Polar Science
December 2024, Volume 42, Pages 101079 (6p.)
https://doi.org/10.1016/j.polar.2024.101079 https://archimer.ifremer.fr/doc/00889/100042/

Mercury Levels in Tissues (Cartilage, Skin, and Muscle) of the Greenland Shark (*Somniosus microcephalus*): Potential Contamination Sources and Implications for Health and Conservation

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Abstract :

The jaws of the Greenland shark have high levels of mercury. Hg of cartilage in comparison with skin and muscle from the same specimen makes it possible to apprehend the distribution of the pollutant in the body. The level of the pollutant between jaw, skin and fresh meat (muscle) shows a strong correlation. The muscle is the most contaminated element in comparison with the skin and cartilage. The species presents the highest levels among different groups of sharks and the results are in accordance with previous studies. Marine ecosystems in the Arctic are globally contaminated by mercury (atmosphere, water, sediments, food web). The methylmercury reduces blood calcium levels, directly affecting the metabolism of cartilage cells. Even if cases of malformations could not be observed in the Greenland shark, numerous cases for other shark species have been documented in connection with heavy metals (e.g., Hg), and in particular for aplacental viviparous with potential morphological anomalies on embryos. The situation of the Greenland shark is worrying due to the conservation status, the fishing catches, the tardive sexual maturity and reproduction, the climate change and the level of mercury on its organism. The results incite to recommend ecological, environmental and fisheries management measures.

Keywords : Greenland shark, Mercury, Potential abnormalities, Conservation, Arctic

1. Introduction

The situation of the Greenland shark is worrying. The species holds a conservation classification of Near Threatened (NT) in Europe (Burgess et al. 2015). However, the reported catches by the Icelandic and Greenlandic fleets surged to more than 43 tonnes in 2010 and 120 tonnes in 2020 (Tonnes Live Weight; FAO areas 14, 14b, 14b2, 5a, 5a1, 5a2) (ICES, 2022).

The Greenland shark, inhabiting the cold waters of the Arctic and North Atlantic Oceans, including the Celtic and North Seas, faces bycatch mainly in Arctic regions, notably by halibut or shrimp fisheries (David et al., 2013). Greenland Shark bycatch ranged from 34.4% for longline, 32.8% for trawl and 18.3% for gillnet respectively in NAFO subarea 0 (Bryk et al., 2018).

The Greenland shark displays aplacental viviparity (Nielsen et al., 2020). Genetic investigations have identified genetically mixed individuals in the Canadian Arctic, sub-Arctic, and temperate Eastern Atlantic areas, suggesting introgressive hybridization with the Pacific sleeper shark (*Somniosus pacificus)* Bigelow and Schroeder, 1944 (Walter et al., 2017). Insights into Greenland shark sexual maturity showed male and female body lengths-at-maturity (Total Length) of 2.84±0.06 m and 4.19±0.04 m, respectively (Nielsen et al. 2020). The species with a large long-live are particularly vulnerable to exposure to mercury biomagnified (Rumbold et al 2014). Several scientific studies confirm the climate change in the Arctic and the polluted environment on mercury included food webs with potential effects of shark health (Braune et al., 2015; MacMeans, 2015; Rodríguez-Romero et al., 2018; Schaefer et al., 2020).

Natural phenomena such as volcanoes, rock erosion, but also forest fires, whether from natural, accidental or human causes, without forgetting industrial activities, especially the exploitation of coal and gold, emit mercury (Hg) into the environment (Veiga et al., 2006; Dabrowski et al., 2008; Witt et al., 2009). Mercury has the particularity of lasting up to two years in the atmosphere before the abiotic oxidation into Hg2+ (Ariya et al., 2015). Hence, the Arctic Ocean is susceptible to mercury contamination, which may stem from sources that are notably remote (Ariya et al., 2004).

