ORIGINAL PAPER

Investigation of mercury concentrations in fur of phocid seals using stable isotopes as tracers of trophic levels and geographical regions

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Abstract Recent studies have shown that the complementary analysis of mercury (Hg) concentrations and stable isotopic ratios of nitrogen (δ^{15}N) and carbon (δ^{13}C) can be useful for investigating the trophic influence on the Hg exposure and accumulation in marine top predators. In this study, we propose to evaluate the interspecies variability of Hg concentrations in phocids from polar areas and to compare Hg bioaccumulation between both hemispheres. Mercury concentrations, δ^{15}N and δ^{13}C were measured in fur from 85 individuals representing 7 phocidae species, a Ross seal (*Ommatophoca rossii*), Weddell seals (*Leptonychotes weddellii*), crabeater seals (*Lobodon carcinophagus*), harbour seals (*Phoca vitulina*), grey seals (*Halichoerus grypus*), ringed seals (*Pusa hispida*) and a bearded seal (*Erignathus barbatus*), from Greenland, Denmark and Antarctica. Our results showed a positive correlation between Hg concentrations and δ^{15}N values among all individuals. Seals from the Northern ecosystems displayed greater Hg concentrations, δ^{15}N and δ^{13}C values than those from the Southern waters. Those geographical differences in Hg and stable isotopes values were likely due to higher environmental Hg concentrations and somewhat greater number of steps in Arctic food webs. Moreover, dissimilarities in feeding habits among species were shown through δ^{15}N and δ^{13}C analysis, resulting in an important interspecific variation in fur Hg concentrations. A trophic segregation was observed between crabeater seals and the other species, resulting from the very specific diet of krill of this species and leading to the lowest observed Hg concentrations.

Keywords Antarctica · Carbon · Denmark · Greenland · Hair · Hg · Nitrogen

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Introduction

Mercury (Hg) is a global contaminant released into the ecosystem from both natural and anthropogenic sources (Pacyna et al. 2006). The gaseous elemental form of this metal is very volatile, and once in the atmosphere, it can be transported from mid to high latitudes over long distances, and thereby reach the polar regions (Brooks et al. 2005). Although Hg is partly re-emitted to the air, it is accumulated in organisms and biomagnified in the trophic chain in polar ecosystems (Atwell et al. 1998; Bargagli et al. 1998). Thus, organisms feeding on species at high trophic levels generally show greater Hg concentrations than those feeding on species at lower trophic levels. Since marine mammals mainly are exposed to Hg through their diet (Aguilar et al. 1999), differences in foraging strategies and feeding habits among species may lead to differences in exposure to Hg, resulting in interspecific disparities in concentrations of Hg.

Biological factors like age, sex or physiological status are known to influence the diet, and thereby, the exposure and accumulation of Hg in many species. However, differences in Hg concentrations can also result from geographical or temporal variations in the environmental levels of Hg. Whether differences in Hg concentrations among species result from differences in diet or environmental levels of Hg can be investigated by analysing naturally occurring variations in stable isotopes of nitrogen (^{15}N and ^{14}N) and carbon (^{13}C and ^{12}C) (Hobson and Welch 1992). The underlying principle is that ratios of heavier *vs* lighter isotopes of nitrogen and carbon in tissues of predators can be traced to those of their prey as these two elements are assimilated through the diet. Consumer tissues are stepwise enriched in ^{15}N relative to those of their prey, and consequently, δ^{15}N measurements can be used to determine the trophic position of organisms (Minagawa and Wada 1984). In contrast, δ^{13}C values vary little through the food chain (<1‰) and can thus help to determine the source of carbon used by primary consumers in a food web and provide information on the foraging habitat of the studied organisms (Fry and Sherr 1984). Furthermore, turnover rates of stable isotopes in tissues vary with the metabolic rate of the tissue (Tieszen et al. 1983), and isotopic analyses can give information on feeding habits over the last days, weeks, months or years, depending on which tissue types are analysed.

Phocid seals are long-lived marine mammals at the top of food chains in polar ecosystems. Using stable isotopes of nitrogen and carbon as tracers of the trophic level and habitat use, respectively, we investigated if concentrations of Hg in seal species from different geographical areas could be used to determine the trophic levels they prey upon. We studied a Ross seal (*Ommatophoca rossii*), Weddell seals (*Leptonychotes weddellii*) and crabeater seals (*Lobodon carcinophagus*) from Antarctic regions, harbour seals (*Phoca vitulina*) and grey seals (*Halichoerus grypus*) found throughout coastal regions of the Northern hemisphere, and ringed seals (*Pusa hispida*) and a bearded seal (*Erignathus barbatus*), which both show Northern circumpolar distributions (Reeves et al. 1992).

While the Ross seal has a varied diet based on squid, fish and krill, and the Weddell seal mainly feeds on fish, but also on various cephalopods and crustaceans, the crabeater seal is specialised on feeding almost exclusively on Antarctic krill (Bowen et al. 2002). In the Northern hemisphere, harbour and grey seals feed on a wide range of organisms, pelagic and benthic fish, cephalopods and crustaceans (Reeves et al. 1992). Finally, ringed seals are believed to feed mainly on a variety of fish and invertebrates such as amphipods, euphausiids and cephalopods, while the bearded seal consumes mainly benthic invertebrates, i.e., clams, crustaceans, cephalopods as well as

some fish (Reeves et al. 1992). Pauly et al. (1998) estimated the trophic levels of several marine mammals mainly by stomach content analysis. Values for trophic levels ranged from 3.3 for the crabeater seal to 4.1 for the Ross seal. Estimated values were 3.4 for bearded seals, 3.8 for ringed seals, harbour seals and grey seals, whereas it was estimated at 4.0 for Weddell seals.

