Mercury in fish from the North East Atlantic: sources, bioaccumulation dynamics and co-occurrence with selenium

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Thesis for the degree of Philosophiae Doctor (PhD)
University of Bergen, Norway
2019

UNIVERSITY OF BERGEN

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Date of defense: 26.09.2019

Year: 2019

Title: Mercury in fish from the North East Atlantic: sources, bioaccumulation dynamics and co-occurrence with selenium

Name: Atabak Mahjour Azad

Print: Skipnes Kommunikasjon / University of Bergen

Scientific environment

The financial support for the PhD position was from the Department of Biology, University of Bergen, Norway. This study was conducted at the National Institute of Nutrition and Seafood Research (NIFES) which was merged with the Institute of Marine Research (IMR) on January 1th, 2019. The funding for the research project was provided by several organizations either directly for this research or indirectly by funding the monitoring projects that generated data utilized in this study: Norwegian Ministry of Trade, Industry and Fisheries, Norwegian Food Safety Authority, The Norwegian Coastal Administration, The Norwegian Seafood Research Fund and the Norwegian Herring Sales Organization.

Acknowledgements

The accomplishment of this PhD work was made possible by the contribution of numerous individuals. Writing this part of the thesis and thinking of all people who helped me is quite overwhelming. First, I would like to thank my supervisors Amund Maage, Sylvia Frantzen and Lise Madsen for their support, guidance and providing advices during my PhD and for having an open mind for my ideas.

I also like to acknowledge Michael Bank; I have really benefited immensely from the discussions I had with you, from your constructive comments on the articles and your invaluable tips on scientific writing. I would also like to thank my other co-authors Bente M. Nilson and Arne Duinker for sharing your knowledge and ideas, David Amouroux and his lab for analyzing the water samples and Ingrid A. Johnsen for data modeling. Your expertise helped me to publish my articles.

I wish to thank my colleagues at NIFES/IMR for contributing to a friendly and good working environment. I would also like to thank my friends including all PhD students and in particular Didrik for sharing the office and good jokes; Marta and Martin for enjoyable conversations during needed coffee breaks, useful scientific discussions, sharing the stressful situations and for all sympathies …

I would like to thank technicians in the Grunnstoff lab for conducting the analyses and particularly Berit Solli for discussion on methods and very friendly people in Prøvemottak and especially Anne Margrethe Aase for helping with sample preparation and always finding solutions.

A special thanks to people who helped me with collecting the materials for this study: Henrik Glenner from University of Bergen, Bruno Danis from University of Brussels and fishermen who collected fish for this study.

I am very grateful to my parents and siblings who supported me during this period and to my friends both in the past and present.

AND finally, my lovely family, you have been indispensable for this PhD; Hoda, I salute your patience. Now, I finished it and I can take over the shift and pass the torch. Sophie and Saqi, you are the most meaningful aspect of my life. I am very looking forward to spending more time with you and from now on in a more relaxed and calm way.

Atabak M. Azad

June 2019

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Abstract

Mercury (Hg) is a global neurotoxin distributed at trace levels in the earth's crust. Although Hg input from anthropogenic sources has been reduced in North America and Europe, in some other parts of the world the emission is still high. Considering the long-range transport and long atmospheric half-life, Hg and particularly its most toxic form monomethylmercury (MeHg), remains an environmental concern at the global level causing threat to both wildlife and human health. In general, seafood is the main source of MeHg exposure to humans and Hg is the main reason for seafood consumption advisories. Therefore, measuring the Hg levels in seafood species and understanding the processes governing the variation of Hg levels are very important for seafood safety and security. Synthesis, bioaccumulation and biomagnification of MeHg are very critical processes controlling the MeHg levels in the environment and the biota.

The main goals of this study were to investigate how Hg levels vary between different fish species as well as between different communities in offshore, fjord and coastal areas of the North East Atlantic Ocean (NEAO). The contribution from different Hg sources and parameters influencing these variations were also investigated.

Large variation in Hg levels between fish species form NEAO was found (Paper I). The pelagic species including Atlantic mackerel (*Scomber scombrus*) and blue whiting (*Micromesistius poutassou*) with mean value of 0.04 mg kg^{-1} ww had the lowest Hg concentrations. Blue ling (*Molva dypterygia*) had the highest Hg levels with a mean of 0.72 mg kg^{-1} ww. Selenium (Se) varied in a smaller range compared to Hg, with mean concentrations from 0.27 mg kg^{-1} ww in Atlantic cod (*Gadus morhua*) to 0.56 mg kg^{-1} ww in redfish (*Sebastes* spp.). The Hg level in fish increased from north towards south in most species and this process was independent of Hg pollution in the environment (sediment). It was hypothesized that a gradual increase in water temperature and primary production duration from the north towards the south are the main parameters governing the intraspecific geographical variation. Fish species collected from fjords and coastal areas contained higher Hg levels compared to the same species sampled offshore. High levels of organic matter and atmospherically deposited Hg washed from

the catchment inducing high MeHg production and high Hg bioavailability in fjords and coastal areas were suggested as the main drivers. The Hg variation between species was mostly driven by Hg trophodynamics and $\delta^{15}N$ as a proxy for trophic position explained the Hg variation between fish species from different areas in NEAO. The results indicated that the fjord and coastal areas and the Barents Sea had lower Hg levels at the base of the food web while demonstrating higher trophic magnification rates compared to the other areas (PhD thesis).

In order to investigate the effect of an industrial point source on environmental Hg levels in a fjord, levels of Hg and MeHg were measured in seawater, sediment and seafood species (fish and crustaceans) close to industrial point source of Hg pollution in Hardangerfjord ecosystem (Paper II). Elevated levels of Hg and MeHg were found in all compartments with increasing levels towards the point source in Sørfjord. However, in predatory species, tusk (*Brosme brosme*), Hg was accumulated at the same level in the sidearm of Eidfjord, where Hg contamination in sediment is low. Thus, organic matter and atmospheric Hg from the catchment area were suggested as other important drivers of Hg variation in biota in fjord ecosystems. In a continuation of this study, a similar investigation was conducted in Sognefjord with no major pollution source. There, Hg in tusk increased from offshore North Sea to the coast and further into outer and inner Sognefjord, while Hg levels measured in sediment samples were at the background level. Measurements of $\delta^{13}C$, as a proxy for energy/carbon source, showed that the contribution of allochthonous carbon to the food web increases towards the inner fjord and explained the majority of the Hg variation in tusk (Paper III).

