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RESEARCH ARTICLE

Mercury in organs of Pacific walruses (*Odobenus rosmarus divergens*) from the Bering Sea

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Abstract The Pacific walrus (*Odobenus rosmarus divergens*) is still used as an important source of protein-rich food by indigenous peoples of Chukotka, Alaska, and other Arctic regions. Total mercury (THg) concentration was measured in eight internal organs of walruses. Samples were taken from 22 individuals (11 males and 11 females). Age of the animals ranged from 1 to 30 years. All the walruses were harvested by local hunters from the coastal waters off the Chukchi Peninsula (Russia) during the autumn of 2011. Total mercury concentration in the samples was determined by atomic absorption method. No statistically significant difference in the level of mercury was found between males and females. Mercury was detected in all the organs of the studied walruses. The highest total mercury concentration was recorded from excretory organs: liver and kidneys. The level of mercury in liver (mean = 1.87 μg/g, range = 0.05–5.87) was by an order of magnitude higher than in kidneys (mean = 0.54 μg/g, range = 0.09–1.64.); in kidneys, it was by an order of magnitude higher than in the rest of the organs. The analyzed organs can be arranged in the order of decreasing Hg concentration as follows: liver >> kidney >> muscle > spleen ≥ heart ≥ intestine > lung ≥ testis. The mercury concentration values in walruses from the coastal waters off the Chukchi Peninsula are lower than those obtained from walruses in Alaska and the Canadian Arctic. Our findings may provide a basis for the further long-term monitoring of the condition of the Pacific walrus population and pollution of the Arctic ecosystem.

Keywords Pacific walrus · *Odobenus rosmarus* · Contaminants · Mercury · Toxic metals · Concentration · Bering Sea · Chukchi Peninsula

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Introduction

The Pacific walrus (*Odobenus rosmarus divergens*) is widely spread in the northern Pacific Ocean (Bering Sea) and in the seas of the Pacific region of the Arctic, mainly in the Chukchi Sea (Smirnov 1908; Ognev 1935; Freiman 1940; Fay 1982; Arsen'ev 1976). This is a migratory species characterized by seasonal mass migrations. In winter, Pacific walruses are distributed in the Bering Sea; in spring and summer, animals migrate to the Chukchi Sea through the Bering Strait. This occurs after the waters of the Bering Sea become free of ice. In the Chukchi Sea, during summer and autumn, walruses actively feed mainly on bivalve mollusks, gastropods, crabs, and other benthic invertebrates. In summer, a small part of the population penetrates into the East Siberian Sea and the Beaufort Sea (Belopol'skii 1939; Freiman 1940; Nikulin 1940; Arsen'ev 1976; Fay 1982; Estes and Gol'tsev 1984). Thus, throughout their life cycle, walruses keep within the Arctic and subarctic ecosystems being far away from sites of industrial activity. Consequently, the direct human impact on these animals and their habitat is limited.

Like other pinniped species, walrus occupies the top of the trophic pyramid, being the terminal link in the benthic food chain. A major part of its diet consists mainly of various benthic invertebrates living a slow-moving or permanently attached lifestyle, among which the most important are mollusks (Arsen'ev 1976; Lowry et al. 1980; Fay 1982).

In the Arctic and sub-Arctic biota, walruses feed on members of the benthic invertebrate community. At the same time, they serve as food to some species of predatory mammals such as polar bear, *Ursus maritimus* (Kiliaan and Stirling 1978; Kochnev 2001), and killer whale, *Orcinus orca* (Melnikov and Zagrebin 2005; Kryukova et al. 2012), and, which is particularly important, to human. In the diet of the indigenous peoples (Chukchi, Eskimo, etc.) inhabiting the northernmost parts of Northeast Asia and North America, this pinniped species has always been one of the main sources of protein-rich food (Suvorov 1914; Arsen'ev 1927; Nechiporenko 1927; Brooks 1953; Nelson 1972; Krupnik 1980; Bockstoce and Botkin 1982; Borodin 2000). The rate of walrus harvesting by the native peoples of Alaska and Chukotka reached its maximum in the 1980s and significantly decreased in the beginning of the twenty-first century (Smirnov et al. 2002; Grachev 2004; Garlich-Miller et al. 2006). However, even today, the walrus traditionally remains a very important item in the diet of indigenous population of the North, and its economic significance as food is still great.

