



Mercury and stable nitrogen isotope ratios in the hair of bearded seals (*Erignathus barbatus nauticus*) from the Sea of Okhotsk

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Abstract

The mercury pollution status in the northwestern Sea of Okhotsk remains largely unexplored. In this study, hair samples were collected from 40 bearded seals harvested between August and October 2021 in the region. Total mercury (THg) concentrations in the samples exhibited a wide range from 137 to 1885 ng/g (median: 407 ng/g). While no significant differences in THg concentrations were found between male and female seals, distinctions were observed between young and potentially mature seals. Stable nitrogen isotope analysis indicated that juveniles and mature adults did not differ, although sample sizes were limiting. The higher THg concentrations in juveniles were attributed to variations in the seals' diets and/or variations in foraging locations during the juvenile stage which likely contribute to THg differences due to greater seasonal migration to offshore habitats. Notably, THg levels in bearded seals from the northwestern Sea of Okhotsk were lower in comparison to other pinniped species in the North Pacific. These findings, representing the first dataset for this pinniped species in the Russian segment of its habitat, contribute insights into mercury exposure in the Sea of Okhotsk mammalian population.

Keywords Bearded seal · *Erignathus barbatus* · Mercury · Stable nitrogen isotope ratios · Pollution · Sea of Okhotsk

Introduction

Mercury (Hg) is a neurotoxic element widely distributed in the environment in trace concentrations. Mercury and all its chemical compounds are hazardous substances (ATSDR 1999). In the pre-industrial period (i.e., before 1800–1840 A.D.), mercury was released into the environment mainly as a result of natural processes, but from the second half of the nineteenth century, anthropogenic sources began to make an increasing contribution to environmental pollution (Driscoll et al. 2013; Lamborg et al. 2014). It is estimated, to date, 92.4% (range from 74.2 to 94.4%) of the total mercury

released into the environment is of anthropogenic origin (Dietz et al. 2009).

Mercury pollution has now become global due to the active transport of this metal around the planet through air mass movement, river runoff, and sea currents (Schuster et al. 2002; Sproveri et al. 2010; Castellini et al. 2012; Cheng et al. 2013; Martin et al. 2017; Liu et al. 2021). The continuous supply of anthropogenic mercury into the marine environment poses a health hazard to all living organisms in marine ecosystems. In general, the marine ecosystem plays the most important role in the global biogeochemical cycle of mercury (Strode et al. 2007; Mason et al. 2012). Once in the water, mercury can enter all living organisms and move freely through the food chains. Mercury accumulates in the body of animals, especially in marine animals located at the top of trophic pyramids (AMAP 2005; Braune et al. 2015; McHuron et al. 2016; Brown et al. 2018). In marine ecosystems, the tops of food pyramids are occupied by marine mammals, including pinnipeds. Due to their trophic level and relatively long lifespan, seals are good bioindicators in studies of environmental pollution by various toxicants and widely used in biomonitoring to assess the state of ecosystems (Bossart 2011; Peterson et al. 2015; McHuron et al. 2016).

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The negative effects of mercury on animal health and seals in particular are well known. An increased amount of mercury in the body can lead to serious illness and death (AMAP 2011; Dietz et al. 2013; McHuron et al. 2016) and even cause population decline (Hyvarinen et al. 1998). Since mercury enters the seals mainly with food (Nordberg et al. 2014), the amount of mercury entering the seals is primarily affected by the trophic level of a particular pinniped species (McHuron et al. 2014). The higher the trophic level of the prey species, the more polluted it is, including with mercury.

Analysis of stable nitrogen isotope ratios ($^{15}\text{N}/^{14}\text{N}$, $\delta^{15}\text{N}$) in animal tissues is used to study nutrition at different trophic levels (Cherel and Hobson 2005; Hobson and Welch 1992; Post 2002; SØReide et al. 2006; Hindell et al. 2012). ^{15}N enrichment occurs with an increase in trophic level, thus using $\delta^{15}\text{N}$ it is possible to determine the food source and trophic position of organisms. The difference in nitrogen isotopic signature between food chain levels can range from 2.5 to 3.5 ‰ (Søreide et al. 2006; Minagawa and Wada 1984; Hobson and Welch 1992; Post 2002; Poupin et al. 2011). The $\delta^{15}\text{N}$ values in animal hair can provide information about the food consumed, thus avoiding invasive testing methods such as gastric or fecal examinations (Peterson and Fry 1987). Previous studies have shown that determination of mercury concentrations and $\delta^{15}\text{N}$ in animal tissues can be useful for studying trophic effects on mercury exposure and accumulation in marine top predators (Aubail et al. 2011; Young et al. 2010; Matias et al. 2022). Quantification of $\delta^{15}\text{N}$ can help determine whether differences in mercury concentrations between species are the result of differences in their diets or mercury levels in habitats.

In recent years, long overdue studies of mercury pollution in pinnipeds from the Russian waters of the northern Pacific Ocean have begun. However, they have so far focused on only two species: Pacific walrus (*Odobenus rosmarus*) from the coastal waters of the Chukchi Peninsula in the Bering Sea (Trukhin and Simokon 2018) and spotted seal (*Phoca largha*) from Peter the Great Bay in the Sea of Japan (Trukhin and Kalinchuk 2018). Similar studies of pinnipeds in the Sea of Okhotsk have not yet been carried out. Moreover, pollution of bearded seals in the Pacific waters of Russia has also not been previously studied.

The bearded seal, or lakhtak (*Erignathus barbatus*), is a circumpolar species of pinnipeds inhabiting the Northern Hemisphere in the Arctic and Subarctic seas. The Sea of Okhotsk is one of the most densely populated areas of the bearded seals in the North Pacific Ocean (Belikov et al. 2017). Two reproductive groups of the bearded seals are distinguished within the Sea of Okhotsk. Throughout the year, they form geographically isolated dense concentrations: one lives in the northeastern part of the sea, and the other lives in the western part, including the Sakhalin Gulf and in the East Sakhalin waters (Fedoseev 2000). The bearded seals

are unevenly distributed in different coastal areas of the Sea of Okhotsk. This is determined by a number of factors, including the ice regime, the orography of the coastline, the prevailing depths, and seasonal availability of different food items. Pups begin to independently obtain food during the period of milk feeding (Chapskii 1938), i.e., less than one month old. The similar pattern of mother and pup foraging dives indicates that pups begin to imitate their mothers' foraging behavior at a very early age (Lydersen et al. 1996; Watanabe et al. 2009). Any differences in the feeding pattern (choice of food) of immature and adult bearded seals have not been described. The densest concentrations of the bearded seal in the Sea of Okhotsk in summer are observed in its western part, in particular in Academy Bay (Freiman 1936; Pikharev 1941). According to Fedoseev (2000), Academy Bay is included in the western reproductive group of bearded seals. In this area, the seals are most numerous in the summer-autumn and remain here at least until the beginning of the ice formation, actively feeding before the winter. The distribution of the bearded seals in the Sea of Okhotsk in winter has only been studied in general terms. The results of aerial observations and satellite telemetry indicate that bearded seals spend the winter throughout the Sea of Okhotsk, in shallow waters covered with dense, almost continuous (9–10 points), ice cover (Kosygin et al. 1984; Solovyova et al. 2021).