Industrial activities associated with resource exploitation in the Arctic, including gold mining, have continued to expand in recent years (Mered, 2020). Gold processing involves the use of mercury and heavy metal pollution of water (Fashola et al., 2016). Kirk et al. 2008 demonstrated the methylation of Hg through analyses of Arctic waters. Concentrations of methylated Hg and Dimethylmercury (Me2Hg) were often low in surface waters (23.8±9.9 and 4.7±4.4 pg L^{-1}, respectively), but they increased with depth (maximum: 178 and 170 pg L^{-1}, respectively; mean: 70.3 ± 37.3 and 56.8 ± 37.8 pg L^{-1}, respectively). Water serves as a significant source of gaseous Hg (gaseous elemental Hg, GEM) in the atmosphere, especially during the ice-free period (Braune et al., 2015). Methylmercury (MeHg) by photodegradation phenomenon and Me2Hg on the surface can contribute to producing GEM deep in the water column (Mason et al. 1998; Chen et al 2003). Subsequently, it undergoes transformation or is reduced to elementary Hg and released back into the atmosphere. Another portion settles as sediments, while yet another part transforms into monomethylmercury (MeHg) and dimethylmercury (Me2Hg) (Braune et al., 2015). The deposition of land-based mercury on marine sediments causes increased microbial Hg methylation and the transfer of MeHg to benthic and pelagic food webs (Ferreira Araujo et al., 2022). The contribution of mercury stems, in part, from the potential release of mercury from permafrost due to the erosion of rivers and coastlines resulting from Arctic warming (Schaefer et al., 2020).

The marine environment is not exempt from this concern. Different mercury forms are present, including elemental (Hg0), inorganic (Hg2+), and organic (CH3Hg+) forms (Clarkson, 1997). Among these, methylmercury (CH3Hg+), the hazardous variant of mercury, gradually accumulates within organisms of marine species included sharks and undergoes an amplification process (known as bioaccumulation) as it moves up the trophic web, from lower to higher levels (Harding et al., 2018, Biton-Porsmoguer et al., 2018; 2022). This susceptibility is linked to their extended lifespans and high trophic level (Gelsleichter and Walker 2010), which can lead to potential morphological abnormalities (Casarini et al., 1997; Rosa et al., 2004; Moore, 2015; Cabanillas-Torpoco et al., 2023).

The Greenland shark exhibits elevated mercury levels, among the highest among marine species analysed in the Canadian Arctic (Chételat and Braune, 2012). The presence of mercury has been demonstrated previously in different organs of the Greenland shark (only in muscle and liver) (McMeans et al., 2007, 2010, 2015; Chételat and Braune, 2012; Corsolini et al., 2014). The biological characteristics of the Greenland shark mentioned above, including longevity, make it a preferential candidate for study the level of contaminating elements in their organism. Further investigations of mercury production have a particular importance in marine arctic environment particularly in zones, which enhance uptake in the food web to evaluate source-receptor impacts (AMAP, 2021). The objective of this study are 1) to measure and confirm mercury concentration within the cartilaginous tissue of the species, specifically targeting the upper and lower jaws; 2) to analyse and compare mercury levels present in skin and muscle samples extracted from the same individual specimens; 3) to examine potential sources of mercury contamination and investigate its potential effects on the morphology and reproductive patterns of sharks; 4) to provide recommendations for conservation strategies aimed at reducing bycatch and minimizing the presence of mercury in the delicate Arctic polar ecosystem. In accomplishing these objectives, the study aims to contribute valuable insights into mercury accumulation dynamics, potential consequences for the species, and imperative conservation actions required in the Arctic environment.

2. Materials and methods

Sampling

The sampling phase was conducted aboard the bottom trawler "Steinunn SF-10." The vessel operated from Hornafjörður, Iceland, during the year 2010. On August 8, 2010, a female Greenland shark was captured in the Greenland Sea, near Denmark Strait (68°016 N - 22°764 W) within FAO fishing area 5.2 (Fig.1). Measuring 434 cm in total length, the specimen was procured and retained on the ship. Samples of both upper and lower jaws muscle and skin were meticulously extracted using knives and scalpels (Fig.1). The tools underwent thorough cleaning with soapy water, disinfection using 90% ethanol, and subsequent rinsing with distilled water between each sample collection. After extraction, all samples were placed within hermetically sealed plastic bags and accurately referenced. Subsequently, the muscle sample was immersed in 70% ethanol, while the jaw (cartilaginous tissue) and skin samples were dried and were securely stored in airtight plastic bags.