As walrus is a traditional food for the peoples of the North, the study of harmful substances including toxic metals, accumulated in organs and tissues of this animal, is of particular interest. One of the most toxic metals is mercury (Hg), which has a negative impact on the health of human and animals (Scheuhammer et al. 2007; Mergler et al. 2007; Dietz et al. 2013). The toxic effects of mercury on human organism have long been known. Mercury causes depression of the central nervous and respiratory systems, disturbs normal functioning of the cardiovascular system, and may induce many other diseases (Donaldson et al. 2010; Mozaffarian et al. 2011; Roman et al. 2011; Virtanen et al. 2012). Excess of this trace element in marine animals often leads to serious health disorders, up to lethal outcome (AMAP 2015; Reif et al. 2015). Studies of previous years have shown that mercury is present in all species of pinnipeds and cetaceans that live in the World Ocean, including Arctic waters (Kemper et al. 1994; Fant et al. 2001; Pompe-Gotal et al. 2009; Nie et al. 2012; Dirtu et al. 2016). But the works considering accumulation of toxic metals (such as lead, cadmium, and mercury) in walruses of the Pacific region of the Arctic are comparatively rare (Taylor et al. 1989; Warburton and Seagars 1993; Wagemann and Stewart 1994; Trukhin et al. 2013, 2015).

In Russia, the problem of mercury exposure of indigenous peoples is poorly studied. In recent years, investigations of toxic metals in human blood have been conducted in Chukotka (Dudarev et al. 2010; AMAP 2015). This problem has been elucidated relatively better in the neighboring regions: in Alaska, the Canadian Arctic, and other polar territories. There, the monitoring of the health of Eskimos is performed regularly (Bard 1999; Donaldson et al. 2010; Dietz et al. 2013). There are also international programs studying the impact of environmental pollution on people living in the Arctic (AMAP 2011, 2015).

Previously, we studied the distribution of lead and cadmium concentrations in the body of Pacific walrus (Trukhin et al. 2013, 2015), but in this work, we aimed to consider another toxic metal, mercury. The following objectives were set up: determination of mercury content in various organs, assessment of the relationship of mercury concentration with animal's age, and identification of regional and inter-annual variations in mercury concentrations. Of particular interest is the problem of the impact exerted by mercury on the health of the indigenous population of Chukotka, whose diet includes walrus as an important component. It is obvious that the quality of food greatly influences the health of not only individuals but also the entire ethnic groups.

Materials and methods

Sample collection

Material for this study was collected on the coast of Mechigmen Bay, Chukchi Peninsula, Bering Sea, from August 13 to October 1, 2011 (Fig. 1). This area is the traditional site of annual aboriginal hunting for walruses that migrate here during the summer and autumn seasons. The nearest Chukchi hunters' village is situated on Cape Akkani. When walruses were swimming in the sea off the cape, they were shot by hunters from motor boats and hauled ashore, where the biological samples from the animals were collected. Prior to sampling, each animal was measured (standard and zoological length, girth, fat thickness on the xiphoid process) and its sex determined. Sample of each organ was a small piece of tissue weighing up to 20 g, which was cut off from

