in the Disko Bay area, the sampling was biased, and mainly juvenile seals were obtained from this area.

Prior to tissue sampling, the animals were measured, and other information such as the date of collection, location, and gender, were recorded. Soft tissues (liver, kidney, muscle) were sampled, and the results of Hg analyses carried out have been presented partly by Dietz et al. (1998) and Rigét et al. (2004, 2007). In addition, the lower jaws were sampled, and later on went through a maceration process at the Zoological Museum in Copenhagen, Denmark, in order to be totally cleaned from flesh. Then, the bone tissues (mandibles) and the teeth were dried and stored in plastic bags or pap boxes until use.

The age of the animals was determinate at the National Environmental Research Institute (NERI, Roskilde, Denmark), by counting annual layers in the cementum of the canine teeth, after decalcification, thin sectioning (14 μm), and staining in toluidine blue as described by Dietz et al. (1991).

The second molar (from the front part) was taken from the lower right mandible for the Hg analytical purpose (n = 285), while the premolar of the same mandible was extracted for the stable isotopes analyses (n = 238).

Prior to analysis, each tooth was immersed in 10% nitric acid for 20s, and rinsed in several ultra-pure Milli-Q water baths. Teeth samples were dried for a minimum of 24h at room temperature, and subsequently stored in cleaned plastic flasks.

2.2. Analytical procedures and instrumentation for Hg analyses

Entire dry molar teeth were analysed for total Hg (Hg). The Hg measurements were performed on the whole molar teeth, at the laboratory of the NERI, using a solid sample atomic absorption spectrometer AMA-254 (Advanced Mercury Analyser-254 from LECO, Sweden). The use of this apparatus does not require a chemical pre-treatment which reduces considerably contamination risks or loss of Hg. The analytical process consists in a drying period at 120°C, prior to a combustion phase at 750°C 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 Hg is measured by UV absorption. The operating times used for this study, i.e. drying time, decomposition time, and waiting time, were respectively 50, 200, and 45s. The instrument is described in detail elsewhere (Hall and Pelchat, 1997).

As there is no commercial reference material with a matrix similar to teeth or bones and certified for Hg, a reference material was made from two commercial Standard Reference Materials (SRMs), the NIST 1400 Bone ash (National Institute of Standards and Technology, USA), and the DOLT-3 (Dogfish liver from the National Research Council of Canada). The Bone Ash SRM does not contain any Hg because it has been calcinated at great temperatures, but it brings the similar matrix to calcified tissues, while the DOLT-3 brings the organic matrix and the certified concentration of Hg. The adding of 0.96 g of DOLT-3 (Certified Hg concentration of 3.37 ± 0.14 in $\mu g.g^{-1}$ dry weight) to 31.39 g of Bone Ash leads theoretically to a Hg concentration of 0.100 ± 0.004 $\mu g.g^{-1}$ dry weight.

The calibration of the customised reference material has been carried out through two different techniques (AMA-254 and cold-vapour Atomic Absorption Spectrophotometry on a Perkin Elmer FIMS 100) in NERI. Moreover, an inter laboratory comparison was carried out for AMA-254 analysis between NERI and CCA (Centre Commun d'Analyses, University of La Rochelle, France). The measurement results of the customised reference material show a good accuracy (i.e. ratio between the recovery measured concentration and the theoretical concentration) of 102%, 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 mentioned above at the beginning and at the end of each

analytical cycle of 10 samples. The Hg measurements of this reference material were in good agreement with the assigned concentration (accuracy of 103.6% and relative standard deviation of 5.6% for n = 80).

The laboratory at NERI participates in the international inter-laboratory comparison exercises conducted by the EEC (QUASIMEME), and the latest 2007 results concerning analyses by AMA-254 were satisfactory (0 < z < 0.5).

All data are presented on a dry weight basis (dw), and the detection limit is 0.01 ng.g^{-1} dw.

2.3. Analytical procedures and instrumentation for stable isotope measurements

Each entire premolar was crushed into small pieces before being ground into homogenous powder using a ball mill (Retsch MM2000) for 2 minutes and the amplitude of 90. Then, powder was stored in small glass flasks. 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. The digested contents were taken up in milli-Q ultrapur quality water, and homogenised before freezing to -20°C. Then, the samples were frozen at -80°C for a short time before freeze-drying. Finally, an aliquot of each homogenised dried sample was taken, weighted, and loaded into tin capsules. Out of 285 individuals, only 238 samples were available for stable isotope analysis.

Relative abundance of stable isotopes of carbon (^{13}C and ^{12}C) and nitrogen (^{15}N and ^{14}N) were determined with an elemental analyser connected on-line to an isotope-ratio mass spectrometer (Micromass, Manchester, UK). Stable isotope results are expressed in delta notation (δ), defined as the part per thousand (‰) deviation from a standard material:

$\delta^{13}C$ or $\delta^{15}N = [(Rsample / Rstandard) - 1] \times 10^3$, where R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$

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

2.4. Data treatment

Prior to the statistical analyses, the Hg data (n = 285) were logarithmic-transformed (base e) to reduce skewness, and fit parametric requirements. Shapiro-Wilk and Bartlett test were applied to test the assumptions of parametric tests, such as analysis of variance (ANOVA) and linear regression analysis. In few cases, the assumptions were not fulfilled because of a couple of high Hg concentration values. However, ANOVA tests are robust to small deviations of the data from the normal distribution (Zar, 2009).