The bearded seal is a typical benthophage. The basis of its diet is benthic invertebrates, as well as some species of benthic fish (Kosygin 1971; Cameron et al. 2018; Belikov et al. 2017). In comparison with other pinnipeds from the North Pacific, the bearded seal feeds at a relatively low trophic level, suggesting a moderate level of contamination with toxic substances. However, this has not yet been confirmed, because bearded seal mercury contamination has not yet been studied in the Far Eastern seas of Russia.

The western Sea of Okhotsk, including Academy Bay, where we conducted our study, belongs to those marine habitats that have been least affected by anthropogenic impact so far. However, mining is planned in some mainland areas bordering this bay. This could lead to the entry of various pollutants into the rivers flowing into the bay. For this reason, it is necessary now to start conducting research to determine the baseline state of the coastal ecosystem before these changes occur. It should be noted that to date there are no data on the distribution and behavior of mercury in the environment of the western Sea of Okhotsk.

The purpose of our study was to quantify mercury contamination of bearded seals in the western Sea of Okhotsk, which is one of the most densely inhabited areas by this species in summer and autumn. The study included: (1) determination of total mercury (THg) concentrations and stable nitrogen isotope ratios ($\delta^{15}\text{N}$) in the hair of bearded seals, (2) identifying the following relationships: THg concentrations

versus $\delta^{15}\text{N}$, THg concentrations and $\delta^{15}\text{N}$ versus sex and age of seals, and (3) comparing our data with similar data for other species of pinnipeds from the North Pacific.

Materials and methods

Area description

Academy Bay is located in the western part of the Sea of Okhotsk (Fig. 1). The bay is shallow (maximum depths: 52 m), the length of its strongly indented coastline is 110 km, the square of water area is 1800 km². Academy Bay with the adjacent water area is separated from the Sea of Okhotsk by the Shantar Islands. The bay is fairly enclosed and was previously called the Shantar Sea, which emphasized its spatial isolation (Zaks 1929; Goncharov 1930; Lotsia 1960).

The waters of Academy Bay are very dynamic with strong tidal currents. They are characterized by significant concentrations of nutrients during the warm season, which causes intensive production of phyto- and zooplankton (Tishchenko et al. 2022). The bottom of the bay apex

is heavily silted (Lotsia 1960). The source of silt is several rivers flowing through valleys with sphagnum bogs and supplying a significant amount of humus matter to the bay (Melnikov et al. 2020). These rivers are a place of mass migration and spawning of salmon. During tidal periods, the water from the bay rises along the rivers against their flow by 35–40 km (Makhinov et al. 2017). The benthic biomass on the northwestern shelf of the Sea of Okhotsk is quite high ($359.5 \pm 44.4 \text{ g/m}^2$); more than 70% are bivalves, echinoderms, sponges, and polychaetes (Koblikov et al. 1990). All this is the basis for the formation of an abundant food source that supports upper trophic level vertebrates.

One of the features of Academy Bay is the lack of settlements on its coast. The nearest villages (Chumikan and Tugur) are located to the west, on the coast of Tugursky Bay and Udskey Bay. The total population in these settlements is a little more than 1000 people, whose main activities are fishing, reindeer husbandry and logging. There are no industrial enterprises here. Thus, there is almost no direct anthropogenic impact on the study area.