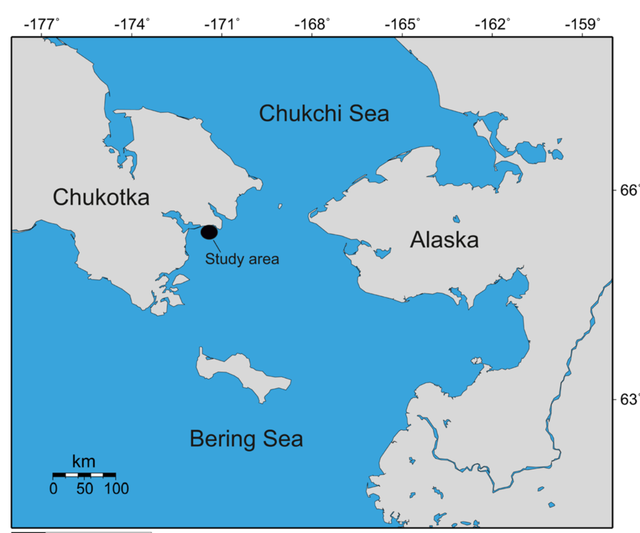

Fig. 1 Study area in the Bering Sea

the organ with a stainless steel knife. Organs such as liver, kidney, heart, muscle, spleen, lung, and intestine tissues were taken for the mercury content analysis. Fragments of testes were also taken from males. Intestine samples were taken from the upper segment of the small intestine with preliminarily removed content. A piece of muscle tissue was taken from the animal's thigh, in the area of the muscle *Musculus biceps*. A total of 176 samples were collected. The samples were dried in a well-heated and ventilated room within the same day. Each dry sample was placed into a separate plastic bag and labeled. Then, the samples were delivered to the laboratory for analysis.

Of particular interest to us was the question if there is a relationship between the mercury concentration and the walrus age. To resolve it, we purposefully selected walruses of various sizes that allowed us to have samples from different age groups: immature young, middle-aged, and old individuals that have reached the senile age. These age groups were determined based on body size and external traits of the animals (such as tusk size, shape, and degree of wearing, as well as presence of age nodules (bosses) on the skin). Samples were collected from 11 females and 11 males. The hunters could sometimes bring ashore several (up to ten) harvested walruses at a time, started dressing their carcasses simultaneously, and immediately utilized fat, meat, and internal organs. In that case, we did not have time to collect all the organs from some of the walruses.

Two teeth from the lower jaw of each walrus were collected for accurate age determination by counting the cement and dentin layers.

All the walruses, from which samples were collected, were physically healthy; they did not have any injuries such as fresh or healed wounds. A visual examination of their internal organs did not reveal any pathologies.

Total mercury analysis

The chemical analysis of the collected samples was conducted at the Applied Ecology and Toxicology Laboratory, Pacific Research Fisheries Center (TINRO-Center), Vladivostok, Russia.

Before analysis, the samples of tissues were dried at 85 °C in a drying oven to a constant weight. A portion of about 100–200 mg of dried samples was used for further analysis.

Total mercury (THg) concentration in tissues was analyzed using a Milestone Direct Mercury Analyzer DMA-80 (tricell). The method is based on thermal decomposition of liquid or solid sample and atomic absorption spectrometry of mercury in vapor.

Mercury concentration was measured as a degree of absorption of light beam intensity in three ranges: 0–10 ng, 10–20 ng, 20–1200 ng. The level of mercury detection (LOD) was 0.0015 ng with an accuracy not exceeding 1.5%.

The direct mercury analyzer was calibrated using the standard Hg solution, listed in the State Register of Approved Measuring Instruments.

Mercury concentration was expressed in micrograms per gram (μg/g) dry weight (dw).

Reliability of THg determination was evaluated by analyzing the standard reference ERM® material, CE278k (Mussel tissue). The obtained value (mean ± 95% uncertainty) was 0.067 ± 0.002 mg THg/kg. This result agreed well with the certified value of total mercury in CE278k, 0.071 ± 0.007 mg THg/kg. The percentage of THg recovery was 94%.

Statistical analysis

Statistical processing of the data was performed using the software packages Microsoft Excel and Statistica 6.0.