Standard parametric tests such as Pearson's correlation analysis, linear regression analysis, and ANOVA, were applied to test for the influence of factors (i.e. age, gender, location, and year) on the Log-transformed Hg concentrations or on the stable isotopic values. If the first runs of ANOVA test showed no significance of the interaction between two factors, the interaction factor was removed, and the test re-run.

The relationship between age and dental Hg concentrations or stable isotopic values were evaluated for each region with a scatterplot using LOWESS (Locally weighted polynomial regression) smoothing. In the same way, the analyses of temporal trends of Hg and stable isotopic values first were evaluated with scatterplots using LOWESS smoothing separately for each region, thus Log-linear regression analysis was applied to describe the linear component, and tested by means of an ANOVA.

The significance level was set to p = 0.05.

The statistical analyses were performed using the free software R, version 2.1.1 (R Development Core Team, 2008).

3. Results

3.1. Concentrations of Hg in molar teeth and influence of the biological factors, gender and age

Dental Hg concentrations ranged from 0.43 to 10.99 ng.g^{-1} dw for ringed seals from CWG and from 0.95 to 57.6 ng.g^{-1} dw for animals from CEG. Arithmetic mean ($\pm$ standard deviation) was of 2.94 $\pm$ 1.99 ng.g^{-1} dw for CWG and 5.75 $\pm$ 6.20 ng.g^{-1} dw for CEG. Details for each location and sampling year are reported in Table 1. The coefficients of variation of dental Hg concentrations were very high for the CEG seals collected in 1999 (191%) and 2006 (197%) (due to two individuals displaying great Hg concentrations in those years, 49.5 ng.g^{-1} dw and 57.6 ng.g^{-1} dw, respectively), while they ranged from 21 to 74% for the other years for both regions.

No gender-related difference in dental Hg concentrations was observed (one-way ANOVA, p = 0.726), hence this parameter was discharged as a variable in the further statistical treatment. The mean age was significantly lesser in seals from CWG (2.7 years old) than in seals from CEG (6.0 years old) (see Table 1) (One-way ANOVA, p < 0.001). Furthermore, no significant difference in mean age was observed for seals in CWG through the years (One-way ANOVA, p = 0.056) whereas the mean age differed significantly among sampling years of the ringed seals from CEG (One-way ANOVA, p = 0.028), with the greatest mean ages in 1986 and 2006, and the lowest in 2000 and 2004.

The plot smoother showed a clear non-linear relationship between age and Hg concentrations, with a decreasing trend in dental Hg concentrations for the first 5-7 years of age of the individuals from both regions (Fig. 2). The slope seemed thereafter to level off in older individuals, and a plateau is reached. Therefore the Linear Regression Model was run on two

age groups of ringed seals, juveniles up to 5 years old and adults above 5 years old. The models showed that there was a significant regression between age and the Log-transformed Hg concentrations for the juveniles ($\leq$ 5 years old) from both CWG ($p < 0.001$), and CEG ($p = 0.024$), with a steeper decrease observed for the West Greenland seals. However, no linear relation was found for adults from CEG ($p = 0.961$), while too few adults were available to test the age relationship for adult ringed seals from CWG.

3.2. Stable isotopes in teeth, influence of gender and age, and relation between elemental and isotopic values

Stable isotopic ratios of carbon and nitrogen determined in the ringed seal teeth exhibited respective mean values of -16.04 $\pm$ 0.52‰ and 16.35 $\pm$ 0.99‰ for CWG, and -17.23 $\pm$ 0.53‰ and 14.90 $\pm$ 1.14‰ for CEG (range of values, see Table 3). Both ratios were correlated across all individuals ($r = 0.4$, $p < 0.001$), but not when investigating CWG and CEG data separately. Gender was shown to have no influence on the stable isotopic values, neither for $\delta^{13}C$ (One-way ANOVA, $p = 0.74$) nor for $\delta^{15}N$ ($p = 0.35$). However, age was correlated significantly with both $\delta^{13}C$ and $\delta^{15}N$ values for seals from CWG ($r = 0.25$, $p < 0.05$ and $r = -0.29$, $p < 0.05$, respectively), and with $\delta^{13}C$ values for seals from CEG ($r = 0.42$, $p < 0.001$) (Fig. 2). The $\delta^{15}N$ age trends were observed on the graphs to be quite similar to the Hg ones while the $\delta^{13}C$ relation with age showed an opposite trend to the Hg and $\delta^{15}N$ ones.

Log-transformed Hg concentrations and $\delta^{13}C$ and $\delta^{15}N$ values were not correlated for the ringed seals from both CWG ($p = 0.15$ and $p = 0.21$, respectively) and CEG ($p = 0.20$ and $p = 0.87$, respectively), when the two regions were studied separately. However, when CWG and CEG data were pooled, relations between Log-transformed Hg concentrations and $\delta^{13}C$ values ($p < 0.001$), and Log-transformed Hg concentrations and $\delta^{15}N$ values ($p < 0.01$), were found to be significant.

These statistical results are summarised in Table 4.

3.3. Spatial and temporal trends

Although the plot smoother showed an overall decreasing trend in dental Hg concentrations for the first 5 to 7 years of age of the seals from both regions, the Hg concentrations measured in the first two years of life of the animals were observed to be quite stable. Therefore, in order to limit the age effect and to avoid the overlapping of the life periods of the seals, we decided to focus on the dental Hg concentrations of the youngest individuals, up to 2 years old, when assessing the spatial and temporal trends of Hg concentrations and stable isotopic ratios.