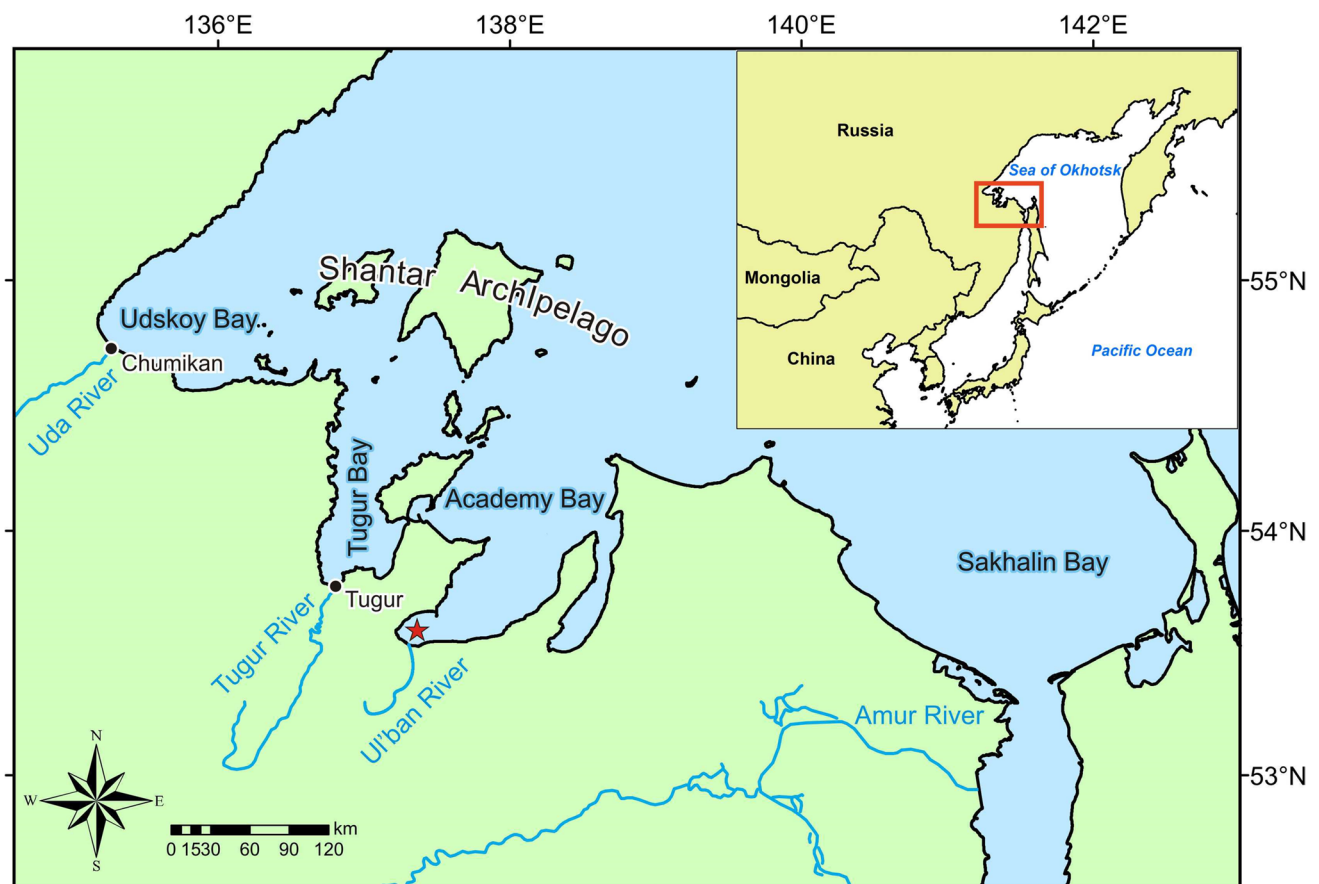


Fig. 1 The sampling site (red asterisk) in Academy Bay, the Sea of Okhotsk

In winter, Academy Bay and the entire adjacent waters are covered with dense ice for 8–9 months. The ice formation begins here in October. The ice remains at least until July, longer than in any other parts of the Sea of Okhotsk (Plotnikov 2002). Annually, after the water becomes free of ice, a large number of marine mammals arrive (cetaceans and pinnipeds). Coastal waters have always been an important area of summer and autumn feeding for them (Goncharov 1930; Pikharev 1941; Berzin and Vladimirov 1988). Among pinnipeds, reproductive-aged bearded seals are most common here. Their habitat is limited to the western Sea of Okhotsk, beyond which bearded seals do not go (Fedoseev 2000).

Animal handling and sampling

Hair samples were taken from 40 bearded seals (14 males, 26 females) legally harvested for the production of seal oil in Academy Bay from August 18 to October 3, 2021. The extraction of seals was carried out by aboriginal hunters in a very limited coastal water area directly adjacent to the mouth of the Ulban River. Reference information about each seal was recorded in the official document “Journal of Biological Resource Extraction” and in individual cards indicating the date of extraction and the details about the animal including its sex and fat mass.

Hair samples were collected from the fore flippers of seals near the claws. The hair cover is denser and longer in this place. The hair was cut to the base with stainless steel scissors.

Age determination

To determine age of seals, we used a well-established method based on the calculation of growth layers in their claws, following a methodology pioneered by Plekhanov (1933) and refined by Chapsky (1952). This approach, while recognized as the simplest and most reliable means of age determination, presents a notable limitation. Claw growth persists throughout a seal's lifespan, with the ends eventually experiencing wear or breakage (Benjaminsen 1973). Consequently, determining the precise age of older bearded seals can be biased negatively. However, the method is particularly effective for younger seals up to the age of sexual maturation, offering a means of assessing age classes: pups, juveniles, and mature adults. The reliability and simplicity of this technique make it a crucial tool for understanding the age dynamics of seal populations, with a specific emphasis on the developmental phases leading up to sexual maturity.

Among the 40 bearded seals included in the study, there was one pup (estimated 5–6 months old); the rest of the seals were aged one year and older. Bearing in mind that the lactation period of the bearded seal lasts less than one month

(Cameron et al. 2010), by the time this young animal was harvested, it had been feeding on its own for several months. All animals included in the sample were divided into two groups: young and adults (Table 1). The first group included seals aged 0–3 years ($n = 10$); the second group included seals aged 4 years and older ($n = 30$). Seals included in the first group (0–3 years) were considered immature, and seals aged 4+ years were potentially sexually mature, since this was the minimum age when ovulation of individual females could end with the first pregnancy (Burns and Frost 1983; Bukhtiyarov 1990; Trukhin 1991). Three years of age is the earliest age when the early maturing females of bearded seals from the North Pacific are able to ovulate for the first time (no more than 10% of the total number of females of this age), but pregnancy does not occur at this age. The female is able to give birth to the first pup at the age of 5 years (Burns 1967; Fedoseev 1973; Bukhtiyarov 1990). The same reproductive parameters characterize the bearded seal living in the Sea of Okhotsk (Trukhin 1991). In this regard, we included all three-year-old females in the group of conditionally immature and pre-reproductive.

Mercury analysis

The hair samples were collected in zip bags and stored in a light-proof bag at ambient temperature. Before the analysis, the samples were manually cleaned of dirt and sand and then thoroughly washed 3 times with a 1% solution of a nonionic detergent (Triton X-100, manufacturer AMRESCO LLC, OH USA) and 3 times with distilled water. After drying in a freeze dryer, the samples were immediately analyzed.

Total Hg concentrations in hair were measured using a RA-915 M atomic absorption spectrometer and a PYRO-915 + pyrolysis attachment (Lumex Ltd., St. Petersburg, Russia) (Sholupov et al. 2004). The detection limit of the method is 2.5 ng/g. The certified reference material ERM-DB001 was used to check the accuracy of the analysis of the mercury concentrations. The recovery for the reference material was $95.0 \pm 3.4\%$ (mean \pm SD). The details of the operation and set-up of this type of mercury analytical complex have been described in many previous studies (Quiñones et al. 2013; Lu et al. 2016; Trukhin and Kalinchuk 2018; Aksentov and Satarova 2020; Ryazanov et al. 2023). Briefly, the sample was placed into the pyrolyzer and heated to 750 °C. As a result, mercury contained in the sample was atomized and entered the AAS detector employing Zeeman modulation polarization spectroscopy with high-frequency modulation. The average weight of the hair sample was 23 mg. The samples were analyzed from 3 to 5 replicates and the mean value was taken for statistical analysis. The average coefficient of variation between the repeated measurements was $4.0 \pm 3.6\%$ (mean \pm SD).

Table 1 Statistics of THg concentrations (ng/g) and $\delta^{15}\text{N}$ (‰) in various samples of the examined bearded seals

Young (1–3 years)		Adults (4+ years)		Total
Male	Female	Male	Female	Male
<p>$N=7$ THg: range: 253–1885; mean \pm SD: 1085 \pm 692; median: 1135; $\delta^{15}\text{N}$: range: 15.0–17.8; mean \pm SD: 16.4 \pm 1.1; median: 16.2</p>		<p>$N=3$ THg: range: 265–618; mean \pm SD: 450 \pm 177; median: 468; $\delta^{15}\text{N}$: range: 15.3–16.3; mean \pm SD: 15.7 \pm 0.5; median: 15.5</p>		<p>$N=23$ THg: range: 137–582; mean \pm SD: 318 \pm 167; median: 298; $\delta^{15}\text{N}$: range: 13.9–16.7; mean \pm SD: 16.0 \pm 0.9; median: 16.3</p>
<p>$N=10$ THg: range: 253–1885; mean \pm SD: 894 \pm 648; median: 552; $\delta^{15}\text{N}$: range: 15.0–17.8; mean \pm SD: 16.2 \pm 1.0; median: 16.2</p>		<p>$N=7$ THg: range: 175–897; mean \pm SD: 453 \pm 227; median: 378; $\delta^{15}\text{N}$: range: 14.1–17.4; mean \pm SD: 15.7 \pm 1.0; median: 15.5</p>		<p>$N=14$ THg: range: 137–1885; mean \pm SD: 701 \pm 626; median: 476; $\delta^{15}\text{N}$: range: 13.9–17.8; mean \pm SD: 16.2 \pm 1.0; median: 16.3</p>
				<p>$N=26$ THg: range: 175–897; mean \pm SD: 453 \pm 219; median: 389; $\delta^{15}\text{N}$: range: 14.1–17.4; mean \pm SD: 15.7 \pm 1.0; median: 15.5</p>
				<p>$N=40$ THg: range: 137–1885; mean \pm SD: 534 \pm 419; median: 407; $\delta^{15}\text{N}$: range: 13.9–17.8; mean \pm SD: 15.9 \pm 1.0; median: 15.9</p>

