

# Tracing Mercury Pollution along the Norwegian Coast via Elemental, Speciation, and Isotopic Analysis of Liver and Muscle Tissue of Deep-Water Marine Fish (*Brosme brosme*)

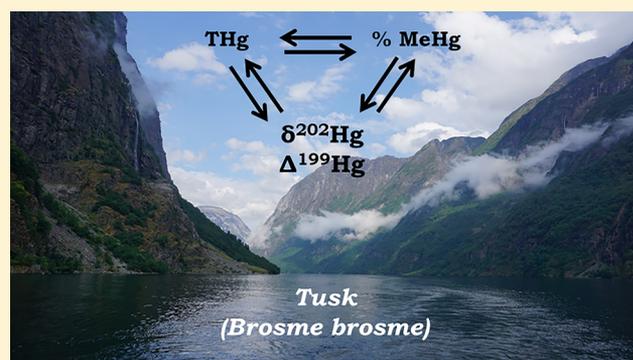
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## Supporting Information

**ABSTRACT:** Liver and muscle tissue of tusks (*Brosme brosme*) have been analyzed for their THg and MeHg concentrations and Hg isotopic signatures for tracing Hg pollution along the Norwegian coast. Clear differences between tissue types and locations were established. At five of the eight locations, the Hg concentration in muscle exceeded the maximum allowable level of 0.5 mg kg<sup>-1</sup> wet weight.  $\delta^{202}\text{Hg}$  values in both tissue types indicated that Hg speciation affects the bulk Hg isotopic signature. Tusk liver seems to be more sensitive to immediate changes and to anthropogenic inorganic Hg, while the muscle rather reflects the Hg accumulated over a longer period of exposure. The  $\delta^{202}\text{Hg}$  values of liver and muscle also enabled different sources of Hg and exposure pathways to be distinguished.  $\delta^{202}\text{Hg}_{\text{muscle}} - \delta^{202}\text{Hg}_{\text{liver}}$  showed a clear correlation with the % MeHg in tusk liver for the coastal waters, but not for the fjords. The absence of significant differences in  $\Delta^{199}\text{Hg}$  values between both tissues of tusk from the same location suggests that in vivo metabolic processes are the underlying reason for the differences in Hg speciation and in  $\delta^{202}\text{Hg}$  values. This work highlights the importance of selecting different tissues of marine fish in future Hg monitoring programs.



## INTRODUCTION

Mercury (Hg) is one of the most important global pollutants. It occurs in the environment as a result of natural and anthropogenic sources and both wildlife and humans are exposed to the harmful effects of this highly toxic heavy metal and its compounds. Anthropogenic Hg emissions resulting from a variety of processes, such as coal combustion, metal refining and manufacturing, cement production and waste incineration, are the main contributors to the worldwide increasing Hg levels.<sup>1</sup> Hg is globally distributed through the atmosphere, atmospheric deposition being the largest source of Hg to the oceans. In aquatic ecosystems, inorganic Hg can be converted into methylmercury (MeHg), the most toxic Hg species. MeHg can be bioaccumulated and biomagnified across food chains,<sup>2</sup> making seafood consumption the prime source of human Hg intake. Norway is the world's second largest exporter of seafood and the related industry is one of the most important pillars of the country's economy.<sup>3</sup> Given its toxicity, there is increasing attention for Hg contamination in seafood, an issue requiring proper attention from the health and food safety authorities.

Several aquatic/marine species, in different trophic positions, have been used over the years for tracing Hg pollution

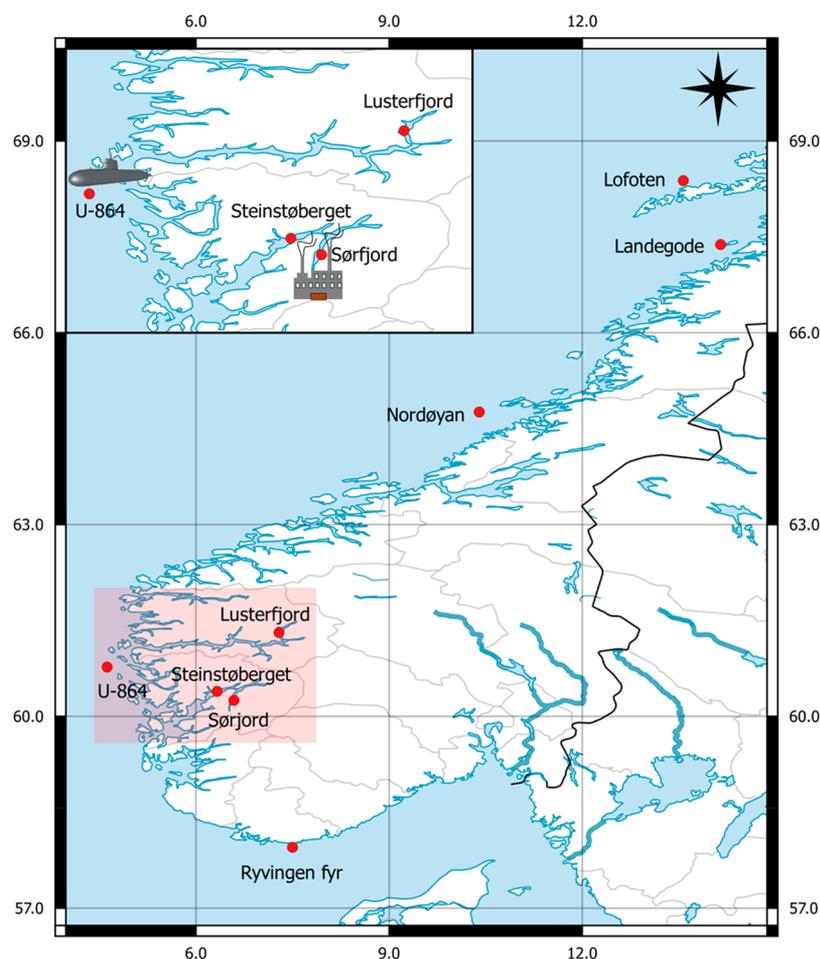
and for improving the understanding of the different pathways of Hg across aquatic/marine food chains.<sup>4</sup> A group of special interest comprises common species that do not migrate over large distances. Tusk (*Brosme brosme*) is such a species. It is a benthic gadoid fish that is distributed widely in Norwegian waters, both in fjords, along the coast and in open ocean areas. Tusk is one of the species of commercial importance for Norway, with catching volumes exceeding 10 000 t per year.<sup>5,6</sup> It is a popular fish for the restaurant market, with its fine taste and texture.<sup>7</sup> It has been reported that tusk tends to accumulate higher Hg levels than other fish species living in the same area, e.g., cod.<sup>7,8</sup> As a result, tusk may be considered as a key species for present and future Hg monitoring programs.<sup>9</sup> In addition to monitoring selected marine species, the study of different tissues from the same species might provide further insight aiming at identifying Hg sources and at unraveling exposure pathways.