A spatial difference in Hg concentrations was found (Two-factor nested ANOVA (location nested sampling year), $p < 0.001$), with significantly greater concentrations in the seals from CEG compared to CWG (Table 1). However, this spatial trend was not consistent among years ($p < 0.001$).

Stable isotopic values differed significantly between regions, with both greater $\delta^{13}C$ ($p < 0.001$) and $\delta^{15}N$ values ($p < 0.001$) in CWG compared to CEG (Table 3). However, while the spatial trend in $\delta^{13}C$ was consistent over time ($p = 0.071$), this was not found to be the case for the spatial trend in $\delta^{15}N$ values ($p = 0.039$).

The temporal trends of dental Hg concentrations of ringed seals are shown on Fig. 3. The temporal trends were very similar for both regions, with an apparent decrease of Hg concentrations until the 1990's, followed by an increase until 2006, although the Hg dental concentrations of CEG seals were actually lesser in 2006 than in 2004. A Linear Regression

Model was applied between 1994 and 2006 for seals from both CWG and CEG, and revealed a significant increase of dental Hg concentrations for this period in seals from both regions ($p < 0.001$ and $p = 0.018$, respectively).

Concerning the stable isotopes, the $\delta^{13}C$ values of the ringed seals from CEG were showed to follow a significant decrease over time (4.7% per year, $p < 0.001$, Fig. 3), while $\delta^{15}N$ values found in those animals were not ($p = 0.65$). Finally, neither $\delta^{13}C$ nor $\delta^{15}N$ values of the ringed seals from CWG did follow a temporal trend ($p = 0.10$ and $p = 0.81$, respectively).

4. Discussion

4.1. Influence of age on Hg concentrations in teeth

Mammalian teeth are composed of hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$, and comprise three types of structures, i.e. cementum, dentine, and enamel. Cementum and dentine grow in well defined layers throughout life, and those layers are known to deposit annually in marine mammals (Miyazaki, 2002). Mercury is believed to be deposited directly from the blood supply or the dentinal tubules into actively growing increments (Haller et al., 1993), and not to be remobilised throughout life of individuals (Eide et al., 1993; Tvinnereim et al., 2000). Despite a low affinity of Hg for the teeth, previous studies have demonstrated that this element can be measured reliably in teeth (Eide and Wesenberg, 1993; Tvinnereim et al., 2000). The dental Hg concentrations found in our study appear to be in the same order of magnitude than those reported in the current literature for marine mammals, i.e. ringed seal, with Hg mean values ± sd of 4.4 ± 1.7 to 8.6 ± 3.7 ng.g^{-1} dw in animals 5-25 years old (Outridge et al., 2009), walrus (*Odobenus rosmarus rosmarus*), with Hg concentrations under 2 ng.g^{-1} dw (Outridge et al., 2002), and beluga (*Delphinapterus leucas*), with Hg mean values

± sd of 70.92 ± 45.35 ng.g^{-1} dw (Kinghorn et al., 2008) and 98.4 ± 109 ng.g^{-1} dw (Outridge et al., 2002).

However, the trends of dental Hg concentrations with the age, described in the literature and in the present study, differ. Indeed, Outridge et al. (2009) found that Hg concentrations measured in the canine tooth (two upper thirds) of Canadian ringed seal was correlated positively with the age of the animal, whereas, in contrary, a significant decrease of dental Hg concentrations was observed the first years of life for the ringed seals in our study ($p < 0.001$ for CWG, and $p = 0.024$ for CEG; Fig. 2). In addition, Outridge et al. (2000) showed that Hg concentrations in the tooth cementum increase with the age in beluga whales, while Kinghorn et al. (2008) did find no significant relation between age and Hg concentrations in the same tissue for the same species. Finally, Outridge et al. (2002) reported an absence of correlation between age and cementum Hg concentrations in the walrus.

From this literature overview on the dental Hg in marine mammals, it seems that age accumulation of the Hg concentrations in the teeth differs within species (in relation to their dentition development), but also with the part of tooth (dentine, cementum or the entire tooth) or even the type of tooth (molar or canine, which may grow at distinct times during the dentition development) on which the Hg measurements are carried out.

Moreover, as marine mammal exposure to Hg occurs mainly via food (Aguilar et al., 1999), the variation in dental Hg concentrations throughout life is expected to be related to changes in dietary regime with age. Ringed seals are diverse feeders on planktonic, nektonic, and benthic organisms, with a diet ranging from pelagic amphipods to different fishes, as polar cod (*Boreagadus saida*) or Arctic cod (*Arctogadus glacialis*) in the Greenland waters (Siegstad et al., 1998). Occasionally, immature ringed seals explore the water column down to

the sea floor, and spend much of their time foraging at depths shallower than 50m (Born et al., 2004), showing a larger preference for crustaceans than adults (Holst et al., 2001). Furthermore, the diving capabilities of ringed seals increase with age, so that older and larger seals dive in general deeper than younger seals, consuming potentially more fish like polar cod while reducing the consumption of crustaceans (Holst et al. 2001; Reeves et al., 1992). However, this foraging behaviour does not explain why adults have lesser Hg concentrations compared to juvenile ringed seals. Crustaceans contain lesser Hg concentrations than fishes in Greenland waters (Dietz et al., 1996), and therefore, a greater crustacean intake of the younger individuals can not be responsible for greater Hg concentrations in teeth of juveniles compared to adult seals. In addition, the significant negative correlations found between age and $\delta^{15}N$ values, for CWG individuals and for all ringed seals (Table 4, Fig. 2), do not match with the above mentioned crustacean-diet for juveniles and fish-diet for adults. Since the mineralisation of the permanent dentition occurs at the foetal stage for the ringed seal (Reeves et al., 1992; Stewart et al., 1998), a consistent explanation to both great Hg concentrations and $\delta^{15}N$ values for the first years of life would be the dietary inputs from the mother during the period of gestation and lactation. Indeed, Hg is known to be transferred from the mother, to the foetus through the placenta, and to the newborn via milk (Grandjean et al., 1994). Both transfers have been reported previously for seals, showing a more quantitatively significant Hg acquisition during the prenatal stage, compared to the postnatal one (Wagemann et al., 1988).