Soft tissues are commonly used for analysis of metals and stable isotopes. However, fur provides a non-invasive way to investigate metals and stable isotopes of seals. Fur is keratin based containing records of metallic and isotopic elements which are deposited there during the annual moult. Mercury is one of the trace elements transported to the fur follicles via the blood supply (Ikemoto et al. 2004) and subsequently incorporated into the hair during periods of growth (Brookens et al. 2007). Hair is widely used as an indicator of blood Hg levels for mammals, and particularly for humans, and has been used as an indicator of exposure to the environmental pollution also in pinnipeds (Yamamoto et al. 1987; Wenzel et al. 1993; Watanabe et al. 1996; Medvedev et al. 1997; Ikemoto et al. 2004; Brookens et al. 2007; Gray et al. 2008).

The purpose of this study was to determine Hg concentrations in the fur of seven species of phocid seals from different geographical areas and to evaluate the influence of biological factors in the bioaccumulation process. Stable isotope analyses were used to evaluate differences or similarities in the diet or in the foraging habitat among species that could explain variations in Hg concentrations. We also evaluated if fur can be used for monitoring of Hg in phocid seals in the future.

Materials and methods

Sampling procedure and preparation

Hair samples of 85 seals were obtained from seven phocid seal species, i.e., Ross seal, Weddell seal, crabeater seal, harbour seal, grey seal, ringed seal and bearded seal. The samples were collected from three distinct geographical areas, Ross Sea, Antarctica (Ant), Denmark (Dk) and Greenland (Gr) (cf. Fig. 1). Samples were taken in the period 1996–2009. Length and weight of the animals were recorded, and age was estimated visually based on size and fur colour, according to three classes (yearling, juvenile and adult). Number of individuals, estimated age and gender are summarised for each species in Table 1.

Samples were stored in polyethylene plastic bags and kept at −20°C. Prior to analysis, fur samples were washed in a diluted standard detergent (RBS 35; Bie & Berntsen A/S, Denmark). Subsequently, the hair samples were rinsed in several ultra-pure Milli-Q water baths and dried for 24 h at room temperature.

Fig. 1 Map of the world showing the three geographical areas, Antarctica (Ant), Denmark (Dk), Greenland (Gr), of fur sampling of the phocid species
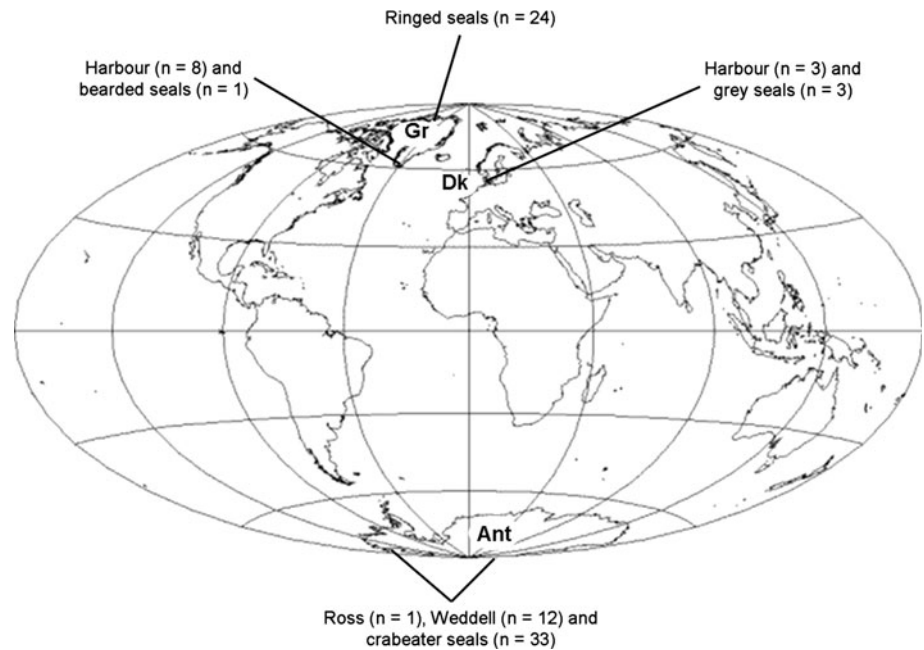
Table 1 Geographical areas and seal species with number of individuals n in parentheses, age classes (*ND* Not Determinate) and genders of the individuals (*M* Male, *F* Female)

Studied area	Species (n)	Age group			
		Yearling M/F	Juvenile M/F	Adult M/F	ND M/F
Antarctica	Ross seal (1)	–	–	–	1/0
	Weddell seal (12)	1ND	5/1	4/1	–
	Crabeater seal (33)	–	11/10 (2ND)	4/6	–
Denmark	Harbour seal (3)	1/0	2/0	–	–
	Grey seal (3)	–	0/2	1/0	–
Greenland	Harbour seal (8)	–	–	5/2	0/1
	Ringed seal (24)	9/6	4/2	1/2	–
	Bearded seal (1)	–	–	1/0	–
	All (85)	10/6 (1ND)	22/15 (2ND)	16/11	1/1