Isotopic analysis

Determination of the isotopic composition in hair samples was carried out in duplicate using an isotope mass spectrometer Delta V Advantage coupled with flow interface ConFlo IV (Thermo Fisher Scientific Inc., Bremen, Germany). Similar analyzers have been used in various studies devoted to the investigation of nutrition through the determination of the isotopic composition in tissues, both in humans and animals: human fingernails (Nardoto et al. 2020), human hair (Fauberteau et al. 2021), seal hair (Aubail et al. 2011), and wild boar hair (Vedel et al. 2022). Reference Material USGS40 (L-glutamic Acid) and Reference Material USGS42 (Isotopes in Tibetan Human Hair) were used in this study. Repeated measurements of reference materials were performed every 10 samples. The standard deviation was $\pm 0.2\text{‰}$ for $\delta^{15}\text{N}$ ($n=9$).

Before analysis, hair samples were ground to a few millimeters, washed 3 times for 40 min in an ultrasonic bath (Derui ultrasonic cleaner DR-MS07) in a chloroform–methanol solution (2:1) to remove lipids (O’Connell et al. 2001). After washing, the samples were dried in a vacuum desiccator for 2 days. The dried samples were weighed and wrapped in tin capsules. The weight of the samples for isotopic analysis ranged from 300 to 360 μg . The isotopic composition (δ) is expressed in thousandths of a deviation (‰) from the standard material:

$$\delta E_n = [(R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}}] \times 1000,$$

where E is the element (N); n is the atomic mass of the heavier isotope; and R_{sample} and R_{standard} are the molar ratio of heavy and light isotopes in the sample and standard, respectively.

Statistical analysis

Statistical analyses were performed using a data analysis software system STATISTICA, version 10 (StatSoft, Inc.). Graphical methods and statistical tests (the Kolmogorov–Smirnov test for normality, the Shapiro–Wilk W test) were used to determine whether sample data were normally distributed. The median with interquartile range (IQR) and the arithmetic mean with standard deviation (SD) were used to describe the THg and $\delta^{15}\text{N}$ data, respectively. We also calculated the arithmetic mean and SD to compare our mercury data with the results of previous studies where medians were not determined. The data for different age and sex categories of seals were compared. We used ANOVA to compare both isotopic data and log-transformed mercury values across samples. Using a two-factor ANOVA, the influence of sex and age

on the values of mercury and $\delta^{15}\text{N}$ was examined. The level of significance for all tests was taken to be $p < 0.05$. We analyzed the interaction between sex and age. Post-hoc test (Tukey HSD) was used to compare the samples. We examined the relationships between mercury concentrations and $\delta^{15}\text{N}$ using the Pearson correlation coefficient ($p < 0.05$).

Results

Mercury in hair

Mercury was detected in all the examined samples. THg concentrations in the seals' hair varied from 137 to 1885 ng/g (dry weight) with an arithmetic mean (\pm SD) of $540 (\pm 419)$ ng/g (Fig. 2, Table 1). The highest concentrations (more than 1000 ng/g) were found in four young (1–3 years old) males.

THg concentrations in young seals (0–3 years, $n = 10$) varied from 253 to 1885 ng/g (mean: 894 ± 648 ng/g), and from 137 to 897 ng/g (mean: 421 ± 220 ng/g) in potentially sexually mature animals (4+ years, $n = 30$) (see Fig. 2). THg concentrations of 137–1885 ng/g (mean: 701 ± 626 ng/g; $n = 14$) were measured in males. THg concentration in females ranged from 175 to 897 ng/g ($n = 26$), with a mean of 453 ± 219 ng/g.

Two-factor ANOVA revealed a significant effect of age on mercury level ($F = 6.5$, $p = 0.02$), but no effect of sex ($F = 0.6$, $p = 0.5$). A significant interaction between sex and age was found ($F = 5.4$, $p = 0.03$). The post-hoc test showed a significant difference in THg concentrations between the following samples: young males vs. adult males ($MS = 0.06$, $df = 36$, $p = 0.02$) and young males vs. adult females ($p = 0.004$) (Table 2). However, no significant differences in THg concentrations were found between young and adult females ($p = 0.9$), adult males and females ($p = 0.5$), young females and adult males ($p = 0.7$), and young males and females ($p = 0.3$).

Stable nitrogen isotopes in hair

Stable nitrogen isotope ratios ($\delta^{15}\text{N}$) in the seals' hair ranged from 13.9 to 17.8 ‰, with a mean of 15.9 ± 1.0 ‰ (Fig. 2, Table 1). The $\delta^{15}\text{N}$ in males ranged from 13.9 to 17.8 ‰ (mean: 16.2 ± 1.0 ‰). The $\delta^{15}\text{N}$ in hair of females ranged from 14.1 to 17.4 ‰, with a mean of 15.7 ± 1.0 ‰. The $\delta^{15}\text{N}$ in the group of young seals varied from 15.0 to 17.8 ‰ (mean: 16.2 ± 1.0 ‰). The $\delta^{15}\text{N}$ in potentially sexually mature seals varied from 13.9 to 17.4 ‰, with a mean of 15.8 ± 1.0 ‰.

Two-factor ANOVA showed that age ($F = 0.3$, $p = 0.6$) and sex ($F = 1.7$, $p = 0.2$) did not affect the value of $\delta^{15}\text{N}$ in hair. No interaction between age and sex was found ($F = 0.2$,