It is suggested that surplus Se may provide protection against Hg toxicity for consumers. In most fish species from NEAO, Hg and Se were correlated and particularly in species with high Hg levels, this correlation was stronger (Paper I). All species from NEAO on average had higher molar concentrations of Se than Hg, and Se health benefit values (HBV_{Se}) were above 2. In predatory species including tusk and blue ling from the inner part of Hardangerfjord, mean Hg levels were above the European maximum level (EUML) and the HBV_{Se} were negative, indicating higher molar concentration of Hg than Se with a relatively high risk for consumers. Although tusk from Sognefjord also

had mean Hg levels exceeding the EUML, while HBV_{Se} values were above 3 in the fillet. Overall in the NEAO, only blue ling had an average Hg level above EUML, and the Hg exposure assessment showed that consumers having two servings of blue ling, tusk and/or Atlantic halibut per week will exceed the tolerable weekly intake (TWI) of MeHg. Consumption of all species from NEAO except Haddock (*Melanogrammus aeglefinus*), common ling (*Molva molva*), tusk and blue ling, on average provide more benefit from essential fatty acids than risk from MeHg.

In the Hardangerfjord and Sognefjord studies both total mercury (THg) and MeHg were measured in tusk fillet and liver. The MeHg to THg ratio (%MeHg) decreased when THg levels increased in tusk fillet and liver in both fjords, indicating MeHg demethylation as a response to MeHg accumulation (Paper II and III). Our results suggest that inorganic Hg (iHg) produced from MeHg demethylation can bind Se and be stored in fish liver. Discovering the details of demethylation process in marine fish may help better understand the Hg fate and cycling in the food web with implication for food safety and security.

List of Publications

Paper I

Azad, A.M., Frantzen, S., Bank, M.S., Nilsen, B.M., Duinker, A., Madsen, L., Maage, A., 2019. Effects of geography and species variation on selenium and mercury molar ratios in Northeast Atlantic marine fish communities. Science of the Total Environment 652, 1482-1496.

Paper II

Azad, A.M., Frantzen, S., Bank, M.S., Johnsen, I.A., Tessier, E., Amouroux, D., Madsen, L., Maage, A., 2019. Spatial distribution of mercury in seawater, sediment, and seafood from the Hardangerfjord ecosystem, Norway. Science of the Total Environment 667, 622-637.

Paper III

Azad, A.M., Frantzen, S., Bank, M.S., Madsen, L., Maage, A. Methylmercury bioaccumulation pathways in tusk (*Brosme brosme*) from Sognefjord, Norway: insights from C and N isotopes. Manuscript.

Abbreviations

AA-CSIA	Amino Acids Compound Specific Stable Isotope Analysis
AI	Adequate intake
ANCOVA	Analysis of covariance
CRM	Certified reference material
DHA	Docosahexaenoic acid
DMHg	Dimethylmercury
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
EFSA	European Food Safety Authority
EPA	Eicosapentaenoic acid
EUML	European maximum level
HBV_{Se}	Selenium health benefit value
Hg	Mercury
Hg^0	Elemental mercury
HQ	Hazard quotient
ICP-MS	Inductively coupled plasma-mass spectrometry
iHg	Inorganic mercury
LC-PUFA	Long chain-polyunsaturated fatty acid
LOQ	Limit of quantification
MeHg	Monomethylmercury
NEAO	North East Atlantic Ocean
NFSA	Norwegian Food Safety Authority
PSP	Point source of pollution
RDI	Recommended daily intake
Se	Selenium
THg	Total mercury
TMF	Trophic Magnification Factor
TMS	Trophic Magnification Slope
TWI	Tolerable weekly intake

1. Introduction

1.1 Mercury species and sources in the environment

Mercury (Hg) is distributed in the earth's crust in low concentrations, and both natural processes such as decay of terrestrial surfaces, forest fire and volcanoes as well as anthropogenic processes may release and recycle Hg to the environment (Sunderland and Chmura, 2000). The main natural ore of Hg, cinnabar (HgS), has been used by humans since ~2400 years ago in Almaden, Spain and anthropogenic release of Hg to the environment has continued via several activities such as combustion of fossil fuels, waste incineration, mining, metal smelting and cement production (Martınez-Cortizas et al., 1999; Sunderland and Chmura, 2000). Some of the anthropogenic Hg emissions stays in the soil, sediment and water close to the operation sites and become point sources of Hg pollution, while some is emitted to the atmosphere and become a part of the global Hg pollution. Precipitation washes off the continental lands and transfers the atmospheric and terrestrial Hg to the rivers, lakes, fjords, coastal areas and finally to the oceans. Mercury emission has increased by 3 fold compared to pre industrial revolution era (Lamborg et al., 2002). It has been documented that between 74 - 94% of Hg in biota from Arctic area originated from anthropogenic sources (Dietz et al., 2009).

Mercury in the environment exists in three major forms: 1) elemental form (Hg^0), 2) inorganic forms (iHg) which can be monovalent state (mercurous Hg; Hg_2^{2+}) or divalent state (mercuric Hg; Hg^{2+}), also denoted as Hg(I) and Hg(II), 3) organic forms which are usually formed when mercuric Hg binds with alkyls and phenyls (Boening, 2000). Elemental Hg is liquid in metallic form, but it can become volatile and evaporate to gaseous elemental Hg and distributed in the atmosphere. Then it can be oxidized into mono or divalent iHg while Hg(II) is the dominant chemical form (Ullrich et al., 2001).

The biogeochemical cycling of Hg consists of four major spheres including the atmosphere, hydrosphere, lithosphere and biosphere which are well connected to each other. While gaseous elemental Hg is the dominant form (more than 95%) in the atmosphere (Lindberg and Stratton, 1998), in the lithosphere, Hg exists mostly as

inorganic form bound to organic matter in the soil. In the hydrosphere, iHg is the dominant species in both water and sediment, although between 10-30% of Hg exist as dissolved elemental Hg (Mason and Fitzgerald, 1993; Wiener et al., 2002). The elemental Hg in the water mostly results from biological reduction of Hg(II) by microorganisms, decomposition of MeHg or released from industrial sources such as chloralkali industry (Mason et al., 1995; Mason and Sullivan, 1999; Mason and Fitzgerald, 1993). The dissolved organic matter (DOM) has been shown to reduce the transformation of the Hg(II) to elemental Hg (Poulin et al., 2019).