Analytical procedures, instrumentation and quality assurance for Hg measurements

The Hg measurements were performed at the laboratory of the National Environmental Research Institute in Roskilde, Denmark, using a solid sample atomic absorption spectrometer AMA-254 (Advanced Mercury Analyser-254 from LECO, Sweden). A homogenised subsample of 5–10 mg of fur was analysed from each individual. The use of this apparatus does not require chemical pre-treatment, which reduces risks for contamination or loss of Hg. The analytical process consists of a drying period at 120°C prior to a combustion phase at 750°C, which leads to desorption of Hg from the samples. The Hg vapour produced is subsequently carried by an oxygen flow to a gold amalgamator and trapped on its surface. The collected Hg is thereafter 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, decomposition, and waiting time, were 30, 150 and 45 s, respectively. The instrument is described in detail elsewhere (Hall and Pelchat 1997).

The accuracy and reproducibility of the method were tested by including human hair (IAEA-086) reference material at the beginning and at the end of the analysis cycle and by running it every 10 samples. Results of quality controls showed a good precision with a relative standard deviation of 3.9%. The accuracy was 96% of the assigned concentration ($n = 9$). The National Environmental Research Institute participates in the international interlaboratory comparison exercises conducted by the EEC (QUASIMEME) and showed satisfactory results in the latest test in 2007 for the AMA-254 ($0 < z < 0.5$). All data are presented on a dry weight basis (dw), and the detection limit is 0.1 ng/g dw.

Analytical procedures and instrumentation for stable isotope measurements

A homogenised aliquot of approximately 0.5 mg of hair from each individual was weighted and loaded into tin capsules.

Relative abundances of stable isotopes of carbon (δ^{13}C) and nitrogen (δ^{15}N) were determined using an elemental analyser (Flash EA 1112 fitted with a "No Blank" option, Thermo Scientific, Milan, Italy) coupled to an isotope ratio mass spectrometer (Delta V Advantage with a Conflo IV interface, Thermo Scientific, Bremen, Germany). Stable isotope results are expressed in delta notation (δ), defined as the part per thousand (‰) deviation from a standard material:

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

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

The analytical precision of the measurements was <0.06‰ for carbon and <0.1‰ for nitrogen, based on replicate measurements of acetanilide as internal laboratory reference material.

Statistical analysis

Prior to the statistical analyses, the Hg data were log-transformed (base e) to reduce skewness and fit parametric requirements. Shapiro–Wilk and Bartlett tests 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 linear regression analysis and analysis of variance were applied to test for the influence of factors (i.e., age category, sex, species and region of sampling) on the log-transformed Hg concentrations or δ^{13}C and δ^{15}N 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. Tukey HSD (Honestly Significant Difference) test was used as a posterior ANOVA test to determine which means were significantly different from one another. Wilcoxon tests were used to test for differences in log-transformed Hg concentrations or stable isotope ratios between two species from the same region or among individuals from the same species originating from two regions. Non-parametric test, Spearman rank correlation, was used to test the relationships between Hg concentrations, δ^{13}C and δ^{15}N values.

The number of individuals was low for some of the species, and only crabeater seals, Weddell seals and ringed seals were represented by more than 10 individuals (Table 1). All species (7 species, 85 individuals) are shown on the graphs, but the bearded seal ($n = 1$) and the Ross seal ($n = 1$) are excluded from statistical analyses due to low sample size.

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).

Results

Hg concentrations and stable isotope ratios

Mercury concentrations in the hair ranged from 0.07 µg/g dw for the crabeater seal to 30.6 µg/g dw for the harbour seal, and mean concentrations increased in the sequence: crabeater seals < Ross seal < bearded seal < Weddell seals < ringed seals < harbour seals (Dk) < grey seals < harbour seals (Gr) (Table 2). Nitrogen stable isotopic ratios in hair of the different seal species varied from 6.02‰ for the crabeater seals to 19.33‰ for the ringed seals (crabeater seals < Ross seal < Weddell seals < bearded seal < harbour seals = grey seals < ringed seals, for mean values), while δ^{13}C values ranged from −25.11‰ for the crabeater seals to −15.06‰ for the harbour seals (crabeater seals < Weddell seals < Ross seal < grey seals < ringed seals < harbour seals (Dk) < bearded seal < harbour seals (Gr), for mean values) (Table 2).

Influence of sex and age classes

Only adult individuals were selected to test the factor sex. However, the three categories of age (yearling, juvenile and adult) were not all well represented for each species (Table 1). Thus, the influence of sex on Hg concentrations was tested only on the crabeater seals, while the influence of age classes was tested on crabeater seals, Weddell seals and ringed seals.