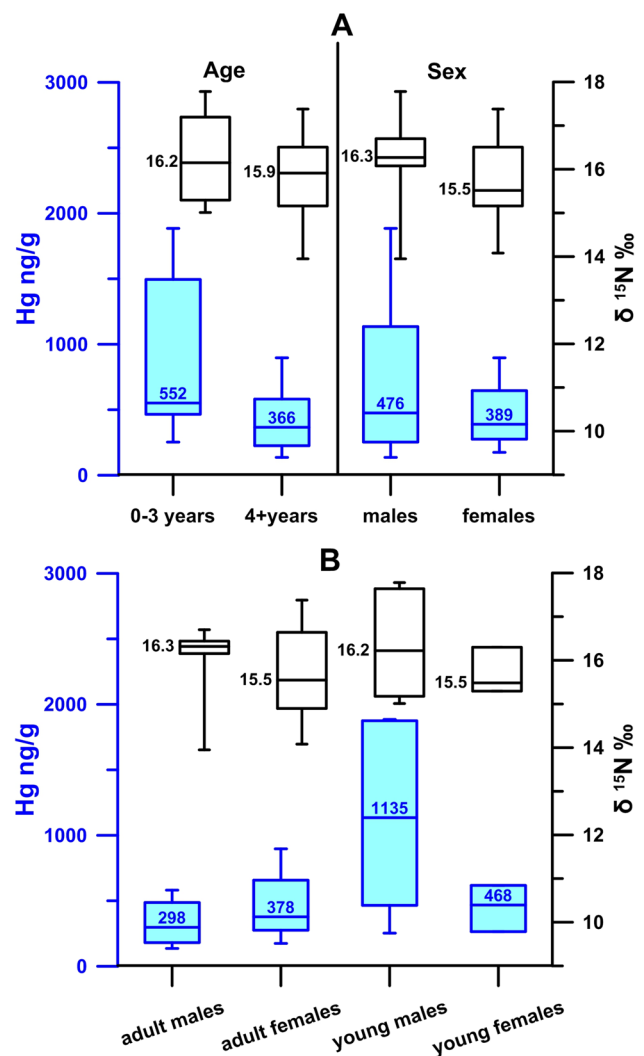


Fig. 2 THg concentrations (in ng/g dw) and $\delta^{15}\text{N}$ values in the hair of seals of different sexes and ages (A) and in age classes by sex (B). The horizontal lines in the boxes indicate the 25th, median, and 75th percentiles. The top and bottom whiskers indicate maximum and minimum values

Table 2 p-levels of the post-hoc test (Tukey HSD: $MS = 0.06$, $df = 36$) with ANOVA comparing log-transformed mercury concentrations between different samples

Samples	Adult females	Young females	Adult males
Young females	0.9		
Adult males	0.5	0.7	
Young males	0.02	0.3	0.004

$p = 0.6$). Significant relationships were found between THg concentrations and $\delta^{15}\text{N}$ both for the entire sample ($R^2 = 0.2$; $p = 0.004$) and for young seals ($R^2 = 0.5$; $p = 0.02$) (Fig. 3).

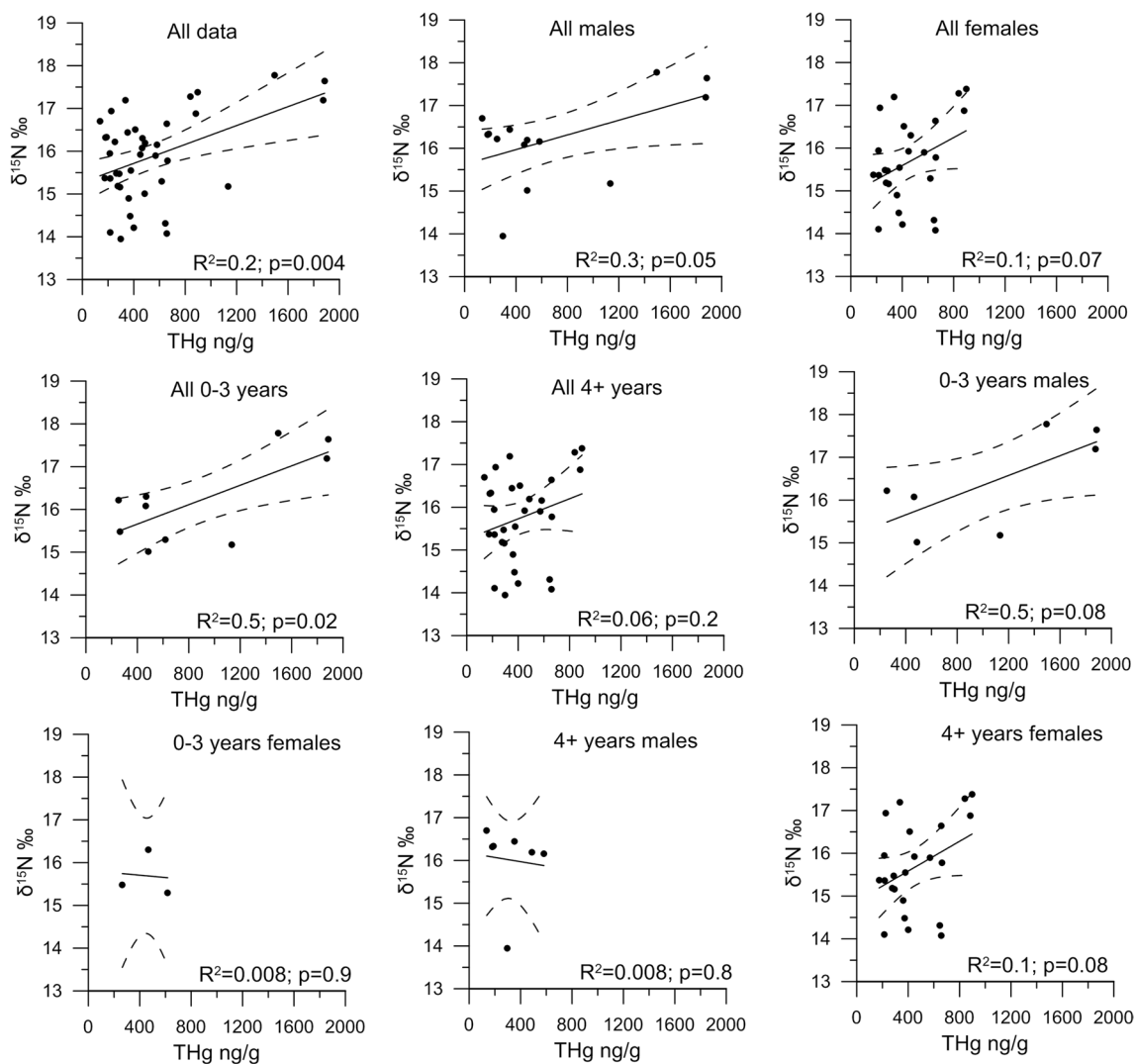


Fig. 3 Relationships between THg concentrations and $\delta^{15}\text{N}$ in the seals' hair. Dashed lines represent 95% confidence interval