Mercury analysis

The total mercury (THg) concentration of the tissue samples was determined using cold vapor atomic absorption spectroscopy (CVAAS) with thermal decomposition and gold amalgamation. The instrument used was the Hydra IIC direct mercury analyzer by Teledyne Leeman Labs (Hudson, NH). Calibration curves were constructed using the Certified Reference Materials (CRM) BCR 414 (phytoplankton), DORM-4 (fish protein), EM-CE278K (mussels) EMR-CE464 (Tuna fish) ranging from 0.276 $mg.kg^{-1}$ to 5.240 $mg.\ kg^{-1}$ dw of THg. Instrument linearity was established across two separate ranges; a low range (0 ng to 30 ng) and high range (75 ng to 200 ng). The high calibration range has a correlation coefficient (r^2) value of 0.9992 and the low calibration range has an r^2 value of 0.9996. The limit of quantitation (LOQ) was set as 10 times the standard deviation of the y-axis intercept of the method calibration curve.

A portion of each tissue sample was placed in a nickel boat and weighed on an analytical balance (±0.0001 g). The amounts of upper and lower jaws, skin and muscle were 0.0127 g dry mass, 0.0175 g dry mass, 0.0213 g dry mass and 0.0142±0.004 g dry mass, respectively. Given the unconventional method of preserving muscle in alcohol for THg analysis and the sufficient sample quantity available for analysis, a total of three separate analyses were conducted. The provided result is the average of the three measurements. Samples were first introduced into the decomposition furnace where they were dried (300 °C, 30 s) and combusted (800 °C, 150 s), then into the catalyst furnace (600 °C, 60 s), and finally through the drying tube and gold amalgamation trap (600 °C, 30 s) before entering the spectrometer. Samples were not homogenized to avoid unnecessary contamination from additional handling and due to the limited available tissue in some cases. Samples were not digested before analysis. For quality assurance, a CRM (EMR-CE464 Tuna Fish) measurement was performed at the end of the six analyses.

3. Results and discussion

Elevated THg levels were analysed in all tissues of the shark's body, ranging from 1.332 mg kg^{-1} dw (upper jaw) to 3.225 mg kg^{-1} dw (muscle) (Fig.2; Table 1). A linear concentration increase trend is confirmed in THg between the lower and upper jaws (cartilage), skin and fresh meat (muscle) (R^2=0.9996) (Fig.2).

The distribution of mercury levels across different shark tissues reveals that muscle accumulates higher levels compared to the skin and cartilage. This disparity may be attributed to variations in the rate of cell turnover, especially notable in cartilage (Marconi et al. 2020) and skin (Meyer and Seegers, 2012), but also with the fact that mercury in fish included sharks is mostly in the methylated form and has a protein affinity (Chouvelon et al., 2018). The mercury levels analysed in this study are consistent with those observed from other specimens of *Somniosus microcephalus* (Table 1). However, it appears that the varying mercury levels obtained are not correlated with the length of the individuals (*P*>0.05, n=19, MacMeans et al., 2010). A multiple test on muscle tissue indicated that concentrations of mercury were higher on Greenland shark, 5.930 mg kg^{-1}, n = 19 (Macnean, 2017), 3.225 mg kg^{-1}, n = 1 (this study) in comparison with specimens of Carcharhiniformes, 1.520 ± 1.900 mg kg^{-1}, n = 1739; Lamniformes, 2.580 ± 4.790 mg kg^{-1}, n = 508; others species of Squaliformes, 1.610 ± 1.040 mg kg^{-1}, n = 415; and Myliobatiformes, 0.383 ± 0.350 mg kg^{-1}, n = 195 (Tiktak et al., 2020). Several factors may explain higher THg values in Greenland shark (i) their feeding at a high trophic position (TP) and (ii) foraging on slightly more contaminated offshore resources in the Arctic (MacMeans et al., 2010). The lack of a correlation between mercury levels and the age (size) of the sharks can be attributed to (i) mercury accumulation in larger, presumably older Greenland sharks not being solely driven by bioaccumulation and (ii) larger specimens not necessarily feeding at a higher TP than smaller sharks (MacMeans et al., 2010).

The limitation of the method used

The measurement of mercury in a sample preserved in ethanol, such as the muscle sample in this case, is not standard. Usually, measurements are carried out on fresh (MacMeans et al.,, 2010) or freeze-dried samples (Biton-Porsmoguer et al., 2018; 2022). Here, the measurements were conducted 13 years after the collection, and the sample preservation method could not be controlled. Alcohol preservation can be used for maintaining tissue samples for various analyses, including mercury measurements. However, certain factors need to be considered. Ethanol itself may contain trace amounts of mercury. Hence, it is crucial to use high-purity ethanol for preservation and ensure that the ethanol used does not introduce additional mercury into the samples.