No difference in Hg concentrations between genders was recorded among the crabeater seals (One-way ANOVA, $F = 0.26$; $P = 0.62$). Figure 2 shows Hg levels for the different age classes for crabeater seals, Weddell seals and ringed seals. Log-transformed Hg concentrations increased significantly with age for crabeater seals (One-way ANOVA, $F = 221.1$, $P < 0.001$), where adults showed a higher mean Hg concentration (0.61 µg/g dw) compared with juveniles (0.11 µg/g dw). However, no significant

Table 2 Mercury concentrations and stable isotopic ratios of nitrogen (δ^{15}N) and carbon (δ^{13}C) (mean values ± standard error SE and range) in fur samples of the various phocid species from the different geographical areas

Area	Species	Hg (µg/g dw)		δ^{15} N (‰)		δ^{13}C (‰)	
		Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
Antarctica	Ross seal	0.48		10.38		−22.31	
	Weddell seal	2.09 ± 0.21	0.63–3.22	13.46 ± 0.21	12.51–14.89	−23.20 ± 0.12	−23.86–(−22.24)
	Crabeater seal	0.26 ± 0.04	0.07–0.99	7.70 ± 0.11	6.02–8.84	−24.28 ± 0.12	−25.11–(−22.63)
Denmark	Harbour seal	7.79 ± 1.99	5.63–11.77	15.75 ± 0.05	15.65–15.82	−16.87 ± 0.36	−17.46–(−16.21)
	Grey seal	10.11 ± 5.47	4.11–21.03	15.76 ± 1.20	13.92–18.03	−17.41 ± 0.68	−18.54–(−16.20)
Greenland	Harbour seal	16.27 ± 2.86	1.49–30.60	15.69 ± 0.16	15.03–16.44	−15.86 ± 0.15	−16.37–(−15.06)
	Ringed seal	3.06 ± 0.49	0.87–10.28	17.64 ± 0.18	15.86–19.33	−17.33 ± 0.16	−19.18–(−15.72)
	Bearded seal	1.97		14.26		−16.71	
All		3.45 ± 0.61	0.07–30.60	12.75 ± 0.47	6.02–19.33	−20.76 ± 0.39	−25.11–(−15.06)

differences among age classes were found for Weddell seals ($F = 1.71$, $P = 0.23$) or ringed seals ($F = 2.70$, $P = 0.09$).

The δ^{15}N and δ^{13}C values also differed significantly among age groups for the crabeater seals (One-way ANOVA, $F = 9.53$, $P = 0.004$ and $F = 113.3$, $P < 0.001$, respectively), with the adults of this species showing lower mean δ^{15}N and δ^{13}C values ± standard errors (7.26 ± 0.27‰, −23.38 ± 0.16‰, respectively, $n = 10$) than the juvenile individuals (7.89 ± 0.07‰, −24.67 ± 0.04‰, respectively, $n = 23$) (Fig. 3). However, this difference in δ^{15}N and δ^{13}C values between age groups was not significant for the Weddell seals ($F = 0.65$, $P = 0.55$ and $F = 4.20$, $P = 0.051$, respectively) and the ringed seals ($F = 0.31$, $P = 0.73$ and $F = 1.30$, $P = 0.29$, respectively). Age-specific differences were not tested for in ringed seals due to small sample sizes in age groups yearling and juvenile.

Influence of the species and the geographical area

The average Hg concentration increased in the sequence: crabeater seals (Ant), Ross seal (Ant), bearded seal (Gr), Weddell seals (Ant), ringed seals (Gr), harbour seals (Dk), grey seals (Dk), harbour seals (Gr) (Table 2). Highly significant differences between species were found for log-transformed Hg concentrations (One-way ANOVA, $F = 84.73$, $P < 0.001$) and δ^{15}N and δ^{13}C values ($F = 648.8$, $P < 0.001$ and $F = 537$, $P < 0.001$, respectively), with the crabeater seal showing the lowest Hg, δ^{15}N and δ^{13}C values of all species (Fig. 4a, b). Tukey HSB tests showed that crabeater seals had significantly lower Hg concentrations compared with the other species, and that Hg concentrations in hair of harbour seals were significantly greater than in Weddell seals and ringed seals (Table 2, Fig. 4a). Moreover, the δ^{15}N values differed among the species, except for between harbour seals and grey seals (Table 2, Fig. 4a), while δ^{13}C values also varied

among the species except for between ringed seals and grey seals (Table 2, Fig. 4b).

No significant difference was found for Hg (Wilcoxon, $W = 6$, $P = 0.7$), δ^{15}N ($W = 6$, $P = 0.7$) and δ^{13}C ($W = 6$, $P = 0.7$) contents in hair between harbour seals from Denmark and Greenland; however, it is worth noticing that the number of individuals was low and age groups were not comparable. Significant differences were found for Hg, δ^{15}N and δ^{13}C values in hair from seals collected in the three regions. Subsequent Tukey tests revealed that Antarctic seals had lower values of Hg, δ^{15}N and δ^{13}C while there were no significant differences between Greenland and Denmark (Table 2, Fig. 4). Significant correlations were also found between log-transformed Hg concentrations and δ^{15}N values (Spearman's correlation, $\rho = 0.68$, $P < 0.001$, Fig. 4a, y = 0.133x−1.673), log-transformed Hg and δ^{13}C values ($\rho = 0.83$, $P < 0.001$, Fig. 4b, y = 0.168x + 3.514) and δ^{15}N and δ^{13}C values ($\rho = 0.76$, $P < 0.001$, Fig. 4c, y = 1.064x + 34.835) including all species.