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**Figure 1.** Map of Western Norway showing the sampling locations where the tusks (*Brosme brosme*) were collected. Fjords: Sør fjord, Steinstøberget, and Lusterfjord. Coastal locations: Ryvingen fyr, U-864, Nordøyen, Landegode, and Lofoten.

Next to elemental and speciation analysis, also Hg isotopic analysis can contribute to an improved understanding of the complex biogeochemical Hg cycle.<sup>10</sup> The isotopic composition of Hg varies between different sources, and different physical and/or (bio)chemical processes are accompanied by Hg isotope fractionation.<sup>11</sup> Mass-dependent fractionation (MDF) was shown to accompany various environmentally relevant processes, such as sorption,<sup>12,13</sup> equilibrium evaporation,<sup>14,15</sup> microbial methylation and demethylation,<sup>16,17</sup> microbial reduction,<sup>18</sup> and photochemical reduction.<sup>19–21</sup> It has also been demonstrated that—at least in some species—different in vivo metabolic processes, such as MeHg demethylation, induce Hg isotope fractionation. While for mammals, the occurrence of in vivo demethylation is widely accepted,<sup>22,23</sup> its occurrence in fish is a matter of more controversy,<sup>24</sup> but several recent papers do claim its existence.<sup>25–29</sup> As a result, a more profound understanding of the different transformations that Hg undergoes in nature is mandatory for tracing the origin of Hg pollution in marine ecosystems by means of Hg isotopic analysis. In addition to MDF, the isotopic composition of Hg can also be affected by mass-independent fractionation (MIF),<sup>30</sup> exhibited primarily by the odd-numbered Hg isotopes (<sup>199</sup>Hg and <sup>201</sup>Hg). MIF has been explained by the nuclear volume effect (NVE) and the magnetic isotope effect (MIE).<sup>31–34</sup> The photoreduction of Hg(II) and the photodegradation of MeHg are considered as the main processes yielding “odd-MIF”.<sup>19</sup> As both MDF and MIF of Hg isotopes

occur in nature, Hg isotopic analysis provides a “multi-dimensional” tracer and a powerful tool for enhancing our understanding of the biogeochemical Hg cycle.

In this work, the possibility of using tusk for tracing Hg pollution along the Norwegian coast has been assessed by means of the determination, speciation and isotopic analysis of Hg in its liver and muscle tissue. The results obtained have also been used to evaluate which organ/tissue is the best suited for monitoring Hg exposure.

## ■ MATERIALS AND METHODS

**Sample Collection and Sample Preparation.** A total of 137 tusk fish (*Brosme brosme*) were caught at eight different locations along the Norwegian coast (see Figure 1 and Table S1 of the Supporting Information (SI) for further information on the sampling zones). Three of the locations are located in fjords: Sør fjord ( $N = 8$ ), Steinstøberget ( $N = 25$ ), and Lusterfjord ( $N = 14$ ). The remaining five locations are spread along the Norwegian coast (south to north): Ryvingen fyr ( $N = 25$ ), U-864 ( $N = 8$ ), Nordøyen ( $N = 25$ ), Landegode ( $N = 25$ ), and Lofoten ( $N = 7$ ). “U-864” refers to a location where a WWII submarine wreck carrying 67 tons of metallic Hg has contaminated the local sediment.<sup>35</sup>

From each fish, liver and muscle tissues were manually separated and homogenized (thus resulting in 274 samples). For the Sør fjord and U-864 locations, the tissues of the individual fish (i.e., 32 tissue samples) were measured

separately (both for elemental and isotopic analysis). For the other 6 locations, samples of liver and muscle of individual fish from each location were pooled and homogenized. After processing, all samples were stored frozen ( $<-20$  °C) until analysis.

Prior to elemental and isotopic analysis, 0.5–1.2 g of sample or certified reference material (CRM) were acid-digested with a 3:1 mixture of 7 M  $\text{HNO}_3$  and 9.8 M  $\text{H}_2\text{O}_2$  in closed microwave vessels using a Milestone (Italy) Ethos One High-Performance Microwave Digestion System (the microwave program was selected following the instructions of the manufacturer and is given in Table S2). The resulting digests were diluted and subjected to ICP-MS analysis (see SI).

In addition, a sediment sample from the vicinity of the Odda Zn smelter was collected to assess a possible link with the occurrence of Hg in fish from the Sørffjord location. Approximately 0.3 g of this sediment sample (two digestion replicates) and of a CRM were also acid-digested (using the same microwave program—Table S2) with a 3:1:1 mixture of 14 M  $\text{HNO}_3$ , 9.8 M  $\text{H}_2\text{O}_2$ , and 28 M HF, and subsequently diluted prior to ICP-MS analysis.