Here, ethanol (70% USP-grade Isopropanol (Isopropyl Alcohol) and 30% USP-grade purified water) was used. It is also reasonable to assume that mercury concentrations could change during preservation, likely due to the loss of mass caused by protein and water loss in samples preserved in ethanol. Hence Gibbs et al. (1974) observed a slight increase in mercury concentrations in fish after a short period of preservation in ethanol. However, when preserved in ethanol, fish samples typically experience an initial loss of mass due to deshydratation, followed by a stabilisation (Shields and Carlson, 1996). In this case, the muscle sample was preserved in ethanol for 13 years. It is highly likely that any initial dehydration induced by the alcohol has reached a state of stabilization. Utilizing a conversion equation holds merit in the endeavour to retrocalculate the mass of ethanol-preserved samples to their initial state under fresh conditions. Nevertheless, it is important to note that these conversion equations tend to possess limited reliability, typically capable of offering only coarse approximations of the live condition (Billy, 1982). Consequently, we have chosen to refrain from their application. We acknowledge that the concentrations measured in the muscle of the ethanol-preserved shark are prone to being higher than the actual concentrations determined based on fresh weight. Shields and Carlson's study (1996) indicates that this overestimation could potentially reach up to 20%. This uncertainty adds to the measurement uncertainties.

The selection of analytical methods for measuring mercury in alcohol-preserved samples could vary from those employed for fresh or frozen samples. Cold vapor atomic absorption spectrometry (CVAAS) has been previously employed for mercury analysis in alcohol-preserved samples (Levengood et al., 2013), thereby confirming the applicability of the method used in this study. The authors analysed the composition of the alcohol in the jars used for specimen storage. Mercury was detected at a very low concentration in the ethanol, confirming that there are minimal mercury transfers from the samples to the alcohol.

Sources of mercury in arctic region for sharks

Several studies have highlighted that prey serves as a primary source of mercury in marine organisms, particularly for top predators like sharks, owing to the phenomenon of bioaccumulation (McMeans et al., 2015; Biton-Porsmoguer et al., 2018; Li et al., 2023). The main prey of the Greenland shark are fish (mainly Teleosts and Elasmobranch as rays), mammals (seals, whales and bears), squids and crustaceans (shrimp and crabs) (McMeans et al., 2010; Nielsen et al. 2013; 2019). The species more contaminated by mercury (muscle) in Arctic were elasmobranch and mammals (as rays and seals, 1.2-2 THg $\mu g\ kg^{-1}$ dw) and teleosts (as ling, blue ling, tusk, 0.12-0.94 THg $\mu g\ kg^{-1}$ dw) (McMeans et al., 2010; 2015).

The results of this study demonstrate 1) the presence of mercury in the cartilage and 2) a strong correlation with the level of THg in cartilage with the skin and the muscle. Numerous studies have focused on the implications of mercury for human health (Ha et al., 2017). It is noteworthy that the flesh of the Greenland shark is consumed in Iceland, known as "Hákarl," and is subjected to a distinctive fermentation process followed by drying for a duration of four to five months (Travel Food Atlas, 2020). The maximum allowable mercury level for shark meat intended for human consumption is set at 1.0 mg/kg of fresh meat[1]. Considering the mercury levels analysed in this study, the shark flesh appears unsuitable for human consumption and could potentially pose a health risk. (Guzzi et al., 2021). Moreover, mercury can also have effect on sharks.

[1] Commission Regulation (EU) 2022/617 of 12 April 2022 amending Regulation (EC) No 1881/2006.

Potential effects of mercury on shark morphology and reproduction

The Elasmobranchs (sharks, skates and chimaeras) are characterized by a cartilaginous skeleton (Seidel et al. 2021). The vertebral centra of elasmobranchs are composed of calcified cartilage, mainly comprised of the calcium phosphate mineral hydroxyapatite [Ca10(PO4)6(OH)2], which is embedded within an organic matrix of proteins, including proteoglycan and collagen (Urist, 1961; Porter et al., 2006). Mercury leads to metabolic disorders and disrupts the proper assembly of the cytoskeleton in marine species. The toxicity of mercury also causes morphological alterations on the development various fish organs, including the gills and olfactory organs. Malformations can involve the craniofacial and skeletal systems with stunted growth, curved spine, and eye deformities (Weis and Weis, 1977; Weis et al., 1981; Jagoe et al., 1996; Ribeiro et al., 1996; Oliveira Ribeiro et al., 2000, Devlin, 2006; Adams et al., 2010).