Presence (absence) of significant differences in mercury concentration between males and females was determined by t test, with $p = 0.05$. To find the relationship of mercury concentration with animals' age, the parametric Pearson correlation (r) was applied. The Pearson correlation was then squared to be expressed in the form of coefficient of determination (r^2); r, r^2, and number of degrees of freedom ($v = n–2$) were used to test the significance of correlation, with $p = 0.05$ (Glantz 2001).

Results

The examination of walrus teeth sections showed that the studied animals were represented by males ($n = 11$) from 1 to 30 years of age (mean age was 12.8 years) and females ($n = 11$) from 2 to 18 years of age (mean, 8.5 years). Thus, the representation of the age groups of animals was rather wide.

Mercury was found in all the analyzed organs of each walrus (Table 1). However, the level of mercury varied considerably both between organs and between individuals. The highest concentrations of THg were in the liver and, somewhat lower, in the kidneys, i.e., in the excretory organs. The mercury concentration in the liver (mean = 1.87 μg/g; range = 0.05–5.87) was higher than in the kidneys (mean = 0.54 μg/g, range = 0.09–1.64), and the concentration in the kidneys was much higher than in the rest of the organs. The analyzed organs can be arranged in the order of decreasing Hg concentrations as follows: liver >> kidney >> muscle > spleen ≥ heart ≥ intestine > lung ≥ testis.

Higher mean values of mercury concentration most frequently occurred in females (in five organs) than in males (in two organs). However, no statistically significant differences in mean mercury concentration between male and female walruses were found in their organs. For this reason, the further analysis was conducted using combined samples that included data of both males and females.

Table 1 Total mercury concentration (μg/g dw) in organs of the walruses from the Mechigmen Bay coast, Chukchi Peninsula, 2011 (combined samples from males and females)

Organ	*n*	Mean	Range	SD	CI
Liver	22	1.87	0.05–5.87	1.54	1.22–2.51
Kidney	21	0.54	0.09–1.64	0.42	0.36–0.72
Heart	22	0.05	0.01–0.07	0.01	0.04–0.05
Muscle	22	0.06	0.03–0.08	0.01	0.05–0.06
Lung	20	0.03	0.01–0.05	0.01	0.02–0.03
Spleen	21	0.05	0.01–0.1	0.02	0.04–0.06
Intestine	21	0.04	0.01–0.08	0.02	0.04–0.05
Testis	11	0.02	0–0.05	0.01	0.02–0.03

The relationship of THg concentration with animal's age was strong and significant for testis only ($r^2 = 0.53$, $t = 3.20$, $v = 9$, $p = 0.05$). In all the other organs, variation in THg, related with the age of walruses, was below 0.1, and the strength of correlation was insignificant.

Discussion

Mercury distribution in organs and relationship of concentration with age of walruses

Mercury was unevenly distributed over the organs of walruses. The highest mercury concentrations were found in the excretory organs: liver and kidneys (Table 1). The higher levels of mercury in the excretory organs can be explained by the detoxifying function of the organs where this metal is accumulated. Presence of free protein thiol group in liver and kidneys leads to a strong fixation of mercury because of the high affinity to various enzymes of SH group of microsomes and mitochondria (Goyer and Clarkson 2001). The process of detoxification and excretion of mercury and other metals also exists, but its efficiency declines with the age of animals (Ikemoto et al. 2004). This explains the age-related accumulation of mercury in walrus testes. Previously, high levels of mercury in liver and kidneys in comparison with other organs of walrus were reported by Wagemann and Stewart (1994). This pattern was also observed in other species of pinnipeds living in the Arctic and subarctic: bearded seal (*Erignathus barbatus*) (Correa et al. 2015; Smith and Armstrong 1978), ringed seal (*Pusa hispida*) (Smith and Armstrong 1978; Riget et al. 2005), and northern fur seal (*Callorhinus ursinus*) (Goldblatt and Anthony 1983).