In the aquatic environment, both elemental and inorganic Hg are volatile and contribute as the major sources of natural Hg emission to the atmosphere (Lehnherr, 2014). At the global scale, the atmosphere is the major transport pathway of Hg from different sources. The atmosphere receives Hg from different sources in the form of elemental Hg or species of Hg(II) and these volatile forms are transported in the atmosphere. Elemental Hg is then oxidized to Hg(II) and deposited together with particulate species of Hg(II) via wet or dry processes to terrestrial and aquatic environments. Although the Hg deposition in Scandinavia, including Norway, has decreased in recent decades, the long range transportation of Hg and legacy Hg in the environment is still redistributing and restoring Hg in Norway (Berg et al., 2006).

On the other hand, the organic mercury is the dominant form in the biosphere. Organic Hg is mostly existing as monomethylmercury (MeHg) or dimethylmercury (DMHg). Methylmercury is toxic to humans and other organisms (Wood, 1974) and seafood is the main dietary pathway of MeHg exposure for humans (Sunderland, 2007).

It has been estimated that approximately 50% of the MeHg in the polar zone originates from methylation of iHg in the water column (Lehnherr et al., 2011). Also, in the Pacific Ocean it has been estimated that MeHg in phytoplankton and the pelagic food web is produced *in situ* in the water column and mostly from anthropogenic Hg sources.

The ultimate source of MeHg to the aquatic biota remains an enigmatic subject that needs more elaboration in the future. Still, many sources including atmospheric deposition, coastal (Hollweg et al., 2009) and deep sea sediments (Ogrinc et al., 2007),

water column production (Topping and Davies, 1981) and freshwater runoff (Kirk and St. Louis, 2009; Schartup et al., 2015) are suggested.

Compared to MeHg, the concentration of DMHg is very low, but in some particular marine environments such as the western Mediterranean Sea and equatorial Pacific Ocean and in sub thermocline water, a significant part of organic Hg is present as DMHg (Cossa et al., 1997; Mason and Fitzgerald, 1993). Authors mentioned that production of DMHg is very small and takes place in the minimum oxygen zone below the thermocline. It has been suggested that DMHg is unstable under natural conditions and it can be converted to MeHg. However, laboratory experiments demonstrate that the stability of DMHg is higher in conditions with high pH and low temperature (Mason and Fitzgerald, 1993). Dimethylmercury is also volatile and readily lost from aquatic systems and therefore not available for accumulation by aquatic organisms (Lehnherr et al., 2011; Morel et al., 1998; Talmi and Mesmer, 1975). Therefore, the organic form of Hg in the biota is considered to be MeHg.

1.2 Mercury methylation

In the past, anthropogenic activities were involved in the MeHg production, either by including MeHg in the desired products such as fungicides or by formation of undesired byproducts from chemical industries. After many MeHg poisoning disasters, such as Minamata (1950's), Iraq (1970's) and the following Minamata convention (2013), the anthropogenic MeHg production has been reduced and natural methylation of iHg in the environment is the dominant source of MeHg (Clarkson, 1993).

The existing knowledge on Hg methylation in the aquatic environment has developed considerably since the 1980s (Regnell and Watras, 2019). Analytical challenges in measurement of low concentrations of MeHg in the seawater was a big obstacle for understanding the MeHg cycle, but recent analytical advances in this field allows for measurements at picomolar concentrations. Later, the methylation and demethylation rates were determined in sediment and seawater using Hg stable isotopes in different species. These rates were compared between different sources, environments and

habitats, providing valuable information on the fate and cycle of MeHg and parameters influencing the methylation of iHg in the environment (Eckley et al., 2005; Hintelmann et al., 1995; Jonsson et al., 2014).

The environmental MeHg levels are the result of net methylation (synthesized MeHg level after sequestration of demethylated MeHg) (Figure 1). Some studies showed high degree of methylation in the sediment (Callister et al., 1986; Olson and Cooper, 1974). However, considering the volume of the water column compared to the thin layer of sediment surface, it is likely that water column methylation may potentially be more important. It has been suggested that MeHg produced *in situ* in deep sea sediment is not a major source for the food web (Hammerschmidt and Bowman, 2012; Motta et al., 2019).

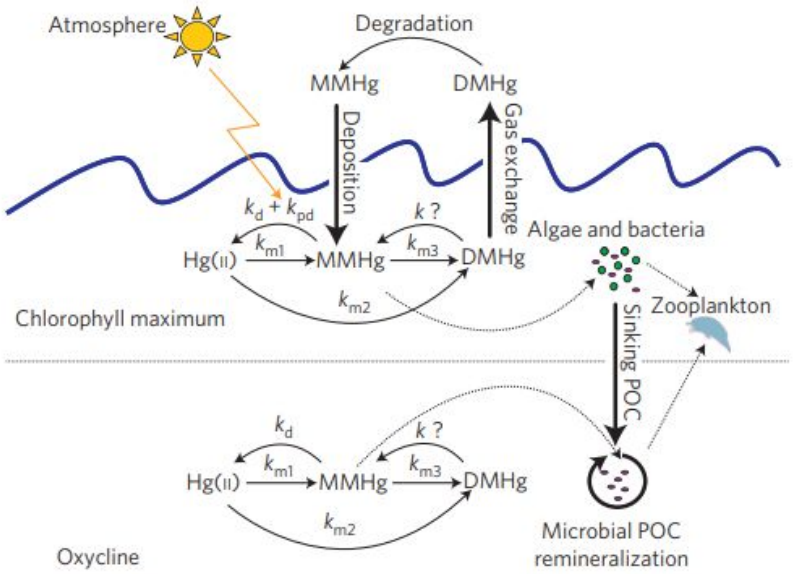

Figure 1. Mercury methylation and demethylation in the marine environment. POC= particulate organic carbon, MMHg = monomethylmercury, DMHg = dimethylmercury, constants km = methylation, kd = demethylation, kpd = photodemethylation. Source: Lehnherr et al. (2011)

Many field studies have showed methylation of Hg in the marine water column in both low oxygen zone (Cossa et al., 2009; Sunderland et al., 2009) and oxic surface layers (Cossa et al., 2011; Hammerschmidt and Bowman, 2012; Lehnherr et al., 2011).