Furthermore, the enriched $\delta^{15}N$ values, measured in the first years of age, tend to support the hypothesis of these maternal transfers. Indeed, $\delta^{15}N$ of a consumer are related to those of its prey (DeNiro and Epstein, 1978), and an enrichment of $\delta^{15}N$, of the order of 3‰ generally, occurs at each trophic transfer (Hobson and Welch, 1992). Thus, the enriched $\delta^{15}N$ values displayed by the youngest seals would reflect their consumer position relative to their

mothers. Similarly to our results, enriched $\delta^{15}N$ values in the early life have been previously

reported in the teeth of other marine species, such as Steller sea lion (*Eumetopias jubatus*)

(Hobson and Sease, 1998), longbeaked common dolphin (*Delphinus capensis*) (Nino-Torres et

al., 2006), and narwhal (*Monodon monoceros*) (Dietz et al., 2004).

In addition, the depleted $\delta^{13}C$ values observed in teeth of youngest individuals (Fig. 2) also

corroborate the idea of a maternal transfer to foetus and young of the year. Indeed, body fat

has been shown to be depleted in ^{13}C compared to other animal tissues due to selective

fractionation (Tieszen et al., 1983), and the depleted $\delta^{13}C$ values of the juvenile seals may

reflect likely the mobilisation and the transfer of the carbon from maternal lipid stores, for the

foetal development and milk production (Dehn et al., 2007; Polischuk et al., 2001). The same

$\delta^{13}C$ trend has been described previously by Hobson and Sease (1998) in the teeth of Steller

sea lions, and explained by the transfer of maternal body lipids during the nursing period.

Following the great Hg concentrations observed in the teeth in early life, Hg concentrations

are observed to drop for individuals from both regions (Fig. 2). This age Hg trend can be

explained by a progressive "dilution" of the dentine deposited at the foetal and weaning stages

by the addition of new post weaning dentine. In addition, the deposition of dentine, and its

subsequent mineralisation, reduces the pulp area, and in some species, the pulp cavity

becomes occluded at some point with subsequent suspension of dentinal accumulation (Hohn,

2002). Thus, the plateau of Hg concentrations reached around 10 years old could actually

reflect the time of the tooth occlusion. Indeed, up to 15-20 dentinal layers have been observed

previously in the canines of ringed seals (Stewart et al., 1996), and the molars being smaller

than canine teeth, it is likely that their occlusion would occur earlier in life.

4.2. Spatial variations of dental Hg concentrations in Greenland

As a general trend, Hg concentrations in teeth of ringed seals from Ittoqqortoormiit (CEG) were found to be significantly greater than those from Qeqertarsuaq (CWG). The same spatial difference in Hg concentrations between CEG and CWG has been reported previously for Hg concentrations in livers of ringed seals (Dietz et al., 1998; Rigét et al., 2005). Such a spatial variation can be caused by either differences in diet or differences in Hg concentrations in the environment among seals of both regions.

Feeding habits of the ringed seal have been documented to vary both spatially and temporally (Reeves et al., 1992; Siegstad et al., 1998), which makes difficult to estimate the dietary Hg exposure of the ringed seals. However, differences in diet have been previously suggested by Dietz et al. (1998), as a likely reason for such a spatial difference in Hg concentrations in ringed seals. In a study on stomach content of Greenland ringed seals, Siegstad et al. (1998) found both arctic and polar cods (65% by weight) to be important food items of this species in the area of Kong Oscars Fjord (CEG), while capelin (*Mallotus villosus*) and redfish (*Sebastes spp*.) (52.2 and 25.2% weight, respectively) have been reported to be among the main food items in the diet of ringed seals from Qeqertarsuaq area (CWG). Nevertheless, it is worth mentioning that these diets may only reflect seasonal preys since they have been determinate from seal stomachs collected in spring for the CWG individuals, and in summer for the CEG ones. Møller (2006) investigated stable isotopes in the CWG food webs and reported greater δ^{15}N values for both Cottidae, arctic and polar cods, than for capelins or redfishes. Thus, although arctic cod exhibits a greater Hg content than other Greenland fish species (Dietz et al., 1996), which would have explained the greater Hg concentrations in teeth of ringed seals from the CEG, our δ^{15}N isotopic data are paradoxically not in agreement with this difference in diet among seals from both areas, since δ^{15}N values were lesser in CEG seals (Cottidae consumers) than in those from CWG (Table 3). On the contrary, δ^{15}N values in teeth would be

the diet's signature of the entire life of the individual. Thus, on a long time period, the seals would be more exposed to Hg in the CEG by eating at lower trophic levels, which implies that the food webs would be more contaminated in the CEG than in the CWG.