The results of the previous studies of mercury in the Pacific walrus did not show any correlation between the concentration of this metal and the age of animals (Taylor et al. 1989; Warburton and Seagars 1993; Braune et al. 2015), probably, due to the lack of young immature walruses in the samples analyzed by these authors.

Similar studies were done on the other species of pinnipeds. There is abundant evidence that the level of mercury in their organs also increases with age (Dietz et al. 1996; Fant et al. 2001; Woshner et al. 2001; Watanabe et al. 2002; Bustamante et al. 2004; Brown et al. 2016).

The found relationship of mercury concentration with age of walruses can be observed in all organs (except for skeletal muscle) (Fig. 2). But in all the analyzed organs, except for testis, it was not statistically significant.

Regional and inter-annual differences in mercury concentration

According to the obtained data, the mercury concentration in walruses from the coastal waters off the Chukchi Peninsula was lower than that in Alaska (Taylor et al. 1989; Warburton and Seagars 1993) and in the Canadian Arctic (Wagemann and Stewart 1994) (Table 2). As some reports show, mercury concentration in the liver of walruses from the Canadian Arctic is significantly lower than in other marine mammals from this region (Braune et al. 2015). It is probable that the ecosystem of the Bering Sea with the adjacent coast of Alaska and Chukotka is cleaner than the Canadian Arctic region. The mercury concentration in tissues of the Atlantic walrus (*O. r. rosmarus*) is comparable to that in the Pacific walrus (*O. r. divergens*) from Alaska and the Canadian Arctic (Born et al. 1981), but higher than in walruses of the Chukchi Peninsula (our data).

Level of mercury in the organs of walruses can vary greatly even between areas located relatively close to each other. Thus, samples collected from walruses at the same time in different parts of the Bering Strait demonstrated a significant variation of mercury concentrations (Warburton and Seagars 1993).

Until the present study, no similar works have been conducted in the Chukotka region, and, therefore, we have no idea how mercury concentration in the organs of walruses in this region could change over a long period of time. However, it is known that in 1986–89, the level of mercury in the organs of these animals in the Bering Strait was about the same as that recorded a few years earlier, in 1981–83 (Taylor et al. 1989; Warburton and Seagars 1993). No trends in mercury concentrations were observed in the Canadian Arctic between 1982 and 2009 (Braune et al. 2015). But the authors of the present paper assume that the number of years of monitoring (9) within this period (1982–2009) was relatively small that was not enough to draw correct conclusions. Changes in the level of mercury in walruses' organs are expected to occur from year to year, as concentration of this metal in the environment has a tendency to vary within a long period of time. For example, the level of mercury in a ringed seal liver sampled in 1987–1993 in the Arctic region was three times as high as that in the samples collected 15–20 years earlier (see review, Bard 1999).