1.2.1 Biotic and Abiotic methylation

Mercury methylation in both terrestrial and aquatic environments is mostly mediated by sulfate reducing bacteria and to a lesser extent by iron reducing bacteria (Gilmour et al., 2013). Recent advances in genetics and discovery of genes involved in Hg methylation (hgc AB) have provided a powerful tool for Hg methylation studies in the environment (Parks et al., 2013). Podar et al. (2015) investigated the presence of hgc AB in more than 3500 available metagenomes as a proxy for Hg methylation potential in different environments. They detected the hgc AB in all studied anaerobic environments. Methylation genes were not detected in any of the 1500 mammalian (including human) microbiomes analyzed, indicating a low chance of Hg methylation in the mammal's gut. On the other hand, Podar et al found methylation genes in the gut of invertebrates and also in habitats including permafrost soil, coastal dead zones, soils, sediments and oxygenated layers in open ocean (Podar et al., 2015). It has been shown that some sulfate and iron reducing bacteria can oxidize elemental Hg to iHg and then convert it to MeHg (Hu et al., 2013). In terrestrial environment, wetlands and hydric soils are considered as hot spots for Hg methylation due to having anaerobic conditions (Amirbahman and Fernandez, 2012).

In freshwater ecosystems, MeHg is mostly produced via anaerobic microbes and from iHg. The methylation process is mainly governed by the activity of microbes and the bioavailability of iHg (Regnell and Watras, 2019). The iHg uptake can take place via passive or active transport. However, Hg methylation in oceanic waters can occur in the surface, it has been shown that the methylation rate is higher in oxygen minimum zone (Regnell and Watras, 2019).

Although some anaerobic, facultative anaerobes, and aerobic bacteria can methylate iHg, anaerobic bacteria are considered more important, and sulfate reducing bacteria

are suggested as the principal methylators in the environment (Compeau and Bartha, 1985; King et al., 2000; King et al., 2001).

Microbially mediated methylation is supposed to be the major methylation pathway in the environment. However, abiotic methylation is possible if suitable methyl donors are available. Photochemical methylation is suggested as a possible pathway to induce alkylation of iHg and sewage effluents, and industrial waste water has been suggested as a source of methyl groups for photochemical methylation (Hamasaki et al., 1995; Wood, 1975). It has been demonstrated that MeHg can be formed abiotically from humic acid and fulvid acid originating from leaf mould and sediment as methyl donor to iHg. This reaction is influenced by temperature, pH and concentrations of iHg and methyl donors (Nagase et al., 1982; Nagase et al., 1984).

1.2.2 Parameters influencing mercury methylation

The methylation rate of iHg is suggested to be a function of iHg bioavailability and the activity of methylating bacteria. A wide variety of biogeochemical parameters are shown to have an effect on the methylation rates in the environment. There is an agreement among scientists that the bioavailable fraction of iHg is more important determining the methylation rate than total Hg concentration. Some studies have found that the MeHg concentrations are independent of total mercury (THg) (Kelly et al., 1995; Lambertsson and Nilsson, 2006), while others have found a connection between THg and MeHg concentration in sediments with legacy Hg pollution (Azad et al., 2019; Rudd et al., 2018).

Physicochemical parameters

The microbial activity increases with temperature and thus, methylation of iHg will increase with temperature. Many studies have shown seasonal variation in methylation rates and measured high MeHg levels in periods with higher temperatures e.g. summer (Hintelmann and Wilken, 1995; St. Pierre et al., 2014; Watras et al., 1995). On the other hand, the MeHg demethylation rate is also increased with increasing temperature (Matilainen et al., 1991). Since there are also seasonal changes in nutrient supply and organic matter, the temperature effect on methylation is complex and interconnected to

other environmental parameters. The synthesis of MeHg has been shown to be higher in conditions with lower pH probably due to higher iHg availability (Golding et al., 2007), or by domination and high activity of methylator bacteria (Winch et al., 2008).

Redox condition is another important parameter for methylation. Generally, there is a consensus that in the natural environment, methylation rates are higher under anaerobic conditions by controlling the chemical speciation and Hg bioavailability. Therefore, the methylation rates are higher in anaerobic/low oxygen sediment and sea water (Ullrich et al., 2001). It has been suggested that microbial demethylation is higher than methylation under aerobic condition (Ullrich et al., 2001).

The methylation rates in freshwater sediments are usually higher than in estuarine and marine sediments. This has been linked to salinity and an inverse relationship between salinity and methylation rate has been reported (Blum and Bartha, 1980; Compeau and Bartha, 1987).

Organic matter

The effect of organic material on Hg methylation is particularly complicated and several studies have shown that organic matter enhances Hg methylation and hence, elevates Hg levels in biota (Chiasson-Gould et al., 2014; Fjeld and Rognerud, 1993; Furutani and Rudd, 1980; Lambertsson and Nilsson, 2006; Le Croizier et al., 2019; Schartup et al., 2015; Taylor et al., 2019). It has been suggested that nutrients associated with organic matter enhance the bacterial methylation activity and may thereby facilitate the bioavailability of iHg to methylator bacteria (Ullrich et al., 2001). The iHg or MeHg bound to terrestrial organic matter may also play a role. On the other hand, several other studies have found an inhibiting effect of organic matter on Hg methylation (Barkay et al., 1997; Driscoll et al., 1995; Grieb et al., 1990; Jackson, 1991).

French et al. (2014) studied 26 lakes in the Canadian tundra and found that dissolved organic carbon (DOC) in water controls the THg and MeHg accumulation in biota. Further, Hg levels increased with DOC up to ca. 8.5 mg carbon L^{-1}, whereas the bioaccumulation was reduced at higher concentrations of DOC (a bell-shaped pattern). The Hg bioaccumulation factor was elevated when Hg was bound to fulvic acid, but Hg

became less bioavailable at higher DOC concentrations (>8.5 mg carbon L^{-1}) where Hg was mostly associated with larger and less available humic acid (French et al., 2014).