Discussion

Differences in THg levels and $\delta^{15}\text{N}$ between sex and age groups

Significant correlations between THg concentrations and $\delta^{15}\text{N}$ were previously found both in the hair of bearded seals and other seal species indicating the influence of the trophic level of prey on the mercury content in predators (Aubail et al. 2011). In our study, we also found significant relationships between THg concentrations and $\delta^{15}\text{N}$ in the entire sample and in the young seals sample (Fig. 3). THg concentrations in males (mean: 701 ng/g; median: 476 ng/g) were higher than those in females (mean: 453 ng/g; median: 389 ng/g), but not statistically different ($MS=0.06$, $df=36$, $p=0.3$). Perhaps this was due to the insufficient number of samples ($n=40$). Also, there were no statistically

significant differences in $\delta^{15}\text{N}$ values between males and females ($MS=1.05$, $df=36$, $p=0.1$), indicating no trophic separation between male and female seals. The absence of differences in $\delta^{15}\text{N}$ by sex was also noted in other organs of bearded seal (Young et al. 2010). Pinniped females generally contain reduced concentrations of mercury in the organs (tissues) in comparison with males (Brookens et al. 2008; Brown et al. 2018). The reason for this phenomenon is the transfer of pollutants from mother to fetus during prenatal ontogenesis through a single circulatory system, and during lactation with milk. During each reproductive cycle, the body of a pregnant and subsequently lactating female loses some amount of toxicants, including mercury. For this reason, the level of mercury in the organs of adult females is often relatively low. Such a phenomenon has not yet been described for bearded seal, but the fact of transplacental and lactational transfer of mercury is established for many

species of pinnipeds (Wagemann et al. 1988; Habran et al. 2011; Castellini et al. 2012; Rea et al. 2013; Nehring et al. 2017; Trukhin and Kalinchuk 2018; Simokon and Trukhin 2021). It is most likely that such dependence is also true for bearded seals. We were unable to determine pregnancy status of the mature females that came to our processing, since internal organs were absent in the bodies of hunter-harvested bearded seals by the time we received the carcasses. Therefore, all seals of this sex were included in the “female” group, regardless of their physiological state.

We compared mercury levels in young (3 years and younger) and potentially matured (4 years and older) seals. Previous studies indicate that mercury levels in adult pinnipeds of the North Pacific are higher than in juveniles (or pre-reproductive individuals) (Elorriaga-Verplancken and Auriolles-Gamboa 2008; McHuron et al. 2016; Peterson et al. 2016; Brown et al. 2016; Trukhin and Simokon 2018), including the bearded seal (Smith and Armstrong 1978). The free movement of mercury through food chains, its ability to bioaccumulate, as well as the relatively long lifespan of pinnipeds can explain this difference. Some surprise of our study was that THg concentrations in young males were significantly higher (mean: 1085 ng/g) than those in adult males (mean: 318 ng/g) and adult females (mean: 453 ng/g). Other researchers have previously obtained similar results indicating a higher mercury level in the muscle tissue and liver of young bearded seals than in adults (Young et al. 2010). However, in our study, no significant difference in THg concentrations was found between young and adult females. It is possible that the age effect could not be detected for female because of a sample size issue. Although THg concentrations were higher in young males than in young females (Table 1), no significant difference was found between these groups (Table 2). We have also found no significant difference in $\delta^{15}\text{N}$ between young males and young females. There is no published evidence of differences in diet between young males and females of bearded seal. We believe that we do not have sufficient evidence to suggest that young males had a different diet than young females that could influence mercury levels in these groups. There was also no significant difference in THg concentrations between adult males and females. Thus, we believe that sex did not effect THg concentrations in each age class.

Organic and inorganic pollutants enter animals in different ways: by absorption through the external integument, with inhaled air, with seawater, but mainly with food (Loseto et al. 2008; Rea et al. 2013; Nordberg et al. 2014; Peterson et al. 2015). Consequently, the mercury content in the seal organs depends on the content of this metal in food. One of the assumptions was that the differences we found in THg concentrations in the hair of young and adult bearded seals could be the result of differences in the diets of seals of different ages. Studies of the bearded seal diets have shown

that such differences do exist (Lowry et al. 1980; Crawford et al. 2015). Isotope analysis of the bearded seal tissues from Hudson Bay showed that the trophic level of young bearded seals was higher than that of adults within the same water area; therefore, young seals were exposed to higher levels of mercury (Young et al. 2010). However, there is no published data indicating a difference in diet between young males and females. In this study, we did not find significant differences between the $\delta^{15}\text{N}$ values in the hair of young and adults, young males, and females of bearded seals from the Sea of Okhotsk, which in theory indicated that they were at the same trophic level and had a similar diet. At the same time, a significant positive relationship was observed between mercury and $\delta^{15}\text{N}$ values in young animals (Fig. 3). In our study, the maximum values of both mercury and $\delta^{15}\text{N}$ were found in this age group. We believe that the lack of significant differences between the $\delta^{15}\text{N}$ values in the hair of juveniles and adults could be due to small sample size.

The second potential explanation was that the differences between the mercury content in bearded seals of different ages might be a consequence of the spatial differentiation of young and adult animals during a certain period of the year due to age differences in habitat use. For example, satellite tracking of tagged bearded seals showed that young animals tended to live near the ice edges, while adult animals lived in the areas with heavier pack ice (Cameron et al. 2018). We assume that young and adult seals fed at the same time in different areas with different mercury levels in the marine environment.

In this study, the area where bearded seals were harvested was very limited spatially. Bearded seals fed on those species of benthic invertebrates that inhabited the inner part of Academy Bay. Prior to the appearance in Academy Bay, bearded seals of different ages could inhabit different areas of the Sea of Okhotsk where they moulted on the ice in late spring and early summer. It was during this period that mercury entered the seals' hair from their bodies. The mercury we found in the hair of young and adult seals entered the hair even before the animals arrived in Academy Bay and reflected the mercury concentration in their bodies during the molting period. The level of mercury in the bearded seals harvested in Academy Bay, in any case, was formed within the exclusive western Sea of Okhotsk, beyond which the seals of this reproductive group, according to existing knowledge (Fedoseev 2000), did not go. Similar conclusions about the distribution of bearded seals in the Sea of Okhotsk were made based on satellite tagging of seals (Solovyova et al. 2021).

It is believed that the bearded seal is a migratory species (Potelov 1969; Burns and Frost 1979; Cameron et al. 2010; Melnikov 2017), although much about the migrations of this species still remains unknown. This circumstance allows for at least a temporary presence of seals in areas where any

anthropogenic activity is carried out. The available information about the bearded seal migrations indicates that their spatial inter-seasonal movements can be quite extensive and seasonal movements tied to habitat preferences. Satellite tagging of bearded seals from the Beaufort Sea, Chukchi Sea and the northern Bering Sea showed that these pinnipeds could widely move in the seas (Frost et al. 2008; Boveng et al. 2012; Cameron et al. 2018). Recent studies of bearded seal migrations in the western part of the Sea of Okhotsk have shown that the length of inter-seasonal movements of these seals is also significant and can exceed 1000 km, and migration routes run through water areas affected by human activities (Solovyova et al. 2021). The Amur River, which flows through the territories of Russia and China and is one of the largest rivers in Asia, flows into the Sakhalin Bay, located 200 km east of Academy Bay. The Amur River basin is under strong anthropogenic pressure due to intensive economic development that includes activities accompanied by noticeable releases of mercury into the environment through poor waste management and accidental discharges (Kot et al. 2010). It can be assumed that bearded seals feeding in summer and autumn in Academy Bay may be more widespread in the western Sea of Okhotsk and visit Sakhalin Bay in other seasons. Be that as it may, mercury concentrations in the hair of the studied animals reflected the degree of mercury contamination of bearded seals from the local reproductive group inhabiting the western Sea of Okhotsk, regardless of which location area within this area was used for breeding, molting, and foraging. Taking into account the different spatial distribution of bearded seals of different ages during the ice period (Cameron et al. 2018), the differences in THg concentrations in the hair of young and adult bearded seals from the western Sea of Okhotsk could be caused by the different mercury content in the areas where the animals of different ages inhabited in the winter-spring period preceding molting. It is possible that during migration, young seals move longer distances, while adult seals are more likely to adhere to a limited home range, especially during the breeding season (mating, birth, and rearing of offspring).