Stable isotopes of carbon provide information about the sources of primary productivity in ecosystems where distinct isotopically sources are present (for example phytoplankton vs. ice algae). Indeed, the ^{13}C enrichment of benthic compared to planktonic algae is substantial enough to allow distinguishing between benthic and planktonic food sources for coastal animals (France, 1995); so that the more depleted δ^{13}C values found in CEG seals could suggest a more pelagic diet for these animals compared to the CWG ones. But most likely, this difference in δ^{13}C values can be attributed to distinct oceanography and characteristic spatial δ^{13}C values of two different ecosystems. Indeed, the West Greenland area is influenced by a mixture of waters from the cold East Greenland Current and the warmer and more saline Irminger Current (a side branch of the Gulf stream, North Atlantic current), while the East Greenland is dominated by the East Greenland Current that brings cold low-saline polar water south along the eastern coast of Greenland (Born et al., 2003). Previously, the temperature has been described as an important factor of variation of δ^{13}C (Rau et al., 1982), resulting in a ^{13}C depletion with high latitudes. Thus, the warmer temperatures of the western Greenland waters may likely lead to more enriched δ^{13}C values of the CWG food web, and explain the greater δ^{13}C values found in the tissues of CWG ringed seals compared to the CEG ones.

Mercury is known to be transported on long distance in the atmosphere, and Pacyna and Keeler (1995) described Europe, Asia and North America as the regions where are located most global and regional anthropogenic emissions of Hg with potential impact on the Arctic environment. The seasonal variability in long-range atmospheric transport to Greenland has been well demonstrated (Kahl et al., 1997), and the influence of this transport may vary

regionally, result in spatial differences in the deposition of atmospheric Hg, and subsequently, contribute to the spatial differences in Hg concentrations in the regional ecosystems of Greenland. However, it has also been reported that some natural geological formations may actually contribute substantial amounts of Hg to the surrounding environment (Painter et al., 1994). Thus, spatial differences in Hg concentrations found in seals and other marine mammals in the global Arctic have been thought to be related directly to the natural input from underlying geology (Rigét et al., 2005; Wagemann et al., 1995). In the same way, the distinct basement rock between CEG and CWG (Watt and Thrane, 2001) could lead to a different natural input of Hg.

4.3. Temporal trends of Hg concentrations in teeth

Significant increases in Hg concentrations were found in teeth of ringed seals from both regions between 1994 and 2006, although for CEG, the dental Hg concentrations of the animals were actually lesser in 2006 than in 2004 (Fig. 3). An increase in Hg concentrations in CWG region has been reported previously in liver of ringed seals for the period of 1999 to 2004 (Rigét et al., 2007). The authors did attribute this increase to likely changes in the feeding behaviour of the animals that they investigated through stable isotopes analysis in muscle tissues. However, the increase was not significant anymore when the period was extended from 1994 to 2004. In addition, no temporal trend was detected in ringed seals from CEG, although some year-to-year differences were observed (Rigét et al., 2004). Mercury concentrations have been shown to increase with trophic levels (δ^{15}N) in Arctic marine food web (Atwell et al., 1998). In our study, the δ^{15}N analyses, which allow investigation of dietary shifts through time, did exhibit no temporal trends neither for the CWG nor for the CEG ringed seals (Fig. 3), so that a change in diet can not be a consistent explanation for the temporal variations of dental Hg concentrations. However, it can not be

excluded that the shift in diet may not be reflected through the $\delta^{15}N$ values, since changes in prey species do not imply necessarily changes in trophic level. Nevertheless, we did observe a significant decreasing temporal trend in $\delta^{13}C$ values in the teeth of seals from CEG (Fig. 3). Since pelagic consumers tend to be more depleted in ^{13}C compared with littoral consumers (France, 1995), this decreasing trend in $\delta^{13}C$ values over time could likely result from a change toward a more pelagic feeding behaviour of the ringed seals. Alternatively, the decrease of $\delta^{13}C$ values over time could reflect the dissolution of isotopically light carbon from anthropogenic CO_2 in the ocean, the so-known Seuss effect (Quay et al., 1992), which results in a reduction of the oceanic $\delta^{13}C$ values, and subsequently, in animal tissues. This isotopically light carbon has been released largely to the atmosphere through the combustion of fossil fuels (Quay et al., 1992), and previous studies on teeth of fur seal and ringed seal have revealed a similar decrease in $\delta^{13}C$ over a century (Newsome et al., 2007; Outridge et al., 2009).

The United States, Europe, Asia and Canada are considered to be the main sources of polluted air reaching Greenland (Boutron et al., 1998). Although Hg anthropogenic emissions to the atmosphere from Europe and United-States have been reduced recently, the Asian Hg emissions have been reported to increase substantially these last decennials, especially from China and India, and mainly to be due to the burning of fossil fuels (Pacyna and Pacyna, 2002). A work carried out by Kahl et al (1997) on air mass trajectories to Greenland emphasised the importance of Asian sources. Thus, the recent Hg increase observed in teeth of ringed seals from Greenland may originate for example, from an increase of Hg emissions from coal combustion partly from China, and subsequent long-range transport of Hg to Greenlandic regions.

5. Conclusion

The present study outlines the important contribution of the prenatal period to the transfer of Hg to the teeth of the ringed seal. Age has been shown to influence Hg concentrations in juvenile seals with a significant decrease during the first 5 years. Although teeth exhibited much lower Hg concentrations than concentrations generally encountered in soft tissues, they appeared to be a good monitor of Hg environmental exposure, showing significant differences in Hg concentrations between both regions and pointing out the increasing temporal trends of Hg concentrations between 1994 and 2006. The ability to investigate temporal trends or to compare geographical areas is useful in the cases where soft tissues are not available. Moreover, teeth constitute a preserved material in which pre-historic temporal trends of trace elements can be measured. However, the results and the comparison with literature also outline the uncertainties and difficulties for interpreting the concentrations and for using such material (e.g. unknown age or type of tooth) as a bio-monitoring media of Hg.