In a study performed in the Gulf of Bothnia, Soerensen et al. (2017) showed that spatial and seasonal variation in seawater MeHg is controlled by organic matter. Although labile DOC increased Hg methylation, the humic content in the water led to decreased Hg methylation, probably by reduction of iHg bioavailability to bacteria. DOM influences the Hg methylation by influencing the cell physiology (as a nutrient) and by governing the bioavailability of iHg to bacteria as a complexing agent (Chiasson-Gould et al., 2014).

In a mesocosm experiment, using Hg isotope tracers in both inorganic and organic forms, Jonsson et al. (2014) showed that iHg bound to thiol group in organic matter had higher availability to methylator bacteria compared to metacinnabar (HgS). They also showed that MeHg in runoff, originated from terrestrial and atmospheric sources, has 5-250 times higher availability to the estuarine biota compared to MeHg formed in the sediment. Further, MeHg from terrestrial runoff has a significant effect on MeHg burdens in estuarine biota. Both autochthonous (marine origin) and allochthonous (terrestrial origin) DOM can enhance the Hg methylation. However, the effect of allochthonous carbon sources significantly enhances the methylation rate more than marine DOM (Graham et al., 2012).

1.3 Mercury in marine ecosystems and food web dynamics

Aquatic organisms can bioaccumulate Hg and particularly MeHg from food, water and sediment, and the uptake rate of MeHg is more efficient than the elimination. Hence, organisms tend to increase their MeHg levels by age. In an *in vivo* experiment, the half-life of Hg in muscle of top predator fish, pike (*Esox lucius*), was estimated to be 3.3 years (Van Walleghem et al., 2007). In the food web, high assimilation and long half-life of MeHg result in increase of the MeHg concentration with the trophic position, defined as biomagnification (Figure 2).

As a result of bioaccumulation and biomagnification, the concentration of MeHg in top predator marine fish species can be 10^6 to 10^7 times higher than seawater MeHg concentration (Azad et al., 2019; Bowles et al., 2001; Kim and Burggraaf, 1999), although the majority (more than 95%) of Hg in the marine environment is in the form of iHg (Wiener et al., 2002).

The concentration of MeHg in seawater results from *in situ* Hg methylation in water and sediment plus the external sources delivered to the marine ecosystem after subtraction of demethylation rate. Phytoplankton can accumulate MeHg in concentrations up to 10^4 times higher than seawater and this process is considered as the main step in MeHg bioconcentration in the marine food web (Pickhardt and Fisher, 2007; Watras et al., 1998).

The food web structure characterization and the source of carbon/energy can be investigated by measurement of ratios of different stable isotopes of nitrogen and carbon (^{15}N / ^{14}N and ^{13}C / ^{12}C) (Cabana and Rasmussen, 1994; Hobson et al., 1994; Pethybridge et al., 2018) (Figure 2 and 3).

The concentration of ^{15}N is enriched along the food web since the heavier isotope (^{15}N) is retained at a higher rate for amino acid synthesis than the lighter isotope (^{14}N) and the lighter isotope has a higher elimination rate. The trophic enrichment factor of $\delta^{15}N$ varies between 2.4‰ and 4.2‰, but overall 3.4‰ is accepted and often used for marine food webs (Jardine et al., 2006; Minagawa and Wada, 1984; Pethybridge et al., 2018). On the other hand, $\delta^{13}C$ has a relatively limited isotopic fractionation (approximately 0.4 ‰) through the food web (Post, 2002) and $\delta^{13}C$ is used to determine the source of carbon in the food web.

Using the trophic enrichment factor of $\delta^{15}N$ ($\Delta^{15}N$) and a baseline species (species with defined trophic position) the trophic position of a biota sample can be determined from measured $\delta^{15}N$ values:

$$TP_{consumer} = \left(\frac{\delta^{15}N_{consumer} - \delta^{15}N_{baseline}}{\Delta\,^{15}N}\right) + TP_{baseline}$$

Proper baseline species is critical when comparing trophic dynamics of different ecosystems, as the source of C and N and hence $\delta^{13}C$ and $\delta^{15}N$ may be different at the base of the food webs (Davenport and Bax, 2002; Hannides et al., 2013; Lorrain et al., 2015; Sackett et al., 2015; Vander Zanden and Rasmussen, 1999). Recently, Amino Acids Compound Specific Stable Isotope Analysis (AA-CSIA) is proposed as a better method for tracking nutrients and contaminants along the food web that may solve the baseline species issue (Lorrain et al., 2009; Pethybridge et al., 2018; Won et al., 2018).

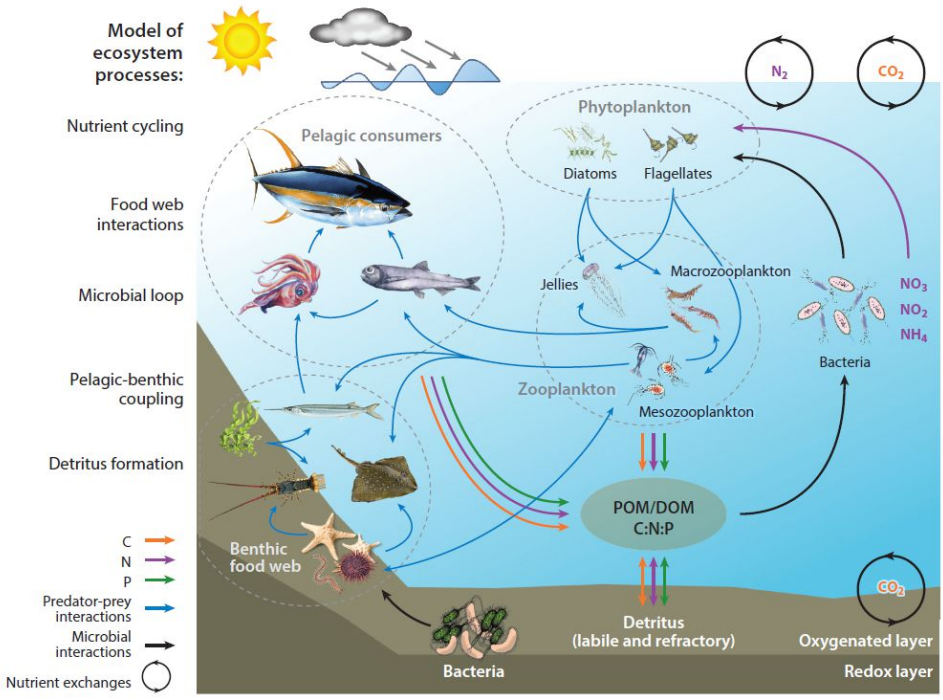

Figure 2. The main ecological cycles explaining the feeding relationship and flow of energy in marine environment. Source: (Pethybridge et al., 2018).