Comparison of our data with data from other regions and other pinniped species

The mean THg concentration in the hair of bearded seals from the Sea of Okhotsk was 540 ng/g with a significant difference between young (mean: 894 ng/g) and adult animals (mean: 421 ng/g). These values were lower than all previously detected mercury levels in the hair of other pinniped species of different ages from the North Pacific (Table 3). We compared our data with data on mercury and $\delta^{15}\text{N}$ in seal hair from other areas previously published by Aubail et al. (2011). Mercury levels in bearded seals from

the Sea of Okhotsk were lower than in harbour seals (mean: 7790 ng/g) and grey seals (mean: 10,110 ng/g) from Denmark, harbour seals (mean: 16,270 ng/g) and ringed seals (mean: 3060 ng/g) from Greenland, Weddell seals (mean: 2090 ng/g) from Antarctica. Our mercury data were at the same level as in Ross seal (480 ng/g in one animal) and higher than in crabeater seals (mean: 260 ng/g) from Antarctica.

Basic values of nitrogen isotopes can vary in different regions, which is due to the physicochemical and biological characteristics of water masses (Jennings and Warr 2003; Hansen et al. 2012; Oczkowski et al. 2016). Previous studies conducted in the Bering Sea and Northeast Pacific Ocean showed spatial variations in $\delta^{15}\text{N}$ values in both producers and consumers (Altabet et al. 1999; Voss et al. 1996, 2001; Schell et al. 1998; Newsome et al. 2010; Zeppelin and Orr 2010). To date, there are no any data on the distribution of nitrogen isotopes in the Sea of Okhotsk, so we compared our data with the $\delta^{15}\text{N}$ values in the fur of seals of various species living both in nearby areas of the Pacific Ocean and in other regions. We estimated the expected diet of bearded seals from the Sea of Okhotsk using data from Hindell et al. (2012), in which the $\delta^{15}\text{N}$ values in the hair of bearded seals from Svalbard (14.9 ± 0.94 ‰) were comparable to our data.

Our $\delta^{15}\text{N}$ data (mean: 15.9 ‰) were consistent with the mean $\delta^{15}\text{N}$ in the hair of gray seal (15.76‰) and harbour seal (15.75‰) from Denmark and harbour seal from Greenland (15.7‰) (Aubail et al. 2011). Lower $\delta^{15}\text{N}$ relative to our data was observed in northern fur seal (14.8 ‰, 14.9 ‰) from different islands of the Bering Sea (Kurle and Worthy 2002), Weddell seal (13.46 ‰), crabeater seal (7.7 ‰), and Ross seal (10.38 ‰) from Antarctica. Higher $\delta^{15}\text{N}$ values (17.64 ‰) have previously been observed in ringed seal from Greenland.

Gray seal, harbour seal, ringed seal are primarily ichthyophagous, with $\delta^{15}\text{N}$ in their hair comparable to or higher than those of bearded seal that feed on benthic organisms. Crabeater seal feeds mainly on krill and $\delta^{15}\text{N}$ in its hair was lower than in bearded seal. Although northern fur seal, Ross seal, and Wedell seal are also primarily ichthyophagous, $\delta^{15}\text{N}$ in their hair were also lower than in the study samples. We speculate that there may be differences in baseline values between different areas and our study area that cause differences between seals at the same trophic level. At the same time, a higher $\delta^{15}\text{N}$ value in bearded seals does not indicate that they are at a higher trophic level than other seals (Young et al. 2010). Benthic food webs may be enriched in $\delta^{15}\text{N}$ compared to pelagic food webs. The $\delta^{15}\text{N}$ values for benthic and pelagic animals are enriched differently (Iken et al. 2005; Macko and Estep 1984). High values of $\delta^{15}\text{N}$ have been noted in benthic invertebrates, most likely due to the decomposition of dead organic material, including organisms of a higher trophic level, which is deposited on

Table 3 Total Hg concentrations (range and mean \pm SD; $\mu\text{g/g dw}^*$, except as noted) in the hair of pinniped species from different parts of the North Pacific Ocean

Age	Area	n	Range	(Mean \pm SD)	Year	References
Bearded seal, <i>Erignathus barbatus</i>						
Male, female, 0–3 yr	Sea of Okhotsk	10	0.25–1.89	0.89 ± 0.65	2021	This study
Male, female, 4+ yr	Sea of Okhotsk	30	0.14–0.90	0.42 ± 0.22	2021	This study
Phoca largha, <i>Phoca largha</i>						
Pups, 2.5–4 weeks	Sea of Japan	138	1.52–6.68	2.88 ± 0.89	2014–2017	Trukhin and Kalinchuk 2018
Harbour seal, <i>Phoca vitulina richardsi</i>						
Pups, within 2 months of age	Central California	26	2.95–40.58	15.96 ± 2.01	2006	Brookens et al. 2008
Adult and juvenile ≥ 1 yr	Central California	138	2.96–144.31	CNP**	2009–2011	McHuron et al. 2014
Pups, ≤ 4 weeks	California	57	2.8–36.9	11.0 ± 0.9	2012	Hoomissen et al. 2015
Adult female	California	27	5.23–26.67	13.10 ± 6.60	2011–2013	Peterson et al. 2016
Adult male	California	10	6.27–144.31	39.85 ± 39.62	2011–2013	Peterson et al. 2016
Juvenile female	California	17	2.96–36.69	16.10 ± 9.07	2011–2013	Peterson et al. 2016
Juvenile male	California	16	7.98–26.06	16.07 ± 5.81	2011–2013	Peterson et al. 2016
Northern elephant seal, <i>Mirounga angustirostris</i>						
Pups, 23 days	California	17	10.07–36.14	21.65 ± 6.54	2011–2013	Peterson et al. 2016
Adult females, late molting	California	48	6.10–32.43	18.74 ± 6.10	2011–2013	Peterson et al. 2016
Adult males, late molting	California	13	14.20–75.23	43.68 ± 8.51	2011–2013	Peterson et al. 2016
Northern fur seal, <i>Callorhinus ursinus</i>						
Adult females	Alaska, St. Paul Island	12	5.89–12.10	7.84 ± 1.78	2000	Beckmen et al. 2002***
Steller sea lion, <i>Eumetopias jubatus</i>						
Pups, up to a year old	Prince William Sound	22	0.90–3.14	1.46 ± 0.64	1998	Beckmen et al. 2002***
Juvenile, 20–22 months	Southeast Alaska	6	0.56–6.75	2.74 ± 2.89	2000	Beckmen et al. 2002***
2–3 months	Southeast Alaska	10	2.20–6.38	4.09 ± 1.62	2000	Castellini et al. 2012
2–3 months	Prince William Sound	12	1.30–21.26	9.09 ± 6.30	2000	Castellini et al. 2012
12–23 months	Southeast Alaska	20	0.77–3.95	1.64 ± 0.87	2000	Castellini et al. 2012
12–23 months	Prince William Sound	16	0.91–3.37	1.78 ± 0.80	2000	Castellini et al. 2012
Pups, up to 2 months	Agattu Island, western	34	3.66–63.95	CNP	2011	Rea et al. 2013
Pups, up to 2 months	Aleutian Islands	6	5.99–59.17	$16.87 \pm \text{CNP}$	2011–2013	Correa et al. 2014
Adult female	California	8	5.40–41.02	16.09 ± 12.86	2011–2013	Peterson et al. 2016
Juvenile female	California	19	0.86–3.18	1.83 ± 0.76	2011–2013	Peterson et al. 2016
Juvenile male	California	12	0.77–3.95	1.78 ± 0.92	2011–2013	Peterson et al. 2016
California sea lion, <i>Zalophus californianus</i>						
Pups, 1.5–2 months	California Bay****	199	6.55–13.7	CNP	1997	Elorriaga-Verplancken and Auriolles-Gamboa 2008
Juvenile, 1–3 yr	Central and southern California	57	0.74–9.57	3.25	2013	McHuron et al. 2016
Adult females	Central and southern California	21	5.1–21.0	10.1	2013	McHuron et al. 2016