Finally, the combined use of the elemental and isotopic analyses provided important complementary information to conclude about the life exposure history of the animals.

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Table 1. Year of capture, number of ringed seals, their mean age (± standard deviation) in years, and their dental Hg concentrations range and mean (± standard deviation) in ng.g^{-1} dw, for the two regions, Qeqertarsuaq (Central West Greenland, CWG) and Ittoqqortoormiit (Central East Greenland, CEG).

		Qeqertarsuaq (CWG)					Ittoqqortoormiit, CEG		
Year	n	Mean age ± sd	Mean [Hg] ± sd	Range [Hg]	Year	n	Mean age ± sd	Mean [Hg] ± sd	Range [Hg]
1982	20	3.7 ± 3.5	3.64 ± 2.69	1.29-10.99	1986	27	7.8 ± 6.6	6.05 ± 1.70	3.4-10.3
1994	26	3.1 ± 2.2	1.20 ± 0.47	0.43-2.20	1994	16	5.7 ± 4.0	2.43 ± 0.95	0.95-4.75
1999	10	2.6 ± 2.8	2.52 ± 1.11	1.25-4.70	1999	19	4.6 ± 2.7	5.63 ± 10.76	1.7-49.52
2000	8	1.4 ± 1.5	2.13 ± 0.46	1.19-2.72	2000	17	4.3 ± 2.4	5.48 ± 2.91	3.68-14.34
2001	15	1.6 ± 1.4	3.26 ± 1.51	2.14-8.19	2001	20	6.6 ± 6.4	6.25 ± 3.27	2.46-13.73
2002	13	1.4 ± 1.2	3.39 ± 1.3	2.03-7.14	2002	19	4.6 ± 2.4	4.91 ± 1.49	2.92-9.29
2004	9	1.0 ± 0.9	4.87 ± 2.5	2.42-10.23	2004	26	4.4 ± 4.1	8.25 ± 5.16	4.55-28.58
2006	14	4.8 ± 7.5	3.94 ± 1.91	1.75-7.07	2006	26	8.3 ± 7.3	5.47 ± 10.79	1.25-57.60
All	115	2.7 ± 3.5	2.94 ± 1.99	0.95-57.60	All	170	6.0 ± 5.2	5.75 ± 6.20	0.43-10.99

Table 2. Analytical quality data for the customised reference material measured concurrently with the tooth samples (units in µg.g^{-1} dw).

	n	Hg concentration mean ± sd	% Relative standard deviation
AMA CCA	8	0.0963 ± 0.0035	3.7
AMA NERI	10	0.105 ± 0.006	5.7
FIMS NERI	5	0.1063 ± 0.0036	3.4
Mean measured concentration*	23	0.1023 ± 0.0064	6.3
Theoretical concentration**	1	0.1 ± 0.0041	4.1

* The mean 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. Range and mean ± standard deviation (sd) of $\delta^{15}N$ and $\delta^{13}C$ values in the teeth of ringed seals from central West Greenland (CWG) and central East Greenland (CEG).

		$\delta^{15}N$ (‰)		$\delta^{13}C$ (‰)	
	n	Range	Mean ± sd	Range	Mean ± sd
All individuals	238	12.11 to 20.26	15.51 ± 1.30	-18.46 to -14.75	-16.72 ± 0.79
CWG	101	14.16 to 20.26	16.35 ± 0.99	-17.14 to -14.75	-16.04 ± 0.52
CEG	137	12.11 to 18.04	14.9 ± 1.14	-18.46 to -15.73	-17.23 ± 0.53

Table 4. Result matrix of the statistics (Pearson's correlation and ANOVA) testing the relations between $\delta^{15}N$ and $\delta^{13}C$ values, age and Hg concentrations in teeth of ringed seals from central West Greenland (CWG) and central East Greenland (CEG).