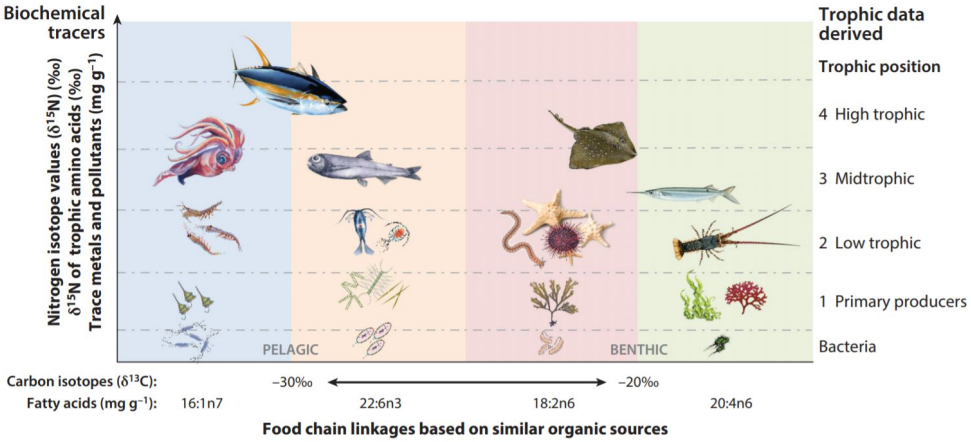

Figure 3. Major feeding relationships and ecological processes in marine ecosystem. Source: (Pethybridge et al., 2018).

It has been shown that $\delta^{15}N$ may increase with fish age as a result of shift to high trophic position prey (Kim et al., 2012). Considering the difficulties and associated uncertainties with aging of the old marine fish for example Greenland halibut (*Reinhardtius hippoglossoides*) (Dwyer et al., 2016) or tusk (Runnebaum, 2017), $\delta^{15}N$ may provide a better estimate of bioaccumulation than age.

Understanding the structure of the food web, and the interaction between predator and their prey as well as sources of energy in the food webs at individual, area, habitat and ecosystem levels provides a very critical dimension of knowledge into bioaccumulation pathways of contaminants. Although the bioaccumulated Hg in phytoplankton originates from seawater, at higher levels of the food web, the majority of Hg comes from the prey (Pethybridge et al., 2018; Won et al., 2018).

1.4 Mercury interaction with nutrients and particularly selenium

Seafood contains several nutrients that may interact with Hg. These interactions can reduce the bioavailability and toxicity of Hg. Among the nutrients, high levels of cysteine (Mok et al., 2014), omega 3 fatty acids (Hojbjerg et al., 1992) and selenium (Se) (Ralston et al., 2008) are documented to reduce the bioavailability and/or toxicity of Hg.

Fish is a high-quality protein source and contains relatively high levels of long chain-polyunsaturated fatty acids (LC-PUFAs), including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Dewailly et al., 2003; Radak et al., 1991; Virtanen et al., 2008).

Methylmercury mainly binds to thiol groups in the proteins (Hightower and Moore, 2003) and this is a key characteristic for MeHg accumulation in muscle tissue. Seafood is a very good source of methionine and cysteine (Coultate, 2009), the sole sulfur containing amino acids present in the protein structure (Brosnan and Brosnan, 2006). Mok et al. (2014) studied the effect of dietary cysteine at levels of 1% and 10% of the diet fed to guppy and demonstrated that cysteine significantly reduced Hg accumulation.

It has been demonstrated that compared with dietary cod liver oil, coconut oil resulted in higher retention of Hg in the body of mice after single dose exposure (Hojbjerg et al., 1992). In another experiment rats were fed with MeHg contaminated fish or fish spiked with chemical MeHg. Results showed that naturally incorporated MeHg led to higher fecal excretion and less tissue accumulation of MeHg and metallothionein induction (Berntssen et al., 2004).

The protective effect of Se on MeHg toxicity in rats was first recognized in the 1960s (Parizek and Ostadalova, 1967). Later, several studies documented that dietary Se can ameliorate the Hg toxicity when they are co-occurred in the diet as chemical forms (Bjerregaard et al., 2011; Ganther et al., 1972; Ralston et al., 2008; Wada et al., 1976) or naturally incorporated in the consumed oceanic seafood (Bjerregaard and Christensen, 2012; Ohi et al., 1976; Ralston et al., 2019; Stillings et al., 1974).

Selenium is an essential trace element which is toxic in high levels. The protective effect of Se against Hg toxicity is linked to different functions of Se including: 1) Selenoproteins may prevent oxidative stress caused by MeHg and overexpression of selenoprotein such as glutathione peroxidases has been documented *in vitro* to ameliorate MeHg induced oxidative stress (Farina et al., 2009). 2) Mercury has higher affinity for Se than the thiol group of amino acids (Berry and Ralston, 2008). Formation of stable MeHg-selenocysteine compounds may block Se bioavailability. When the concentration of MeHg is high, cellular available Se level is reduced and the antioxidant

activity of the selenoenzymes will be impaired. Still, available Se from the diet or body supply can compensate for the reduced Se in HgSe or MeHg-selenocysteine and preserve the Se dependent enzyme function in the central nervous system (Peterson et al., 2009a).

The molar ratio of Se:Hg is an important toxicological risk indicator and a ratio above one is suggested to be protective against Hg toxicity in human and fish (Burger et al., 2012; Peterson et al., 2009b; Ralston, 2008), but the details of Hg and Se interaction mechanism is not fully understood. Determination of Se:Hg molar ratios may provide a more accurate and physiologically relevant indicator for MeHg toxicity in the body (Peterson et al., 2009a; Ralston et al., 2019; Ralston et al., 2008).