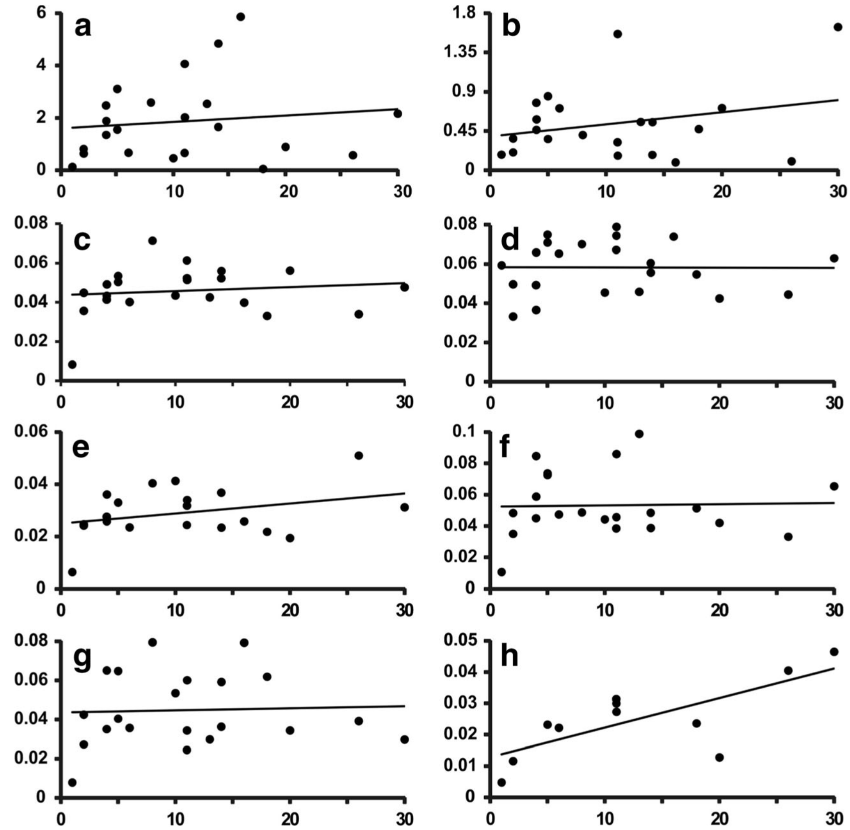

Fig. 2 Mercury concentrations in organs of the Pacific walrus **a** Liver. **b** Kidney. **c** Heart. **d** Muscle. **e** Lung. **f** Spleen. **g** Intestine. **h** Testis. *X*-axis is age (years) and *y*-axis, concentration (μg/g dw)

Walrus as a food source for the native people of the Arctic and the degree of human exposure to mercury and other heavy metals

In the Russian Arctic, indigenous people living in coastal areas of the Chukchi Peninsula have traditionally harvested walruses each autumn to preserve meat and fat for the upcoming cold season. Walruses are hunted at coastal rookeries or, more often, at sea, where they are shot with hunting guns from motor boats, during their summer and autumn migrations to the feeding grounds (Arsen'ev 1927; Krupnik 1980; Smirnov et al. 2001). The products from walruses are frozen or preserved by fermentation and then used as food throughout the subsequent winter and spring. The current rate

Table 2 Total mercury concentration (μg/g dw) in organs of walruses in the Bering Sea and in some adjacent regions of the Arctic (our results compared with data reported in the literature)

Region	Year	Liver	Kidney	Muscle	Reference
Canadian Arctic, Foxe Basin	1982–1988	4.52 ± 3.60* 0.03–19 (117)	1.37 ± 0.53 0.32–3.2 (112)	0.42 ± 0.50 0.05–5.0 (113)	Wagemann and Stewart 1994
Canadian Arctic, Northern Quebec	1990	6.84 ± 5.48 0.5–16 (13)	No data	No data	Wagemann and Stewart 1994
Bering Strait, Alaska	1981–1984	4.5** (62)	No data	No data	Taylor et al. 1989
Bering Strait (Diomede, St. Lawrence Islands)	1986–1989	4.17 ± 6.07 0.27–37.01 (53)	1.10 ± 1.06 0.29–7.56 (50)	No data	Warburton and Seagars 1993
Chukchi Peninsula	2011	1.87 ± 1.54 0.05–5.87 (22)	0.54 ± 0.42 0.09–1.64 (21)	0.06 ± 0.01 0.03–0.08 (22)	This study

*The first row is mean ± SD; the second row, range; the third row (in parentheses), number of samples.

**Mean value, data were converted from wet weight using the formula proposed by Warburton and Seagars (1993): (mg/kg dry weight = mg/kg wet weight / (1–(% moisture / 100)) of walrus harvesting in Chukchi Peninsula is about 1000 individuals per year. Thus, in 2010–2014, local hunters annually killed 819–1051 (mean 990) walruses in Chukotka (D. Litovka, pers. comm.).