*Dry weight of hair

**CNP calculation not provided

***Wet weight of hair

****Generalized data from eight rookeries

the ocean floor (Macko and Estep 1984; Iken et al. 2005; Young et al. 2010).

For an approximate calculation of the main food source for bearded seals, the isotopic enrichment factor per trophic level for nitrogen was used (3.4 ‰, Søreide et al. 2006). Using isotope data for potential prey of bearded seals (Hindell et al. 2012), we calculated that the prey of bearded seals

from the Sea of Okhotsk could be the following organisms with the range of $\delta^{15}\text{N}$ from 10.5 to 14.4 ‰: benthic gastropods, decapods, pelagic, demersal, and benthopelagic fish. Hindell et al. (2012) found that benthic gastropods and decapods were the most common prey in the Kongsfjorden/Krossfjorden region of Svalbard. It should be noted that our estimate of the bearded seals' diet is approximate and for

an accurate estimate it is necessary to consider the isotopic composition of tissues of both predators and their prey, collected from the same area over a short period of time.

There are no settlements on the coast of Academy Bay and no anthropogenic mercury sources on its shores or in adjacent areas of the Sea of Okhotsk due to the lack of human development of this area. The remoteness of the study area from industrial centers, and the absence of large settlements on its coast initially suggested a low level of toxic pollution of the marine environment. Nevertheless, we found mercury in all samples.

The bearded seal has a circumpolar distribution in the Northern Hemisphere, and its numbers are quite high (Belikov et al. 2017). This pinniped species is very accessible for studying within its habitat. There is information on mercury levels in some tissues and organs (liver, kidneys, muscles, and heart) of bearded seals from the Bering Sea and the Pacific sector of the Arctic (Smith and Armstrong 1978; Dehn et al. 2005; Quakenbush and Citta 2009; Correa et al. 2015). However, there are no studies on mercury levels in the hair of bearded seals from the North Pacific. This makes it impossible to compare our results with those for this species from other areas of the Pacific Ocean. There is only one report indicating the mercury level and $\delta^{15}\text{N}$ in the hair of a single adult male bearded seal from Greenland was equal to 1970 ng/g and 14.26 ‰, respectively (Aubail et al. 2011). These values correspond to the range of THg concentrations and $\delta^{15}\text{N}$ we obtained for bearded seals from the Sea of Okhotsk.

At the same time, comparison of our data with those for other pinniped species from the North Pacific showed that, in general, the mercury level in the bearded seal hair was lower than in the hair of other seal species (Table 3). Most of the pinniped species from the North Pacific are mainly ichthyophages with a high trophic level. Compared to them, the bearded seal, whose diet is based on invertebrates, has a relatively low trophic level. Consequently, the intake of mercury into its organs (tissues) should be lower. According to the results of a comprehensive study conducted in the Atlantic, the mercury content in the bearded seal hair was lower than in mainly fish-eating pinnipeds: gray seal, harbour seal, and ringed seal (Aubail et al. 2011).

On the other hand, we sampled hair from bearded seals living in the part of the Sea of Okhotsk without anthropogenic sources of mercury pollution. However, mercury was detected in all samples. The presence of mercury in all analyzed samples is explained both by the bioaccumulation and biomagnification of this element and by its global distribution in the oceans, due to the active migration as a result of transport by air masses and sea currents to areas not directly affected by any man-made effects (Bard 1999; Schuster et al. 2002; AMAP 2011; Dietz et al. 2013; Kalinchuk et al. 2021). In addition, almost all marine animals, including pinnipeds,

are themselves a source of active transport of organic and inorganic pollutants, including mercury (Cossaboon et al. 2015; Khristoforova et al. 2015; Lukyanova et al. 2015; Tsygankov et al. 2018; Grajewska et al. 2020).

Conclusion

Our study of mercury contamination of bearded seals is the first for this seal species in the Russian Far East and the first for marine mammals inhabiting the Sea of Okhotsk. A significant difference in mercury concentrations depending on age was found. However, stable nitrogen isotope analysis showed no differences in the trophic level of seals of different age and sex. At the same time, a significant relationship was noted between the mercury content and the stable nitrogen isotope ratios in the hair of young seals, suggesting the effect of the trophic level of food on the mercury content. It is possible that differences in $\delta^{15}\text{N}$ were not detected due to the small sample size. Significant differences in mercury levels between young and adult seals were presumably due to differences in diet and/or the spatial differentiation of juveniles and adults during the pre-harvest period before arriving at Academy Bay. It is likely that young seals could live in areas with higher levels of mercury in the marine environment. The average total mercury content in the hair of bearded seals was expectedly low compared to other pinniped species living in the North Pacific. We explain this by two main factors: the low level of trophic relationships of the bearded seal, whose diet consisted mainly of representatives of epifauna and infauna, as well as the remoteness of the studied seals habitats from the industrial centers. However, this may change radically over time due to increased human demand for the raw materials of the northwestern coast of the Sea of Okhotsk. This necessitates long-term study in order to determine the dynamics of background mercury concentrations in the region. In this regard, the bearded seal can be considered as a sentinel species in the study of pollution of the coastal ecosystem of the Sea of Okhotsk. Our results, as basic information, can be of fundamental importance in the study of contaminants in North Pacific pinnipeds.

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Zolotukhin: sampling and methodology. All authors read and approved the final manuscript.

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Data availability Data will be made available upon request.

Declarations

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