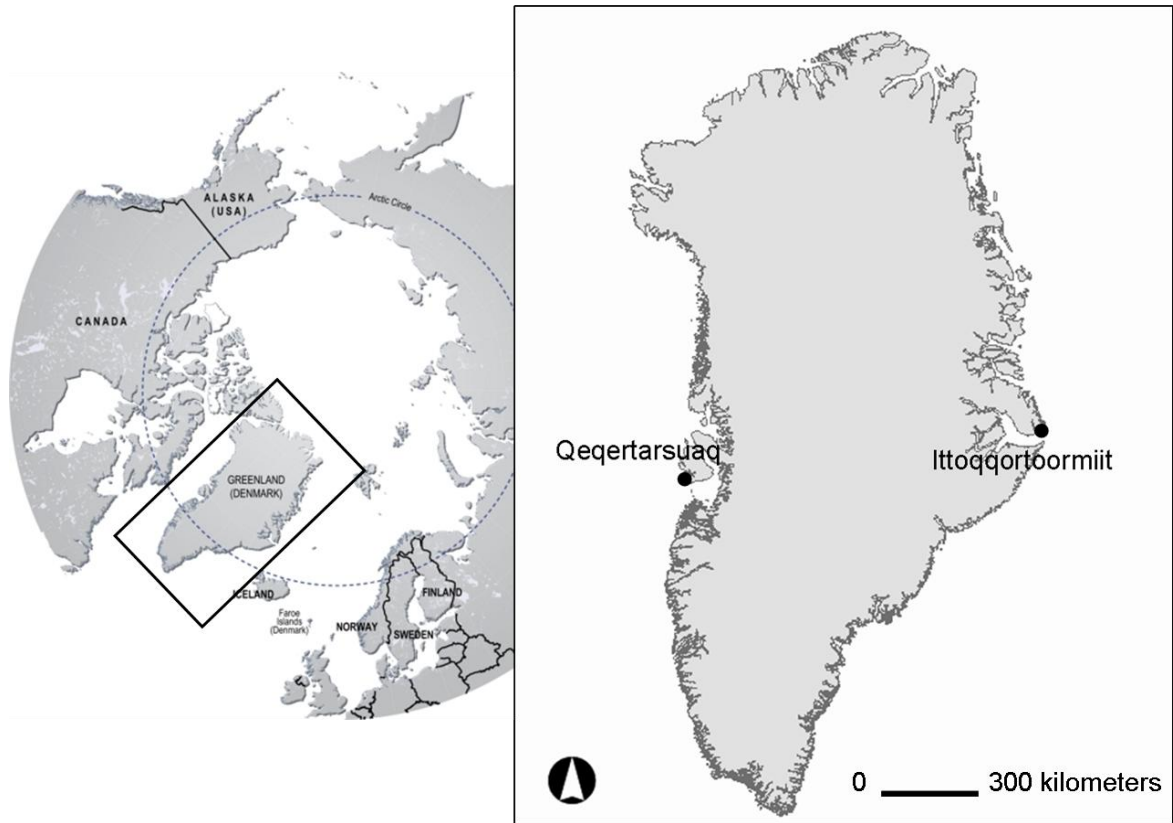|	$\delta^{15}N/\delta^{13}C$	Age/$\delta^{15}N$	Age/$\delta^{13}C$	Log Hg/$\delta^{15}N$	Log Hg/$\delta^{13}C$
All individuals	0.399***	-0.341***	-0.028	-0.191**	-0.392***
CWG	-0.057	-0.289**	0.25**	0.125	-0.145
CEG	-0.011	-0.162	0.417***	0.014	-0.109