**Reagents and Standards.** Pro-analysis 14 M  $\text{HNO}_3$  and 12 M HCl (ChemLab, Belgium), further purified by sub-boiling distillation in PFA equipment, 9.8 M  $\text{H}_2\text{O}_2$  (Fluka, Belgium), ultrapure 28 M HF (Fisher Chemicals, Great Britain), and high-purity water obtained from a Milli-Q Element water purification system (Millipore, France) were used for sample digestion and subsequent dilution. For Hg isotopic analysis, pro-analysis  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$  (Sigma-Aldrich, U.K.) was used for Hg introduction via the reduction of  $\text{Hg}^{2+}$  into elemental Hg.

Appropriate dilutions of 1000  $\text{mg L}^{-1}$  stock solutions of As, Cd, Cr, Cu, Hg, In, Ni, Pb, Rh, Se, and Zn (Inorganic Ventures, The Netherlands) were used as calibration standards for elemental analysis. For isotopic analysis, the Hg and Tl isotopic reference materials (NIST SRM 3133 and NIST SRM 997) were used for mass bias correction. An in-house standard solution with well-characterized Hg isotopic composition (Inorganic Ventures, The Netherlands)<sup>35,36</sup> and several CRMs with a matrix composition similar to that of the samples of interest were used for QA/QC.

**Elemental and Isotopic Analysis.** A ThermoScientific (Germany) Element XR single-collector sector-field ICP-MS instrument (SF-ICP-MS) was used for elemental analysis; additional information is provided in the SI and in Table S3. For THg quantification QA/QC (method detection limit of 1  $\mu\text{g kg}^{-1}$ ), different CRMs (BCR CRM 464 tuna fish, NRC–CNRC DORM-4 fish protein, TORT-3 lobster hepatopancreas and NIST SRM 2704 Buffalo river sediment) were analyzed for their Hg content (average recovery of 97%—Table S4).

MeHg speciation was performed via isotope dilution gas chromatography ICP-MS (GC-ICP-IDMS) using an Agilent (U.S.A.) 6890N gas chromatograph coupled to an Agilent Technologies (Japan) 7500a ICP-MS instrument. The extraction procedure and the details on the GC-ICP-MS method are described elsewhere.<sup>37</sup> For QA/QC, various CRMs—NRC–CNRC TORT-2 lobster hepatopancreas, DORM-3 dogfish muscle, DOLT-4 dogfish liver, and ERM CE464 tuna muscle—were used. The results were found to be in good agreement with the corresponding certified values ( $z$ -scores  $< 1.5$ ).

The results obtained upon elemental analysis are expressed as their average  $\pm$  standard deviation in the case of the pooled

samples. For the Sørffjord and U-864 locations (for which individual fish were measured instead of pooled samples), however, median, minimum and maximum concentrations are reported. All concentrations were expressed on an “as-is” basis or as “wet” values (per unit of wet tissue weight), as also the maximum allowable concentration is expressed on a wet weight basis.

A ThermoScientific (Germany) Neptune multicollector ICP-MS (MC-ICP-MS) instrument with an HGX-200 cold vapor and hydride generation unit for sample introduction (Teledyne Cetac Technologies, U.S.A.) was used for Hg isotopic analysis (this setup is described in detail elsewhere,<sup>38</sup> while the instrument settings are provided in Table S5).

Instrumental mass discrimination was corrected for by using the combination of the “Baxter approach”—using Tl (NIST SRM 997) as an internal standard—and external correction in a sample-standard bracketing (SSB) approach using the Hg isotopic reference material NIST SRM 3133.<sup>36,38</sup> A description of the CVG-MC-ICP-MS measurement protocol, including QA/QC, is provided in the SI and in Table S6.<sup>35,36</sup>

Mass-dependent fractionation (MDF) and mass-independent fractionation (MIF) are reported in delta ( $\delta^{xxx}\text{Hg} \text{‰}$ ) and capital delta ( $\Delta^{xxx}\text{Hg} \text{‰}$ ) notation, respectively (the corresponding equations are given in the SI).

## RESULTS AND DISCUSSION

**Mercury Distribution between Liver and Muscle Tissue of Tusk.** Concentrations of THg and MeHg in liver and muscle tissue of tusks collected along the Norwegian coast are shown in Table S7 and Figure S1. In addition, also those of other environmentally relevant metals—As, Cd, Cr, Cu, Pb, Ni, Se, and Zn—were determined to evaluate possible links with the occurrence of Hg in fish tissues. These possible links are indicated along this manuscript, while the overall results are described in the SI and are shown in Table S7 and Figure S2.

Total Hg (THg) concentrations ranged from 0.11 (Lofoten) to 27 (Sørffjord)  $\text{mg kg}^{-1}$  in liver tissue and from 0.20 (Lofoten) to 2.6 (Sørffjord)  $\text{mg kg}^{-1}$  in muscle tissue, while MeHg concentrations ranged from 0.050 (Lofoten) to 3.5 (Sørffjord)  $\text{mg kg}^{-1}$  in liver tissue and from 0.18 (Lofoten) to 1.6 (Sørffjord)  $\text{mg kg}^{-1}$  in muscle tissue. MeHg and THg concentrations correlated for both liver (Figure S3A) and muscle (Figure S3B) tissues (Spearman’s correlation,  $r = 0.976$  and  $0.970$ ,  $p = 0.000$  for liver and muscle, respectively). In addition, a strong positive correlation was found between  $\text{THg}_{\text{liver}}$  and  $\text{THg}_{\text{muscle}}$  (Figure S3C) and  $\text{MeHg}_{\text{liver}}$  and  $\text{MeHg}_{\text{muscle}}$  (Figure S3D), although a clear deviation from the general behavior is observed for the Sørffjord location, i.e., the most polluted area, for which liver concentrations were particularly high compared to the muscle concentrations (Spearman’s correlation excluding the Sørffjord data points,  $r = 0.964$ ,  $p = 0.000$ , and  $r = 0.955$ ,  $p = 0.001$ , for THg and MeHg, respectively).