Discussion

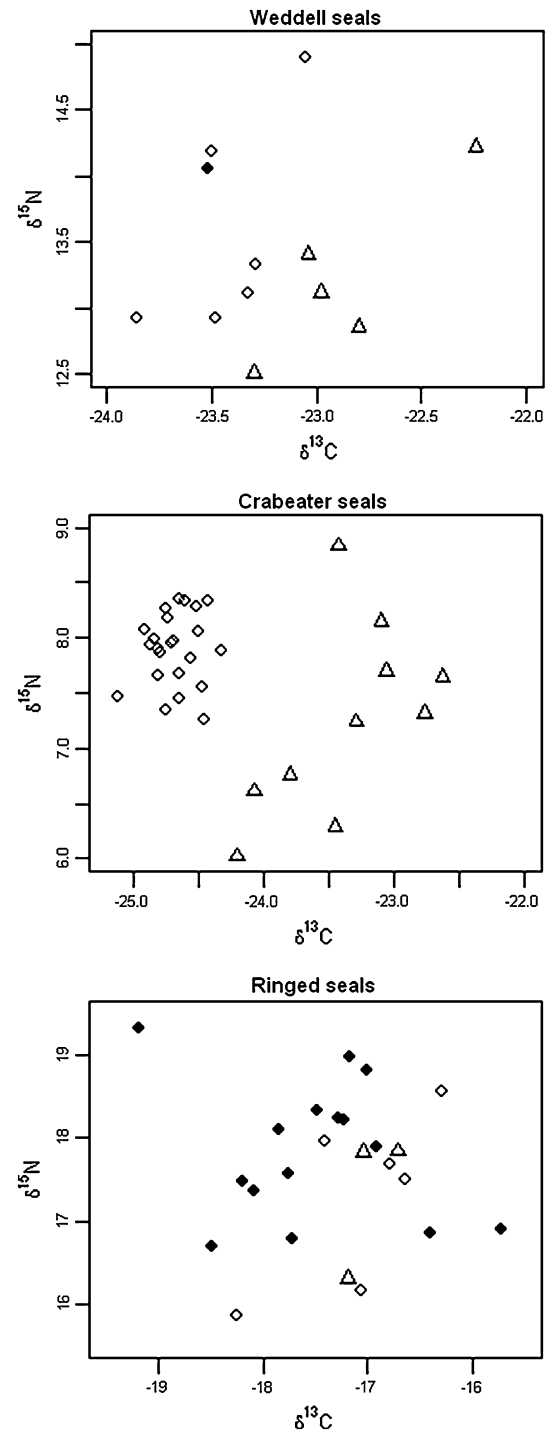Influence of sex and age groups on Hg concentrations in fur

The influence of gender on Hg concentrations varies among studies and species. Thus, a study carried out on fur from Baikal seals *Phoca sibirica* showed no difference in Hg concentrations between genders (Watanabe et al. 1996), whereas Wenzel et al. (1993) observed higher levels and a greater variability in Hg concentrations in fur from female compared with male harbour seals. In the same way, greater concentrations of Hg were found in fur from adult male Pacific harbour seals *Phoca vitulina richardii* and leopard seals *Hydrurga leptonyx* as compared with females
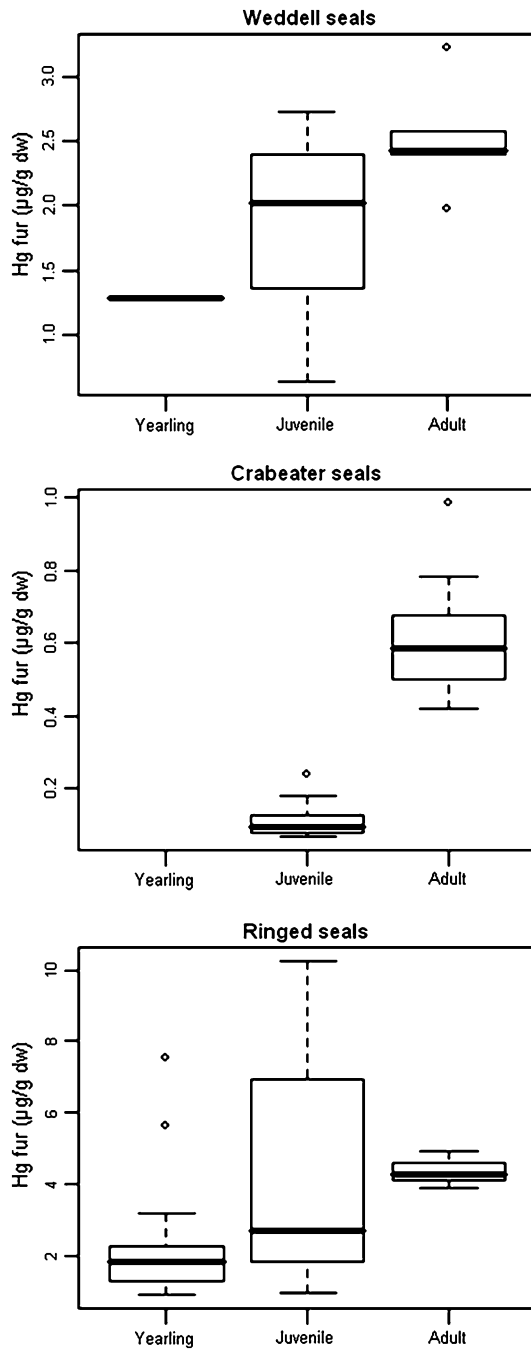
Fig. 2 Comparisons of Hg concentrations in fur (in µg/g dw) among age classes for the Weddell seals, crabeater seals and ringed seals. The *upper* and *lower hinges* represent the quartiles, the *vertical lines* represent the maximum and minimum data values and the *bold line* represents the median value while the points are outliers