1.5 Seafood as methylmercury source for humans and consumption advisories

Fish and seafood are the most important sources of MeHg in the human diet (Sunderland, 2007). In populations characterized by a high fish intake, associations between moderate prenatal Hg exposure and impaired neurodevelopment in the offspring have been demonstrated (Axelrad et al., 2007; Crump et al., 1998; Grandjean et al., 1997). Although in some of these studies such as Faroe study, pilot whale with negative HBV_{Se} (-120) and high levels of organic contaminants was a significant part of their diet (Ralston et al., 2016).

In Norway and Europe, the trade of seafood products is regulated for Hg levels and required to be below 0.5 mg kg^{-1} ww for most of marine species or 1.0 mg kg^{-1} ww for specific large predatory species (EC, 2006). Since Hg exposure results from the Hg levels in foods as a function of intake level, the European Food Safety Authority (EFSA) has set a tolerable weekly intake (TWI) at 1.3 μg kg^{-1} body weight.

However, seafood also contains beneficial and essential nutrients, and a review of the epidemiological literature concludes that the results of low-level MeHg on neurodevelopmental outcomes are inconsistent (Karagas et al., 2012). Further, if seafood intake is below 350 g per week during pregnancy, the risks from the lack of

nutrients may be greater than the risks of harm from exposure to trace contaminants for the offspring (Hibbeln et al., 2007).

A health survey performed in 36 Arctic communities between 2007 – 2008 demonstrated that seafood consumption is linked to higher intake of proteins, protein related micronutrients, vitamins A and C, and lower intake of carbohydrates, saturated fat, fiber and lower sodium:potassium ratio (Egeland et al., 2011).

2. Study Objectives

The main aim of this thesis was to explore Hg accumulation in different fish species from different areas in the Northeast Atlantic including offshore areas and Norwegian coast and fjords, with particular emphasis on geographical variation within fish species. We aimed to describe the main parameters driving Hg variation between species and within single species. Additionally, Se was measured and the potential interaction between Hg and Se at individual level of different species and also at the habitat and areas scales were studied. To reach the above-mentioned goals, this study was conducted in six parts to:

1. Evaluate how Hg level varies between commercially important fish species and the geographical variation within each species from different areas of NEAO and to identify the parameters influencing the Hg levels in different species and the spatial variation within species (Paper I).
2. Investigate the Hg trophodynamics and carbon source in different areas of NEAO using stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) analysis (this thesis).
3. Evaluate the effect of Hg point source on different compartments of ecosystem in a polluted area (Hardangerfjord) and its bioavailability to seafood species (Paper II).
4. Evaluate the effects of the fjord characteristics on the Hg accumulation in food web in a fjord ecosystem with no significant point source (Paper III).

In paper II we found that Hg contamination increases towards the inner part of Sørfjord and the pollution source, whereas the Hg levels in tusk were highest in Eidfjord where the sediments were less Hg polluted. In the next step, the effect of increasing runoff from catchment towards the inner parts of the Sognefjord and particularly increasing organic carbon on bioavailability of MeHg to the food web and MeHg levels in top predator fish were studied.

5. Evaluate the Hg exposure assessment and risk-benefit from consumption of seafood from NEAO (Paper I and this thesis)
6. Study the MeHg metabolism in species with high Hg contamination (Tusk) and possible demethylation of MeHg (Paper III).

3. Methodological considerations

3.1 Measurement of total mercury, methylmercury and selenium in biological samples

The concentration of Hg and Se in biological samples were determined using inductively coupled plasma-mass spectrometry (ICP-MS) following microwave digestion. First, samples were digested using concentrated HNO_3 (65%) and H_2O_2 (30%) in a microwave oven (Milestone Microwave digestion system MLS-1200 MEGA Microwave Digestion Rotor - MDR300/10). The concentrations of Hg and Se were determined using quantitative ICP-MS (Agilent7500 with collision cell and ICP-ChemStation software) and a standard curve. Germanium (Ge), thulium (Tm) and rhodium (Rh) were used either individually or in combination as an internal standard. Gold (Au) was added to stabilize the Hg signals. The method is a Nordic and European standard (CEN, 2009; NMKL, 2007) and was described in detail by Julshamn et al. (2007).

For quality assurance, certified reference materials (CRM) 1566 (oyster tissue) from the National Institute of Standards and Technology (Gaithersburg, USA) and lobster hepatopancreas (TORT-2, TORT-3) from the National Research Council (Ottawa, Canada) were included in each sample run. The recoveries of both Hg and Se ranged from 80% to 120% for the whole period of analysis (2006–2015) and reproducibility (% RSD) from five-day analyses of reference materials showed a variation in the results less than 10% on analysis values above limit of quantification (LOQ) of the method. The same method was used for all biological samples in this study analyzed for Hg and Se between 2006 to 2015 and produced data were therefore comparable for whole period.

Methylmercury was measured using an isotope dilution method and gas chromatography coupled with ICP-MS. For details, refer to Valdersnes et al. (2012). The internal method reproducibility for MeHg (RSD) was between 1 and 12% and the Z-score for different CRM's was better than |1.5|. The method was validated in different seafood matrices.

When %MeHg was determined (MeHg concentration ÷ THg concentration × 100), we obtained values above 100%, particularly in the fillet. Values above 100% is theoretically not possible. However, considering the measurement uncertainties of both methods (giving a total measurement uncertainty of around 50%), this can be expected. Since THg and MeHg were measured using separate methods and different sub-samples were analyzed, the baseline signal of the instruments at different times and homogeneity of samples can explain a significant part of this issue. Using a method that simultaneously measures both THg and MeHg would have improved this issue. Considering the high number of samples analyzed in this study and the fact that %MeHg was only used for comparison between sites, we believe that the data can be used with some cautions for our purposes.