Overall, liver and muscle of tusks collected in the fjords show higher THg concentrations than those from coastal locations. This observation was also found for the majority of the other environmentally relevant metals, and it can most likely be attributed to local anthropogenic sources and/or to the poorer water circulation in fjords compared to open ocean areas. Also, the specific morphology of the fjords gives rise to higher accumulation of pollutants, potentially trapped at great depths and for longer periods within the fjord.<sup>39</sup>

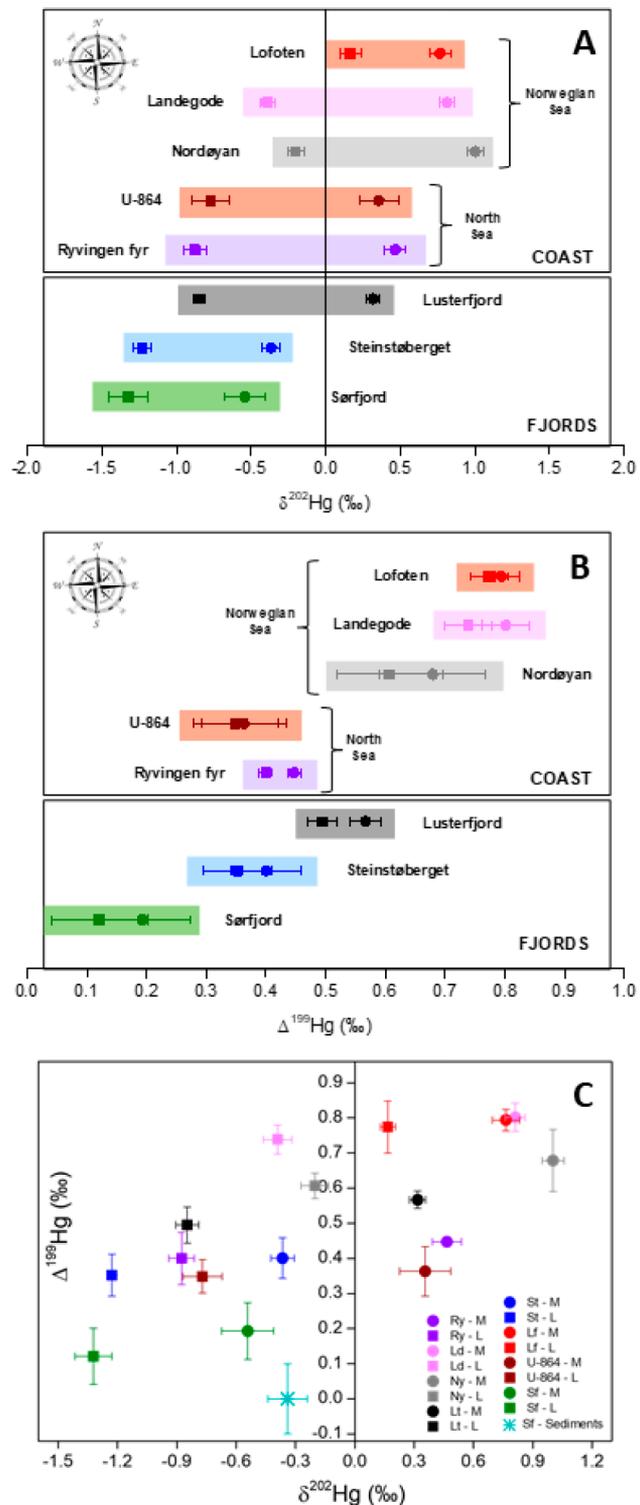
THg concentrations were also found to be significantly higher in liver than in the corresponding muscle tissue for tusk from the three fjord locations (THg<sub>liver</sub>/THg<sub>muscle</sub> ratio ranging from 1.8 to 4.3), while for tusk from the coastal locations, the THg concentrations in muscle were rather similar to and sometimes even slightly higher than the corresponding levels in liver (THg<sub>liver</sub>/THg<sub>muscle</sub> ratio ranging from 0.55 to 1.4). The liver-to-muscle ratio has already been used to identify the degree of Hg pollution in a specific ecosystem (THg<sub>liver</sub>/THg<sub>muscle</sub> > 1 indicates an increase in Hg uptake).<sup>40,41</sup> Similarly, significant differences were found between both tissue types in the case of the other environmentally relevant metals, with liver tissue generally showing much higher concentrations than muscle tissue. The THg concentrations observed in tusk from the fjords indicate an unusually high Hg intake, greatly affecting the liver as a vital organ owing to its key metabolic role in, e.g., Hg redistribution, excretion and/or detoxification.<sup>23,42–44</sup>

Also MeHg levels revealed differences as a function of location and tissue type. Approximately 100% of the Hg present in muscle tissue of tusk was in the form of MeHg,<sup>42</sup> while in liver tissue only 14 to 52% of the Hg was in the form of MeHg. For liver tissue of tusk, the lowest % MeHg<sub>liver</sub> corresponded with the most polluted area (Sørfjord), while the highest % MeHg<sub>liver</sub> was found for the least polluted area (Lofoten); assessment of the degree of pollution was based on the THg concentration in tusk. In fact, a negative correlation was found between THg and % MeHg<sub>liver</sub> (Spearman's correlation,  $r = -0.786$ ,  $p = 0.021$ ), indicating that a high degree of Hg pollution is accompanied by a low % MeHg in tusk liver. A reduction of the MeHg fraction in fish tissues as a function of the amount of anthropogenic Hg was found to be in good agreement with previous literature on contaminated freshwater lakes and it can most likely be attributed to direct iHg intake and/or differences in Hg metabolism (e.g., higher MeHg demethylation rate<sup>41</sup>) in heavily polluted areas.<sup>45</sup> The differences in Hg speciation between liver and muscle tissue of tusk may stem from a different distribution of the different Hg compounds (iHg and MeHg) (i) as originally taken up and/or (ii) as present after partial demethylation of MeHg.<sup>23</sup> Marine fish primarily accumulate Hg via the diet (mainly in the form of MeHg), although some direct absorption via the gills, particularly in highly polluted areas, cannot be ruled out.<sup>46</sup>