Fig. 3 Relations between $\delta^{15}N$ (‰) versus $\delta^{13}C$ (‰) values for the three age classes (*filled diamond* yearling, *open diamond* juvenile, *open triangle* adult) for the Weddell seals, crabeater seals and ringed seals

in both species (Brookens et al. 2007; Gray et al. 2008). Gender-specific differences in Hg concentrations could be the result of sexual dimorphism or dissimilarities in dietary intake or dietary preferences between male and female seals. This could be partially linked to the different energy requirement for gestation and lactation. Crabeater seals do not show any marked sexual dimorphism (Shirihai 2007), and no significant differences for Hg concentrations between male and female crabeater seals were found, which would be consistent with comparable exposure for both genders in this species.
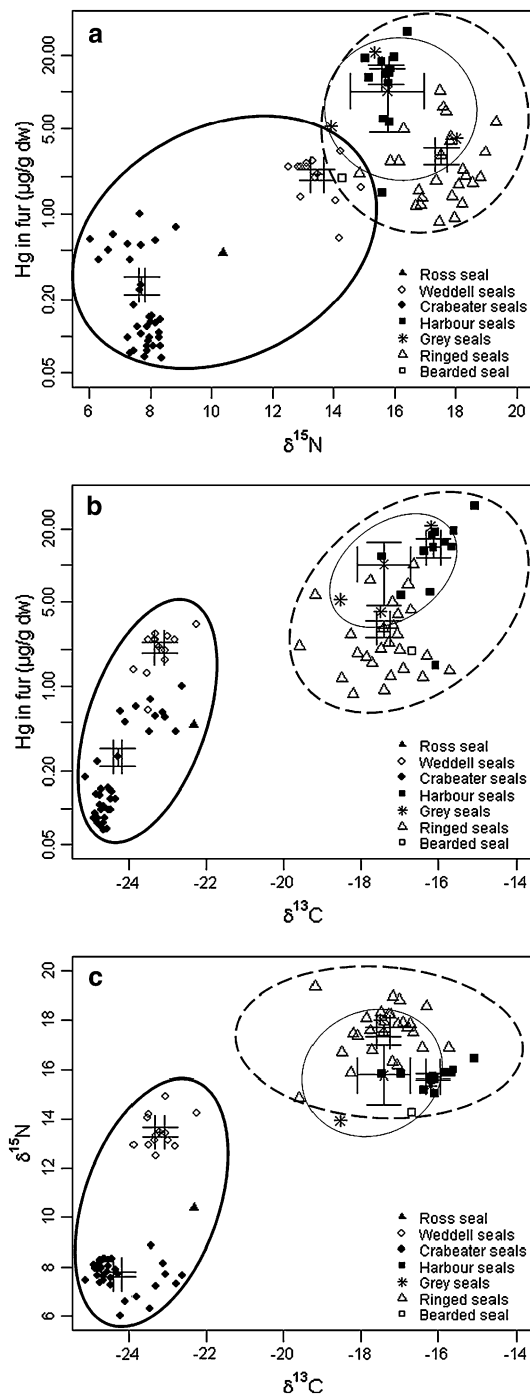
Fig. 4 Relations between Hg concentrations (in μg/g dw) and $\delta^{15}N$ values (in ‰) (**a**), Hg concentrations and $\delta^{13}C$ values (in ‰) (**b**), and $\delta^{15}N$ and $\delta^{13}C$ values (in both ‰) (**c**) in fur of the seven phocid species, with means ± Standard Errors. The three geographical regions are shown by *circles*, *solid thin* for Denmark, *dashed* for Greenland and *solid thick* for Antarctica. Note that the Hg concentrations are plotted on a logarithmic scale

Concentrations of Hg increased significantly with age in crabeater seals, but not for the Weddell seals or the ringed seals (Fig 2). Such age-related differences have been previously reported for harbour seals (Wenzel et al. 1993;

Brookens et al. 2007) and ringed seals (Medvedev et al. 1997). Increasing concentrations with age in our study likely result from a difference in foraging habits between juveniles and adults, which was confirmed by the significant difference in $\delta^{15}N$ and $\delta^{13}C$ values among age classes detected for the crabeater seals (Fig. 3). The greater variability of $\delta^{15}N$ values for the adults compared with the juvenile crabeater seals suggests that older individuals feed on a wider range of prey than younger seals. In addition, the distinct $\delta^{13}C$ values displayed by the juvenile individuals and the adults indicate that they have different feeding habitats. Since $\delta^{13}C$ values are generally greater in benthic or sea-ice algae than in pelagic algae (Fry and Sherr 1984; Tamelander et al. 2006), our results, with significantly lower $\delta^{13}C$ values in the juveniles, suggest that juvenile individuals may forage mainly krill in the water column. Previous findings indicate that despite the high degree of specialisation of this species on a single prey resource, i.e., krill, crabeater seals have the behavioural plasticity to forage successfully under a wide range of environmental conditions, for example, at varying depths and duration of dives and selecting prey in a greater panel if necessary (Burns et al. 2004). Similarly to our study, higher $\delta^{13}C$ values have previously been observed in adult and subadult crabeater seals compared with juveniles and pups by Zhao et al. (2004), though no statistical difference between age classes was found in that study.