The effects of mercury on bone metabolism have also been demonstrated for teleosts and in particular for the activity of osteoclasts (cell responsible for the dissolution and absorption of bone) and osteoblasts (cell which secretes the substance of bone) (Suzuki et al., 2004). The methylmercury reduces blood calcium levels (calcemia), directly affecting the metabolism of bone cells at the scale (Suzuki et al., 2004). Osteoclasts on bone and those on cartilage also have similar basic molecular characteristics (Larrouture et al., 2021). Yachiguchi et al., (2014) reported a decrease in TRAP (Tartrate-Resistant Acid Phosphatase) and ALP (alkaline phosphatase) expression in marine fish exposed to Methylmercury or inorganic mercury. The ALP and TRAP are known as marker enzymes for osteoblasts and osteoclasts, respectively (Bonuci and Nanci 2001). Mercury would therefore inhibit the activity of both osteoclasts and osteoblasts (Rodríguez and Mandalunis 2018).

The observation of sharks during boarding and the study of the literature did not make it possible to identify malformations in Greenland shark. However, many observations of morphological abnormalities in sharks have been documented for many years. Thirteen studies have been published since 1963 reporting malformations on the blue shark (*Prionace glauca*) (Linnaeus, 1758) in different marine areas (North and South Atlantic Ocean, Pacific Ocean, California Gulf, Mediterranean Sea) (Cabanillas-Torpoco et al., 2023). Hoenig and Walsh (1983) noted four cases of vertebral lesions in three species of sharks, Sandbar shark *Carcharhinus plumbeus* (Nardo, 1827, Limon shark *Negaprion brevirostris* (Poey, 1868) and Sand tiger shark *Carcharias Taurus* Rafinesque, 1810). The spines had fused centra, ribs, and neural arches, additional deposition and erosion of calcified material in the centra, and in one case compression of the centra (Hoenig and Walsh 1983). Missing fins in an adult tawny nurse shark, *Nebrius concolor* (Lesson, 1831) were reported by Taniuchi andYanagisawa (1987), abnormal bicephalism in blue shark and tope shark (*Galeorhinus galeus*) (Linnaeus, 1758) by Ramirez-Amaro et al. (2019). The complete absence of pelvic fins in a milk shark (*Rhizoprionodon acutus*) (Rüppel, 1837) was reported in the Persian–Arabian Gulf (Moore 2015). The causes of such deformities were unknown.

The environmental degradation or pollution (effects of contaminants) can explain the malformation presence in sharks (Casarini, 1997; Rosa et al., 2004; Mancini et al., 2006). In this context, the presence of high levels of mercury (Hg) have been confirmed by several studies (Barrera-García et al., 2012; Rodríguez-Romero et al., 2018) and genetic alterations abnormalities in a blue shark embryo related with initial stages of development (Mancini et al., 2006; Rodríguez-Romero et al., 2018). Moreover, Van Hees and Ebert (2017) have demonstrated the maternal mercury transfer on the leopard shark *Triakis semifasciata* Girard, 1855 (an aplacental viviparous species as Greenland shark). Embryos were found with potentially harmful mercury concentrations in their muscle tissues.

4. Conclusion

Marine ecosystems in the Arctic are globally contaminated by mercury (atmosphere, water, sediments, food web). Hg analyses performed on the specimen showed a contamination in different parts of his organism (cartilage, skin and muscle) with potential risks for the individual and human health. Recent studies demonstrated the maternal mercury transfer for aplacental viviparous and connected mercury with potential morphological anomalies on sharks. Arctic temperatures will increase more than the global average (Johannessen et al., 2004). Estimated aerobic scope for polar species will indicate their adaptive capacity and predictions of food web shifts (Steiner et al., 2019). Studies on mercury levels on elasmobranch should to be encouraged to analyse the state of embryos and better understand the causes of malformations. These results could be very interesting to list the species most affected and for their survival rate and the conservation of the species.

However, considering the substantial amount of data that corroborates the elevated mercury levels found across various tissues of the Greenland shark (including cartilage), as well as the observed morphological anomalies in numerous other species (raising suspicions of environmental pollution), coupled with the declining population in Europe (Burgess et al., 2015), shouldn't precautionary measures be implemented as a principle of safeguard?