When the ingested Hg has been absorbed in the intestine, it is transported through the bloodstream to vital organs, such as liver, which, along with the intestine, has been identified as a key organ for MeHg demethylation.<sup>28</sup> The different Hg compounds can then be (partly) accumulated in the liver and/or (partly) redistributed to other organs and tissues, such as muscle. MeHg can cross cellular membranes more readily compared to iHg, allowing MeHg to reach the muscle tissue where it can bind tightly with cysteine-rich proteins. Therefore, muscle tissue acts as a reservoir of the MeHg resulting from the MeHg intake coming from the diet or the remaining MeHg after partial demethylation, while the iHg thus produced and/or the iHg fraction directly introduced into the fish in highly polluted areas accumulates in liver tissue. The different accumulation patterns and the preferential excretion of iHg compared to MeHg explain the differences in MeHg fractions between liver and muscle tissues of tusk.<sup>23,46</sup>

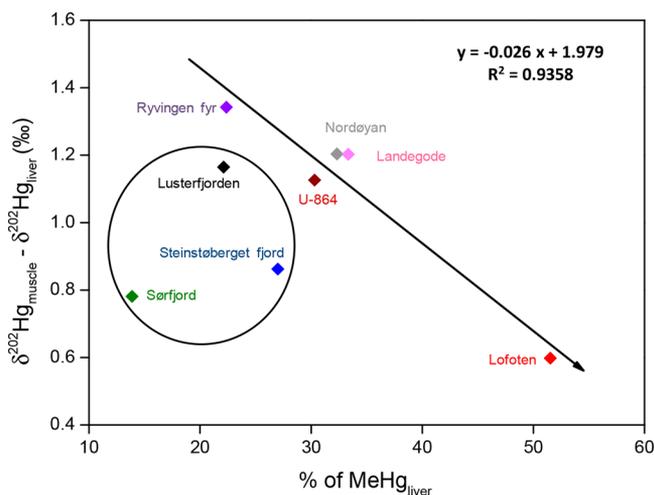
In addition to THg concentration and MeHg speciation, the isotopic composition ( $\delta^{199}\text{Hg}$ ,  $\delta^{200}\text{Hg}$ ,  $\delta^{201}\text{Hg}$ ,  $\delta^{202}\text{Hg}$ ,  $\Delta^{199}\text{Hg}$ ,  $\Delta^{200}\text{Hg}$ , and  $\Delta^{201}\text{Hg}$ ) of Hg can provide added value (the Hg

isotope ratio results are provided in Table S8 and are summarized in Figure 2). Figure 2A shows how  $\delta^{202}\text{Hg}$  (MDF) varies between the different locations and between the two tissue types, with values ranging from  $-1.32$  to  $0.17$  ‰ for liver tissue (average of  $-0.65$  ‰) and from  $-0.54$  to  $1.00$  ‰ for muscle tissue (average of  $0.35$  ‰). Overall, significant



**Figure 2.**  $\delta^{202}\text{Hg}$  (A),  $\Delta^{199}\text{Hg}$  (B), and  $\Delta^{199}\text{Hg}$  vs  $\delta^{202}\text{Hg}$  (‰) for liver (squares) and muscle (circles) tissues of tusk (*Brosme brosme*) for the different locations studied in this work.

variations were observed between the different locations and between the two different tissue types. The lowest  $\delta^{202}\text{Hg}$  values correspond with the highest Hg concentrations (Sørfjord), while the highest  $\delta^{202}\text{Hg}$  values were found in the less polluted areas, i.e., Lofoten and Nordøyen; these two locations are situated on the Norwegian Sea coast, where a high level of Hg accumulation is not expected as it is relatively remote from most sources of Hg pollution. This suggests a certain correlation between  $\delta^{202}\text{Hg}$  values and THg concentrations. The existence of this correlation was indeed confirmed for liver (Spearman's correlation,  $r = -0.786$ ,  $p = 0.021$ ), while such a correlation was not found for muscle (Figure S4). Liver tissue always showed a lower  $\delta^{202}\text{Hg}$  (more enriched in the lighter Hg isotopes) than the corresponding muscle tissue (by 1 ‰ on average). In a previous work,<sup>23</sup> liver tissues of marine mammals (seals and whales) were also found to be enriched in the lighter Hg isotopes compared to muscle tissues. In this work, the difference in  $\delta^{202}\text{Hg}$  values between the two tissues ( $\delta^{202}\text{Hg}_{\text{muscle}} - \delta^{202}\text{Hg}_{\text{liver}}$ ) ranged from 0.60 to 1.34 ‰. These differences showed a clear negative correlation with the % MeHg in liver for the coastal locations (Figure 3).



**Figure 3.**  $\delta^{202}\text{Hg}_{\text{muscle}} - \delta^{202}\text{Hg}_{\text{liver}}$  as a function of the % MeHg in liver for the eight locations studied in this work.