Differences in diving and foraging behaviour between adults and juveniles and ontogenetic shifts in diet have previously been documented in several species of phocid seals (Lowry et al. 1980; Reeves et al. 1992; Burns et al. 1998; Holst et al. 2001; Born et al. 2004). Generally, newly weaned pups forage less efficiently and have more limited diving capacity compared with older seals, leading to differences in diet (Reeves et al. 1992; Holst et al. 2001). Similarly to crabeater seals, distinct $\delta^{13}C$ values were observed between juvenile and adult individuals of ringed seals and Weddell seals, suggesting that juvenile seals of those species tend to explore different foraging habitats than the adults. One such example is the dichotomy among Weddell seal yearlings, where some individuals predominantly feed on benthic species in shallow waters, whereas others mainly forage in the same deep-water pelagic zone as the adults do (Burns et al. 1998).

Differences among geographical areas and species (feeding strategies)

Phocid seals renew their fur once a year over a relatively short period, the annual moult, which occurs at different times of year depending on species (Berta 2002). The hair grows rapidly and discontinuously, and concentrations of Hg measured in the hair reflect the availability of Hg to the

growing pile via blood during the annual moult (Berlin 1979). Most seals moult within a two-months period (Stutz 1967), and Hg concentrations in fur result from the diet composition during or prior to the moulting period. In addition, the moulting period occurs from late spring to late summer in both hemispheres. As a result, months during which it occurs differ, and species from the Northern hemisphere tend to moult between May and September, whereas species from the Southern hemisphere moult between December and February (Reeves et al. 1992; Shirihai 2007). Thus, seasonal variations in the origin of Hg, its long-range atmospheric transport and its deposition, including Mercury Depletion Events or MDEs which have been shown to occur at the respective springtime in both Arctic (Schroeder et al. 1998) and Antarctica (Ebinghaus et al. 2002), may have a great influence on the Hg exposure and bioaccumulation in the seals.

Levels of Hg concentrations in fur of species from Antarctica did not exceed 3.5 μg/g dw which was relatively low compared with those from Greenland and Denmark, and earlier studies of Hg levels in hair from Antarctic pinnipeds have not reported concentrations exceeding 11 μg/g dw (Yamamoto et al. 1987; Gray et al. 2008). Although this difference can be related to the fact that the various species have different feeding habits, the global distribution pattern of released Hg may also contribute to those differences in Hg concentrations observed in seals from the Northern and the Southern hemispheres. Indeed, the total deposition pattern of Hg (dry and wet deposition) in the 2000s shows that concentrations of Hg are much lower in the Southern hemisphere than in the Northern hemisphere (AMAP/UNEP 2008).

The variation in stable isotopic values between species from Northern and Southern polar ecosystems could likely reflect geographical differences in the $\delta^{15}N$ and $\delta^{13}C$ values at the base of food webs. Phocid seals from Northern ecosystems generally show greater $\delta^{15}N$ values and significantly greater $\delta^{13}C$ values than those from Southern waters (Fig. 4). However, $\delta^{15}N$ values reported for producers do not differ between both hemispheres. Horton et al. (2009) determined a mean value of approximately 6.5‰ (range between 4 and 8‰) for phytoplankton and 6‰ for ice algae (range between 4 and 7‰) from different studies carried out in the Arctic. Mincks et al. (2008) reported similar mean values in the West Antarctic Peninsula Shelf, ranging from 3.3 to 7.5‰ for phytoplankton and from 4.5 to 6‰ for ice algae. Thus, the $\delta^{15}N$ values found in our study would imply a greater number of steps in the Arctic food web and thus higher trophic levels for seals. Earlier studies have shown that Hg concentrations in biota increase with the trophic levels (i.e. $\delta^{15}N$ values), i.e., predators feeding on high trophic levels usually show higher Hg concentrations than those feeding on lower

levels (Atwell et al. 1998; Campbell et al. 2005). The biomagnification of Hg in the food web is also demonstrated in our study by the greater Hg concentrations as well as $\delta^{15}N$ values exhibited by the Northern hemisphere species.

Concerning carbon stable isotopes ratios, Rau et al. (1982) reported distinct latitudinal trends in plankton $\delta^{13}C$ between the Northern and Southern oceans, with $\delta^{13}C$ values of the Southern ocean decreasing more and faster towards the South Pole than towards the North Pole. The isotopic results of our study illustrate these latitudinal variations of $\delta^{13}C$ values between Northern and Southern oceans, the phocid seal species from Antarctic waters showing lower $\delta^{13}C$ values of approximately 4‰ relative to the Arctic species (Table 2). In addition, some of the variations in $\delta^{13}C$ values could also be due to the influence of the different foraging habitats of the species, which imply various carbon sources, i.e., pelagic, sympagic or benthic. It seems indeed that the sympagic or ice-associated algae, which exhibits approximately mean $\delta^{13}C$ values of −20 to −18‰ in both polar regions (see review in Mincks et al. 2008; Horton et al. 2009) would influence more the $\delta^{13}C$ values of the Arctic seals (mean value of −16.95 ± 0.94‰ (sd)), and especially the ringed seals, than the Antarctic ones (−23.95 ± 0.81‰). Thus, it would indicate a greater participation of the Northern species in the sympagic food web than that of the Southern ones. As a result, according to the type of food web and prey species on which seals feed, the Hg exposure and bioaccumulation would be different.