* $0.01 < p < 0.05$

** $0.001 < p < 0.01$

*** $p < 0.001$

Figure 1. Map showing the two regions in Greenland from where ringed seals were sampled.
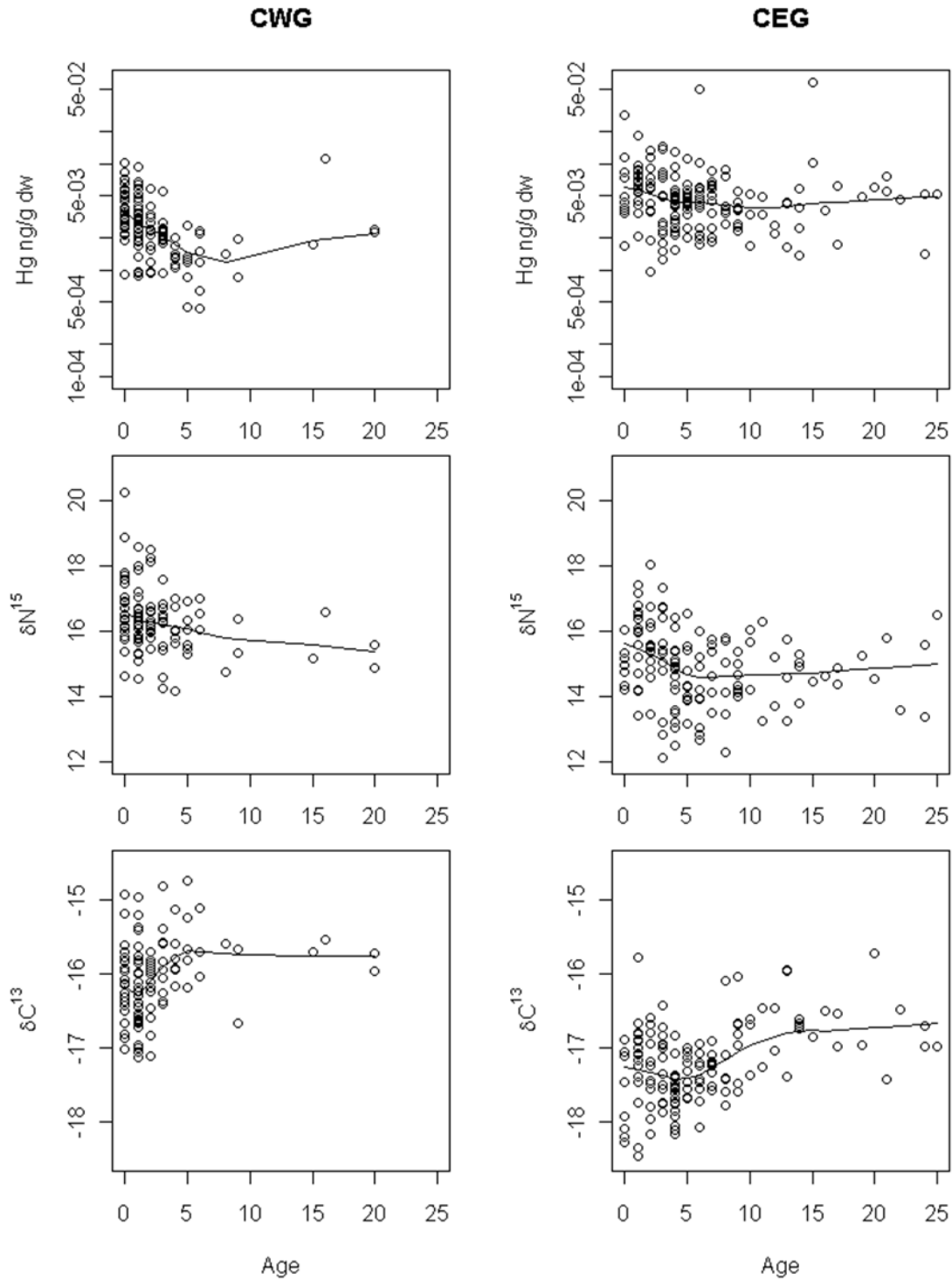
Figure 2. Age (in years) vs. Hg concentrations (in ng.g^{-1} dw), δ^{15}N and δ^{13}C values (in ‰) in teeth of ringed seals from central West Greenland (CWG, left figures) and central East Greenland (CEG, right figures). The black lines are LOWESS smoother (robust, locally weighted scatterplot smoothing system based on the LOWESS algorithm). The y-axis for Hg concentrations is logarithmic-scaled.
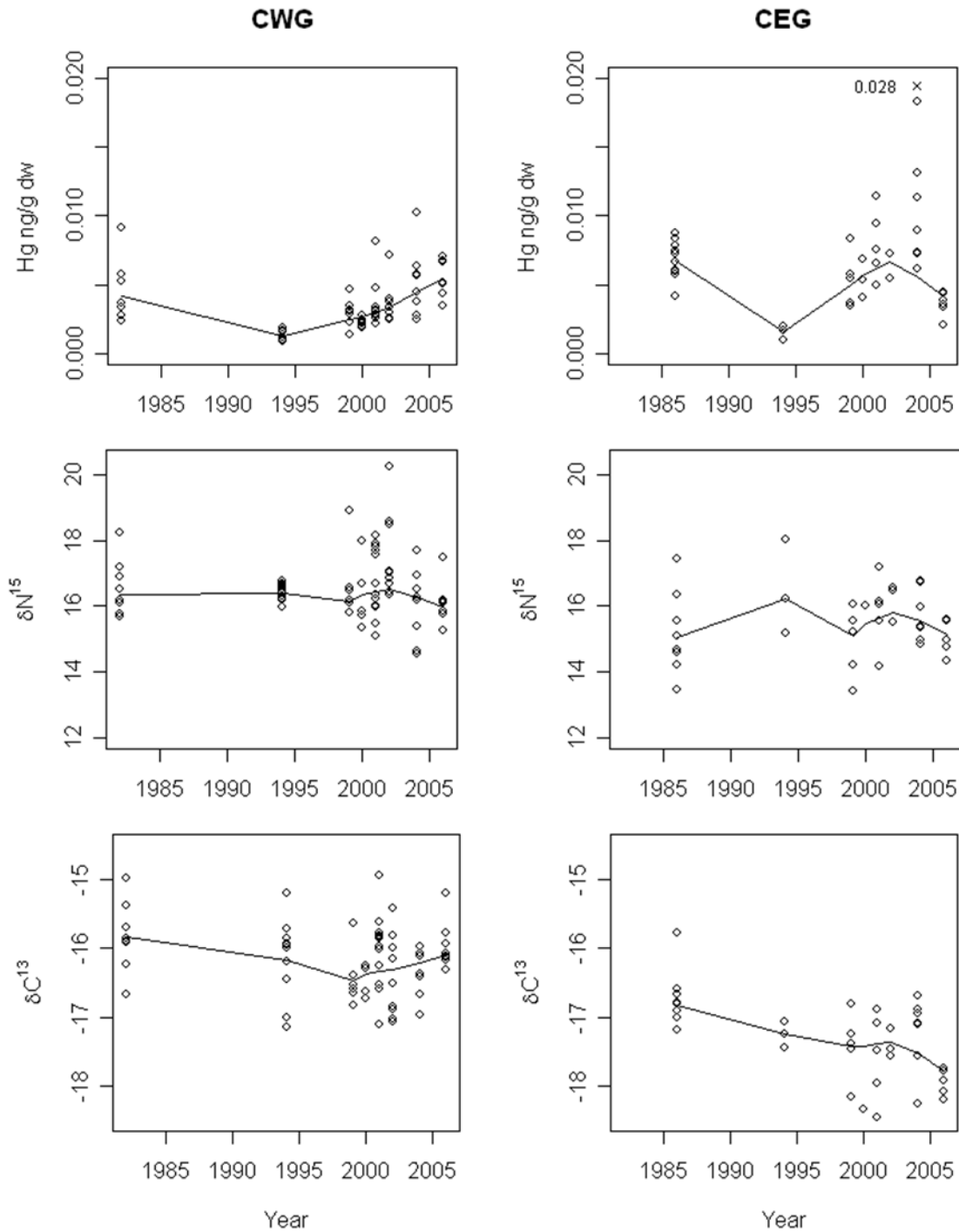
Figure 3. Temporal trend of Hg concentrations (in ng.g^{-1} dw), δ^{13}C and δ^{15}N values (‰) in teeth of ringed seals from central West Greenland (CWG, left figure) and from central East Greenland (CEG, right figure) ringed seals. The smoothing lines (robust, locally weighted scatterplot smoothing system based on the LOWESS algorithm) represent the fitted non-linear trends of the values. The y-axis for Hg concentrations is logarithmic-scaled.