When fitting a straight line through the data for the coastal locations only, i.e., excluding the data for the three fjords, an  $R^2$  coefficient of 0.936 and a  $p$ -value of 0.007 were obtained. The deviation from the line for the fjord samples indicates the additional uptake of iHg and/or higher Hg demethylation rates with increased exposure, i.e., in heavily polluted areas.<sup>26,41</sup>

Study of the additional MIF affecting the odd-numbered isotopes of Hg (Figure S5) can shed some light onto the origin of the differences in Hg speciation and isotopic signatures for liver and muscle tissues of tusk.  $\Delta^{199}\text{Hg}$  as a function of location and tissue type ranged from 0.12 to 0.77 ‰ in liver tissue and from 0.19 to 0.80 ‰ in muscle tissue (Figure 2B and Table S8). The  $\Delta^{199}\text{Hg}$  values were found to be related with the THg concentration, with a lower extent of MIF in the highest polluted areas (Sørfjord) and a higher degree of MIF in the two northernmost locations, Landegode and Lofoten. This was found to be in good agreement with the low MIF shown in heavily polluted areas.<sup>47</sup> In contrast to the situation for MDF, no significant differences in  $\Delta^{199}\text{Hg}$  values between liver and muscle tissues were observed, barely affected by the location studied. The absence of significant differences in  $\Delta^{199}\text{Hg}$  values

between liver and muscle of tusk from the same location suggests that in vivo metabolic processes, such as MeHg demethylation, are the underlying reason for the differences in Hg speciation and in  $\delta^{202}\text{Hg}$  values between both tissue types. Also the experimental  $\Delta^{199}\text{Hg}/\Delta^{201}\text{Hg}$  ratio of  $1.15 \pm 0.06$  (Figure S6) was found to be close to the value characteristic for MeHg photodegradation ( $\sim 1.2$ ).<sup>19</sup> We observed a gradient in  $\Delta^{199}\text{Hg}$  from open ocean to the coast, suggesting that the MeHg has undergone different degrees of photodemethylation before it entered the food web. Therefore, the  $\Delta^{201}\text{Hg}$  values were used to estimate the % of MeHg photodegraded, with values ranging between 6 and 24% (see the SI for further insights into the use of MIF Hg isotopic signatures).<sup>19,22,48–50</sup>

As indicated before, while in vivo demethylation of MeHg in mammals is accepted, for fish it is a matter of controversy. Perhaps the relatively high position of tusk within the food chain might be responsible for the differences observed between tusk and small fish species commonly used in feeding experiments. Thus, the differences in Hg isotopic composition between liver and muscle may result from in vivo metabolic processes, such as MeHg demethylation, and they are linked to a difference in Hg speciation between the two tissue types, greatly affecting the corresponding bulk Hg isotopic signatures (Figure S7).<sup>23</sup> In addition, the mixture of Hg from various sources and/or of different Hg compounds (especially in highly polluted areas) cannot be ruled out, although based on the consistency of the  $\Delta^{199}\text{Hg}$  values between both tissues of tusk from the same location (see below), this possibility is rather unlikely.

**Potential Links between Mercury Occurrence in Tusk and Anthropogenic Sources.** Since the level of THg in tusk muscle (the edible part) exceeded the European limits (established at  $0.5 \text{ mg kg}^{-1}$  (w.w.))<sup>51</sup> in five of the eight locations studied in this work, i.e., in all of the fjord locations and in two of the five coastal locations (Ryvingen fyr and Landegode), the link between potential anthropogenic Hg sources and the occurrence of mercury in liver and muscle tissues of tusk was assessed. As a result of their specific topography (e.g., poorer water circulation and/or higher contribution of natural terrestrial runoff), elevated concentrations of Hg (and other pollutants) are expected for fjord locations compared to coastal and open ocean areas. In this work, this was observed for fish tissues from these fjords, although at an even higher extent in the case of the Sørfjord, which can be attributed to a higher input from anthropogenic Hg sources at this location.

Two well-established Hg sources were hypothesized to be related with the occurrence of Hg in tusk from the Sørfjord and U-864 locations (fjord and open ocean area, respectively). In previous works, high local Hg pollution was reported in fish, soils and mushrooms from the surroundings of the Sørfjord location.<sup>7,52–55</sup> This was mainly attributed to the direct impact of a Zn smelter located in Odda, a small town situated at the southern end of the Sørfjord, since Hg is an important associated element in Zn ores.<sup>56</sup> Thus, Hg contamination in this area may stem from byproducts of the roasting process of Zn ores, e.g., atmospheric emission of  $\text{Hg}^0$  and/or  $\text{Hg}^{2+}$  and subsequent deposition, and/or (accidental) release of contamination into the fjord water e.g., during unloading of Zn ore material at the dock or by residues from the smelting process.<sup>56–59</sup> In this work, the highest THg concentrations in liver and muscle tissues of tusk were found in the Sørfjord, while tusk livers also showed significantly higher concen-

trations of Pb than for the other locations (5–10-fold higher), illustrating the anthropogenic pollution affecting this area. In addition, a 2-fold higher concentration of Zn was observed compared to all the other locations, supporting the influence of the Zn smelter located in Odda on the fjord ecosystem. This elevated concentration of Hg and other metals, such as Pb and Zn, was also found in fish and sediments from other ecosystems affected by smelting and mining activities.<sup>60–62</sup> Also, the low % MeHg in liver (14%) pointed to an anomalously high Hg pollution, as discussed in the previous section.