In order to protect the environment, the precautionary approach shall be widely applied by States according to their capabilities. Where there are threats of serious or irreversible damage, lack of full scientific certainty, explained in this case in part by the difficulty of studying this Green land shark at different biological stages but also by the geographical area and sampling conditions, shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation (Principle 15 of the 1992 Rio Declaration States) (United Nation, 1992).

One of the objectives of this work was to provide recommendations for conservation strategies aimed at reducing bycatch, detailed by gear previously. The proposal measures could be, 1) Islandic and Greenland fleets must take the necessary measures to develop the turtle excluder devices and Nordmore grates and release specimen still alive; 2) Protect the central Arctic Ocean beyond national jurisdiction with a precautionary fisheries moratorium; 3) Contribute effectively to the fight against global warming and reduce any industrial production that is a source of mercury (and any other pollutant) in the Arctic Ocean and 4) Initiate research programs to better understand the areas of movement and distribution of the species, which will inevitably be reduced by climate change and in order to limit any fishing activity there.

Declaration of competing interest

The authors have no competing interests to declare that are relevant to the content of this article.

Acknowledgments

The author (SBP) thanks the crew and the captain of the bottom trawler (IMO 9226475) who accepted his presence on board for several days and who authorized the samples taken from the specimen in the Greenland Sea. SBP also thanks the co-authors from IFREMER for the analyses of the samples without which this study could not be carried out.

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FIGURES CAPTIONS

Fig. 1. Position of the Greenland shark fished and sampled off Northwest Iceland marked by red cross. A. Skin. B. Upper jaw. C. Fresh meat. D. Lower jaw. Female. Total Length 434 cm. Greenland Sea. August 2010.

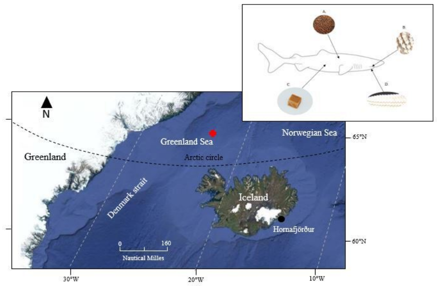
Fig.2. Hg level (mg kg^{-1} dw) in selected tissues of the Greenland shark: cartilage (upper and lower jaw), skin and muscle (fresh meat).

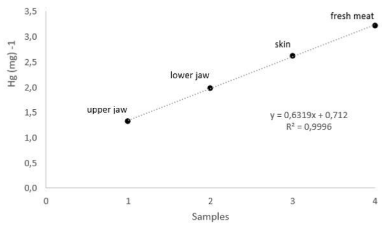
TABLES

Table 1. Range of total Hg levels (mg kg^{-1} ww) in the Greenland shark (*Somniosus microcephalus*) in the Arctic and adjacent seas; - No data. dw: dry wet; ww: wet weight; dry weight (dw) concentration = 5 ww concentration (Cresson et al., 2014).

Regions	Greenland shark							
	TL (cm)	upper jaw	lower jaw	muscle	skin	liver	ww/dw	References
Greenland Sea	434	1.332	1.985	3.225	2.625	-	dw	*This study*
Cumberland Sound	236-325*	-	-	-	-	4.92±0.58	dw	McMeans et al. 2007
Islandic waters	415.6±25.2	-	-	5.93±0.59	-	5.32±2.73	dw	McMeans et al. 2010
Pangnirtung, Baffin Bay	293±1.1	-	-	1.715±0.457	-	-	ww	Chételat and Braune 2012
NE Greenland fjords	-	-	-	4.10-6.91	-	-	dw	Corsolini et al. 2014
Labrador Sea	273.3±31.5	-	-	3.54±1.02	-	-	dw	McMeans et al. 2015

TL = Total Length of individuals (cm); *fork length (cm); Hg = Total Hg concentration (mg kg−1)


A.
B.
C.
D.
N
Greenland Sea
Norwegian Sea
65°N
Greenland
Arctic circle
Denmark strait
Iceland
Hornafjörður
0
160
Nautical Milles
60°N
30°W
20°W
10°W



3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
Hg (mg) -1
fresh meat
skin
lower jaw
upper jaw
y = 0,6319x + 0,712
R² = 0,9996
0
1
2
3
4
Samples

Declaration of competing interest

The authors have no competing interests to declare that are relevant to the content of this article.