The Chukchi and Eskimo consume walrus meat, fat, and almost all the internal organs on a permanent basis. In addition, in many Chukchi villages, walrus meat and fat is only feed for sled dogs; thus, being an important item of the economy of these peoples. Walrus hunt is part of the aboriginal culture; walrus has always been a character of many local fairy tales and legends.

Toxic metals, including mercury, are ingested by people consuming walruses as food, because human is the terminal consumer in the food chain. Thus, marine mammals can be considered, to some extent, an indicator of both pollution of ecosystem and human health problems (Bossart 2011). The data collected from the regions of the Canadian Arctic, neighboring Chukotka, show that there is a further increase in the mercury concentration in marine biota. Due to this fact, researchers raised concern about the health of the ecosystem and the native people inhabiting it. For example, when examining Canadian Eskimos eating traditional food (walruses), they found higher values of mercury concentration in the blood of these people as compared to the ones obtained for the entire Canadian population, with a three to tenfold difference between them (Laird et al. 2013; AMAP 2015).

Similar studies in the Chukotka region revealed mercury concentrations in kidneys and liver of walruses and gray whales (*Eschrichtius robustus*) exceeding the safety limits two–four times; concentrations in seals' organs were three to 100 times as high (AMAP 2015). Even low concentrations of mercury in some of the walrus organs may probably pose danger to humans, because, as noted above, walrus is traditionally an important item in the diet of the indigenous peoples of Chukotka (Trukhin et al. 2015). Therefore, intake of even small doses of mercury occurs continuously and causes this metal to accumulate in the human body. Previously, we conducted a similar work to study concentrations of another toxic metal, cadmium (Cd), in walrus organs. Having a cumulative effect, this metal reaches dangerous values in kidneys of old walruses. This gave us a reason to recommend to the native people of Chukotka to avoid eating meat and organs from old animals (Trukhin et al. 2013, 2015).

There is the threshold (maximum permissible) level of total mercury in muscle and liver tissues of marine mammals, accepted in the Russian Federation: 0.5 mg/kg wet weight (Hygienic requirements … 2009). The values of mercury concentration in walruses from Chukotka did not exceed this level if expressed in terms of wet weight. But it should be kept in mind that mercury is accumulated in the tissues of mammals in various forms, including methylated one, methylmercury. The latter is the most toxic form of mercury, concentration of which in muscles of marine mammals may reach 50% THg; in liver, only 3–12% (Wagemann et al. 1998). Due to its high toxicity, methylmercury undoubtedly aggravates the negative effect of mercury on the health of the indigenous population, in whose diet of marine mammals and fish constitutes a major part (Booth and Zeller 2005; AMAP 2011). Thus, the health examination of the native people in Chukotka, with the accent on concentration of mercury and other toxic metals in their foods, should be improved in the future.

Conclusion

Marine mammals, including walrus, are sensitive indicators of the condition of marine ecosystems, as these mammals, characterized by a long lifespan, are the terminal link in the benthic trophic chain. The trophic transfer of Hg up a marine food web is well known (Braune et al. 2015). In walrus, all these factors cause various toxic metals to accumulate in organs to high concentrations throughout the lifespan. In addition, all marine mammals build up substantial fat reserves, in which other types of toxic substances, lipophilic pollutants, are deposited (Ross 2000; Wells et al. 2004; McHuron et al. 2014). In this regard, walrus, like other pinniped species, can be considered as an indicator of environmental pollution in the Arctic.

As a result of this work, we obtained the first data on mercury concentrations in tissues of Pacific walruses from the areas of the Chukchi Peninsula inhabited by indigenous peoples. No similar studies have been conducted previously in the western Bering Sea. Mercury was present to a greater or lesser extent in each organ of all the walruses studied, including juveniles. We believe that the results obtained can serve a basis for organizing a long-term monitoring of the ecological condition of the coastal ecosystem in the Chukchi Peninsula. Due to the considerations above, the further study of concentrations of pollutants in the Pacific walrus is strictly recommended.

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