In addition, the lowest  $\delta^{202}\text{Hg}$  ( $-1.32 \pm 0.09$  and  $-0.54 \pm 0.13$  ‰, for liver and muscle, respectively) and  $\Delta^{199}\text{Hg}$  ( $0.12 \pm 0.08$  and  $0.19 \pm 0.08$  ‰ for liver and muscle, respectively) were found in the Sør fjord.  $\Delta^{199}\text{Hg}$  values tend to be low in highly polluted areas owing to the large amount of anthropogenic iHg.<sup>47</sup> With the Zn smelter located in Odda as the main source of Hg pollution within the Hardangerfjord, tusk collected in the Sør fjord will most likely be affected to a higher extent because of the closeness to the contamination source. During Zn ore roasting, Hg present in the ore is fractionated, resulting in different Hg compounds with varying isotopic signatures, e.g., solid waste enriched in the heavier Hg isotopes and emissions of lighter  $\text{Hg}^0$  and/or  $\text{Hg}^{2+}$  to the atmosphere.<sup>58,63</sup> Thus, the Hg isotopic signatures of liver and muscle tissues of tusk can be the result of (i) mixing of Hg from multiple sources, such as direct release into the seawater, atmospheric deposition and/or freshwater runoff and of (ii) different *in vivo* metabolic processes and their associated variations in the isotopic composition. In order to assess the influence of the Zn smelter, marine bottom sediments were collected from an area close to where Zn ore was unloaded and waste material was collected. These sediments were found to be strongly affected by Hg contamination (THg concentration of  $1.40 \pm 0.25$  mg kg<sup>-1</sup>), and isotopic analysis of Hg showed  $\delta^{202}\text{Hg}$  and  $\Delta^{199}\text{Hg}$  values of  $-0.34 \pm 0.04$  and  $0.00 \pm 0.03$  ‰, respectively. These values were found to be similar (both for MDF and MIF) to those reported on in literature for sediments impacted by a Zn smelter via atmospheric deposition, and contaminated with byproducts of ore roasting (e.g., Hg waste calcine) and/or mining.<sup>58,61,64,65</sup> In this work, slightly more negative  $\delta^{202}\text{Hg}$  values were obtained for muscle tissue of tusk from the Sør fjord location relative to the sediments collected near the Zn smelter (Figure 2C), which can be explained by the net MDF accompanying the methylation and demethylation processes.<sup>66,67</sup> The small differences in  $\Delta^{199}\text{Hg}$  values can be explained by the input of iHg from atmospheric deposition and/or the contribution of the diet in an open marine Hg isotope composition, i.e., MeHg with high  $\Delta^{199}\text{Hg}$  anomalies. Thus, on the basis of the Hg isotope ratio results obtained in this work, the Hg pollution affecting the tusks collected at the Sør fjord location can most likely be attributed to the impact of the Zn smelter.

In the case of Steinstøberget (outer part of the Hardangerfjord),  $\delta^{202}\text{Hg}$  values ( $-1.23 \pm 0.02$  and  $-0.37 \pm 0.06$  ‰, for liver and muscle, respectively) also pointed toward contamination from the Zn smelter as the main source of Hg pollution. This can most likely be attributed to the water current going from the inner to the outer part of the Hardangerfjord and to the predominant winds going from south to north in this area (i.e., following the direction of the fjord as shown in Figure 1).<sup>53</sup> However, the slightly higher  $\Delta^{199}\text{Hg}$  values ( $0.35 \pm 0.06$  and  $0.40 \pm 0.06$  ‰ for liver and

muscle, respectively) seem to indicate a lower impact from the smelter, while the Sør fjord (with depths of ~350 m) and Steinstøberget (with depths of ~150 m) also show a difference in depth; Figure S8 shows the  $\Delta^{199}\text{Hg}$  values and the depth at which the tusks were caught for all the locations studied in this work.

As indicated above, from the eight locations studied, three areas did not show a high level of Hg pollution. Surprisingly, the U-864 location was found to be one of them. The U-864 is a German submarine that was carrying 67 tons of metallic Hg in its keel when it was torpedoed and sunk in the proximity of Bergen (Norway) at the end of World War II.<sup>35,68</sup> The U-864 Hg contaminated the surrounding sediments with metallic Hg,<sup>69</sup> but it is not clear yet whether this Hg has entered the food web. In a previous work from the same authors,<sup>35</sup> Hg isotopic analysis of brown crab (*Cancer pagurus*) tissues showed that  $\delta^{202}\text{Hg}$  values in the brown meat (predominantly consisting of the digestive system and the gonads) of crabs from the wreck location were shifted toward the isotopic signature of the metallic Hg collected at the vicinity of the submarine wreck, while such differences were not found for claw (muscle) meat (Figure S9). This shift in the Hg isotopic signature was hypothesized to be related with the feeding habits of this crab species, allowing for direct ingestion of metallic Hg. On the basis of (i) the THg and MeHg concentrations, (ii)  $\text{THg}_{\text{liver}}/\text{THg}_{\text{muscle}}$  ratio, and (iii) % MeHg in liver, of tusks from the U-864 location, we did not find any evidence of anomalously high degree of Hg pollution at this location. This can be attributed to the lower methylation rate of metallic Hg (if any at the location studied) compared to that of  $\text{Hg}^{2+}$ .<sup>70,71</sup>  $\delta^{202}\text{Hg}$  values for liver and muscle of tusks from the U-864 location were also compared to those obtained for crab tissues (Figure S9). In contrast to the previous work, the Hg isotopic signatures of liver and muscle of tusk do not seem to be affected by those of contaminated sediments from the wreck location and/or with that of metallic Hg from the submarine. Furthermore, no significant differences in  $\delta^{202}\text{Hg}$  and  $\Delta^{199}\text{Hg}$  values were found between tusk from the U-864 location and from Ryvingen fyr. It needs to be noted that higher Hg pollution was found for Ryvingen fyr (the location further south in the North Sea) compared to the U-864 location, and that the oceanic currents flowing from south to north in this area are not expected to allow the transport of the U-864 Hg from the wreck site to the Ryvingen fyr location. On the basis of the aforementioned results, we suggest that, so far, the metallic Hg from the U-864 wreckage has not entered the food chain.