Thus, the sequence of Hg impregnation was harbour seal > grey seal > ringed seal > bearded seal > Weddell seal > Ross seal > crabeater seal, which may reflect the influence of differences in diet of these species. The crabeater seal showed the lowest Hg (0.26 ± 0.04 μg/g dw) and $\delta^{15}N$ values (7.70 ± 0.11‰) in fur of all investigated seals, which is the result of its very specific diet, almost entirely composed of krill (Bowen et al. 2002). Of the three species from the Southern ocean, the Weddell seal exhibited the greatest Hg concentration (2.09 ± 0.21 μg/g dw) and $\delta^{15}N$ value (13.46 ± 0.21‰). These high values reflect its diet consisting of pelagic fish, which are believed to be the predominant prey items of the Weddell seal in McMurdo Sound, near our sampling area, although this species has also been reported feeding on cephalopods and crustaceans (Burns et al. 1998).

Among the phocid seal species from the Northern hemisphere, harbour seals had greater Hg levels than ringed seals, although a bit lower $\delta^{15}N$ values were found in ringed seals (Table 2). Harbour seals and ringed seals are small pinnipeds, feeding on a large variety of organisms, including fish, cephalopods and various crustaceans (Reeves et al. 1992). Pauly et al. (1998) reported the

harbour seal being on a slightly higher trophic level (TL = 4) than the ringed seal (TL = 3.8). Our sample of Greenlandic harbour seals consisted of adults, whereas ringed seals from Greenlandic waters were mainly represented by yearlings. The elevated δ^{15}N values showed by the ringed seals in our study (17.64 ± 0.18‰) may be caused by the fact that the diet of yearlings partly included milk, which would give this pattern, as also found for Steller sea lions (*Eumetopias jubatus*) (Hobson and Sease 1998). This maternal input to ringed seal yearlings is supported by the greater δ^{15}N values displayed by this age group compared with the adult one. Similarly, the greater Hg concentrations in adult harbour seals could result from that they feed on larger prey with greater burdens of Hg than ringed seal yearlings.

No significant difference in Hg concentrations, δ^{15}N or δ^{13}C values was found between harbour seals and grey seals from Danish waters, which is in concordance with results from Pauly et al. (1998), who estimated both species to the same trophic level at 4. The grey seal feeds on schooling fish, squid, cephalopods and occasionally sea birds (Barros and Clarke 2002), and a great variability in both Hg concentrations and stable isotopic ratio values was observed for this species, reflecting a wide range of prey from various foraging areas, but likely also the low sample size.

Finally, no significant difference was found in Hg contents in hair among harbour seals from Greenlandic and Danish waters, but the sample size was small for this species, and samples from Greenland and Denmark were not comparable since the Greenlandic sample of harbour seals consisted of adults, whereas the Danish sample was composed of a yearling and juvenile animals. However, whereas all individuals showed similar δ^{15}N values, suggesting a more or less similar diet, greater δ^{13}C values found in the Greenlandic harbour seals compared with Danish ones may reflect either a difference in the foraging strategy, related to the age dissimilarity, or a difference in the isotopic carbon signature of the two geographical areas. Furthermore, this difference could also highlight the influence of the ice-associated producers on the Greenlandic seals δ^{13}C values, as shown by the greater δ^{13}C value of ice algae compared with the phytoplankton one (Tamelander et al. 2006; Horton et al. 2009). The greater δ^{13}C values of the Greenlandic harbour seals compared with those from Danish waters may thus underline the role of the sympagic food web in the Arctic marine ecosystem.

Conclusion

This study showed consistent variations in Hg concentrations and δ^{15}N and δ^{13}C values among species and geographical regions. This demonstrates that fur is a good

tissue for monitoring exposure to Hg in phocid seals and for reconstructing their exposure history through their diet, especially with the non-destructive and invasive sampling protocols relative to this tissue. The significant correlation between Hg concentrations and δ^{15}N values highlighted the biomagnification of Hg in the food webs. The comparison of both hemispheres showed the greater Hg concentrations in the Northern one, which would be the result of a greater contamination and/or somewhat longer food chains in the Arctic. Our study confirmed the tight link between Hg exposure and feeding habits (as revealed by δ^{15}N and δ^{13}C values) of seals. Thus, measurements of Hg contents in organisms in an ecosystem can provide information on the trophic levels of species and age groups. In the most optimal way, Hg analysis should be combined with isotopic tracers, although the seasonal and vertical variations in δ^{15}N and δ^{13}C values in the water column and with latitudes may somehow introduce a bias in the interpretation of the results. The use of these analytical tools contributes to a better understanding of local ecosystem functions permitting investigations of partitioning of habitat use and trophic levels of similar species co-existing within the same ecosystem.

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