In addition to the three fjords, higher concentrations of Hg in tusk from Ryvingen fyr and Landegode were found compared to that of the other coastal locations. The differences between these two locations and the other three locations displaying Hg concentrations below the allowable limit might be related to the higher input of Hg coming from other Hg sources and transported by oceanic currents. For instance, Hg transported from the areas of Denmark and Oslo could elevate the level of Hg at Ryvingen fyr, while at Landegode, Hg could accumulate as a result of geographical constraints, limiting the circulation of water and hampering the removal of discharged waste (Figure 1). A further evaluation of the Hg isotopic signatures of tusk from the different locations seems to indicate the occurrence of three groups (Figure 2). These groups correspond with the two locations of (i) the Hardangerfjord (the Sør fjord and Steinstøberget), (ii) the two locations in the

North Sea (U-864 and Ryvingen fyr), and (iii) the three locations in the Norwegian Sea (Nørdoyan, Landegode and Lofoten). Within the same group, the Hg isotopic composition of muscle was found to be more consistent than that of liver.

**Selection of Fish Tissue for Monitoring Mercury Exposure.** Addressing the appropriate tissue type(s) is a crucial issue for Hg monitoring programs. However, the selection of the best suited organ/tissue still remains challenging, especially given the dynamics of Hg within the different body compartments, their different affinities toward the respective Hg compounds and/or the occurrence of in vivo metabolic processes.<sup>41</sup> To address and/or minimize these issues, liver and muscle tissues of tusks from different locations have been measured in this work for their THg and MeHg concentrations and Hg isotope ratios, a combination that allowed one to do the following:

- use the  $\text{THg}_{\text{liver}}/\text{THg}_{\text{muscle}}$  ratio or liver/muscle index to identify heavily contaminated areas,
- assess the degree of Hg pollution by measuring the % MeHg in liver (also the % MeHg in muscle has been reported on for this purpose),
- provide insight into in vivo MDF and metabolic processes, such as MeHg demethylation, based on the comparison between  $\delta^{202}\text{Hg}$  for liver and muscle tissues, and
- evaluate the absence of in vivo MIF relying on the differences in  $\Delta^{199}\text{Hg}$  for liver and muscle, therefore providing additional information on different sources of pollution and/or different Hg compounds that can be characterized by a different degree of MIF.

Obviously, the measurement of different tissue types provides further information, but also increases the cost and time of the monitoring program. Therefore, it is important to realize the type of information provided by analyzing specific tissue types and the corresponding limitations. Liver is a highly responsive organ toward iHg exposure and is a target organ for contaminants, or in other words, it is able to accumulate higher Hg concentrations than other tissues. Liver also plays an important role in Hg detoxification and Hg metabolism, and is one of the key organs responsible of MeHg demethylation and subsequent redistribution of the two main forms of Hg. Thus, iHg in liver can originate from direct uptake by the organism and/or from the demethylation of MeHg. Given its importance, changes in Hg exposure are generally reflected first in the liver before they are evidenced in other organs and tissues, such as muscle. Some studies have indicated the faster turnover of this organ upon exposure and have noted the faster re-equilibration of the Hg isotopic signatures, thus better retaining the source signatures.<sup>72–74</sup> However, the occurrence of in vivo metabolic processes, such as MeHg demethylation, and the associated isotope fractionation might hide or completely erase the original source signature.<sup>74</sup> Because of this reason, Masbou et al.<sup>44</sup> suggested that liver  $\delta^{202}\text{Hg}$  is not a suitable tissue/tracer, as it is strongly influenced by hepatic metabolic MDF, and recommended the use of liver  $\Delta^{199}\text{Hg}$  owing to the absence of in vivo MIF and the capability to preserve the isotopic signatures. However, muscle is the target tissue for MeHg accumulation and the main reservoir of this highly toxic Hg species, but it is not a good site for iHg deposition.<sup>42</sup> Muscle is characterized by a slower response toward sudden changes in Hg concentrations and reflects the Hg accumulated over a longer period of exposure, making it

less sensitive to immediate changes in Hg exposure.<sup>40,41,74</sup> These differences between both tissue types have also been observed during this work, in which a higher degree of variability of the Hg isotopic signatures of tusk livers was found compared to that of muscles, thus pointing to the more sensitive behavior of liver as a response toward Hg exposure. Muscle tissue was found to be more consistent between groups, which was especially visible for the locations from the Norwegian Sea.

Although the following conclusions need to be handled with care due to the complexity of the biogeochemical Hg cycle and of each specific marine ecosystem, liver might be seen as the preferred organ of study when Hg contamination stems from inorganic forms, while one may assume that muscle is to be recommended to assess organic Hg pollution due to the storage capabilities of this tissue. However, in vivo metabolic processes occurring within the fish body can strongly influence the Hg finally stored in the muscle, and it is therefore important to investigate all these processes into a greater detail. It also needs to be noted that muscle is the edible part and its study is thus of high interest for seafood safety. In addition to the different Hg species, muscle can be recommended for lightly contaminated areas ( $\text{THg}_{\text{muscle}} > \text{THg}_{\text{liver}}$ ), while for heavily polluted locations, liver tissues provide additional information.<sup>40</sup>

Overall, important knowledge gaps still remain in our understanding of the biogeochemical Hg cycle, but elemental, speciation and isotopic analysis of Hg in (i) different species collected at the same location, (ii) the same species collected at different locations, and especially (iii) in different tissues of a selected species, seem to be a powerful approach for dealing with this important environmental and human threat.

## ■ ASSOCIATED CONTENT

### 📄 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acs.est.8b04706](https://doi.org/10.1021/acs.est.8b04706).

Figures S1–S9 and Tables S1–S8; elemental analysis protocol; Hg isotopic analysis protocol; determination of other environmentally relevant metals in tusk; and  $\Delta^{199}\text{Hg}/\Delta^{201}\text{Hg}$  ratios and estimation of the extent of MeHg photodegradation before its incorporation to the aquatic food web (PDF)

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The authors declare no competing financial interest.

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