3.2 Geographical comparison of mercury and selenium levels in fish from NEAO

In this part, available data produced during several surveillance and monitoring projects at National Institute of Nutrition and Seafood Research (NIFES)/Institute of Marine Research (IMR) were compiled and inter and intra species variation of Hg and Se were investigated. It has repeatedly been shown that Hg concentration correlate with fish size and therefore for comparison of the same species across spatial range, it is critical to remove the effect of size as a covariate. In this study, analysis of covariance (ANCOVA) was applied whenever the parameter correlated to size and then least squares mean concentrations were used as size corrected element level (e.g. Hg), which was then used for correlation with other influential environmental parameters. This approach was used for all spatial comparisons in paper I, II and III.

3.3 Tusk as bioindicator of mercury contamination in food web

There are many marine species used as sentinel or bioindicators of Hg pollution in the marine environment. Species such as blue mussel and different species of macroalgae are good examples of indicators that are ideal due to their stationary occurrence. However, most of these species belong to lower trophic positions and do not accumulate

high levels of Hg, thus they do not exhibit small changes in Hg pollution. Among several commercially important fish species, tusk can accumulate very high levels of Hg compared to other fish species from NEAO (Paper I) and they are at the same time distributed in all different geographical areas (Barents Sea, Norwegian sea, North Sea and Skagerrak) and habitats (offshore, costal area and fjords) (Paper I). In paper III, we showed that tusk from all habitats and 9 of 10 sites can be discriminated based on $\delta^{13}C$ indicating that this fish has a relatively small vagility or home range meaning that it is a good representative for the sampling location and no large migration is reported for this species (Runnebaum, 2017). Tusk is a commercial species that is consumed as seafood and the high trophic position of this species (McMeans et al., 2010) make it a good bio/eco indicator for Hg pollution and Hg risk assessment in different habitats of NEAO.

Considering possible MeHg demethylation and organ redistribution in tusk, to test the Hg bioavailability in polluted areas or areas with high Hg availability, the Hg levels in fillet can be misleading and Hg burden of the whole organism need to be considered. For more details refer to section 4.7 in this thesis.

3.4 Sediment sampling

Sediment samples analyzed in paper II were collected using grab and scuba diving to 20m depth of water in Hardangerfjord. The sampling method did not allow for undisturbed sediment samples and therefore it was not possible to separate the top layer (oxic) from the rest of sediment column. In this case a mixture of top 15 ± 2 cm sediment layer was homogenized, and Hg species (THg and MeHg) were measured. Thus, data produced from these samples were interpreted with caution in paper II and mainly used to determine the spatial extent of Hg distributed from the point source and to compare the Hg species concentrations between sites.

3.5 Stable isotope measurements

For the determination of carbon and nitrogen stable isotopes, after combustion of samples in presence of O_2, the produced N_2 and CO_2 were analyzed using an elemental analyzer (ECS 4010, Costech Analytical, Valencia, CA). Then produced gases were separated with a 3m gas chromatography column and analyzed with a continuous flow isotope ratio mass spectrometer (Delta PlusXP, Thermofinnigan, Bremen) (Brenna et al., 1997).

Carbon and nitrogen isotopic results were measured in per mill (‰) relative to VPDB (Vienna Pee Dee Belemnite) and N_2 in air, respectively:

$$\delta^{13}\text{C or } \delta^{15}\text{N (‰)} = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000$$

where R denotes the ratio between $^{13}C/^{12}C$ or $^{15}N/^{14}N$, respectively.

For quality assurance, replicates of reference material of protein standard B-2155, were included in each sequence of analysis. The $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) values of the standard were as follows: -26.9‰ and 5.9‰, respectively.

Average (±1SD) results for 16 analyses of the B-2155 standard analyzed together with the samples were as follows: $\delta^{13}C$ VPDB: -27.14‰ ± 0.14 and $\delta^{15}N$ AIR: 6.09‰ ± 0.15. All carbon and nitrogen stable isotope analyses were conducted at Stable Isotope Core Laboratory, Washington State University, USA.

3.6 Mercury trophodynamics in food webs from different areas

In order to study and compare the trophodynamics of Hg in food webs in different areas of NEAO, a sub-sample of five individuals of each fish species from different areas were randomly selected from the top length quartile. The similar boarder definitions for different areas as in paper I was applied. Samples were selected from the 25% largest fish of each species in order to minimize the possible variation in $\delta^{13}C$ and $\delta^{15}N$ and to avoid large variation in groups when the sample size was small. It has been shown that fatty tissue is relatively more depleted in $\delta^{13}C$ compared with proteins and

carbohydrates and therefore, variation in fat content can cause a significant bias to the interpretation of $\delta^{13}C$ between species (Post et al., 2007). In this part of the thesis, several fish species with large variation in fat content in their fillet were analyzed for stable isotopes. Thus, fat contents needed to be considered. When the lipid content of the marine animals was more than 5%, equal to C:N ratio more than 3.5, fat extraction prior stable isotope analysis or normalization of crude data based on C:N ratio is recommended (Post et al., 2007). A significant part of samples used in this study had C:N ratio above 3.5 and therefore data were normalized for C:N ratio. Marine organisms showed strong correlation between C:N ratio and fat content and between fat content and $\delta^{13}C$, allowing to normalize the $\delta^{13}C$ based on following formula (Post et al., 2007):

$$\delta^{13}C_{normalized} = \delta^{13}C_{untreated} - 3{:}32 + 0{:}99 \times C{:}N$$

In paper III, only one species (tusk) was compared between different sites for $\delta^{13}C$. Since tusk fillets are lean and all individuals except one had C:N ratio less than 3.5, untreated $\delta^{13}C$ data was used.

For comparison of Hg trophic magnification between different areas of NEAO, the slope of simple linear regression between log-transformed Hg and $\delta^{15}N$ was used as Trophic Magnification Slope (TMS). Although TMS and Trophic Magnification Factor (TMF) calculated based on trophic position instead of $\delta^{15}N$ is widely used in this field, trophic position was not used here to avoid bias related to baseline species. There were two possibilities in this study: 1) using different species such as capelin, herring and mackerel in different areas as baseline species. 2) using blue mussel collected from fjord and coastal areas of different offshore areas and use them for baseline of offshore food webs. Both possibilities will have their own bias and limitations that introduce more uncertainty and thus, $\delta^{15}N$ was used for TMS calculations (this thesis).

On the other hand, calculation of THg at the base of the food web is challenging and since in trophodynamic studies usually empirical measurement of primary producer is missing, it is often estimated based on available data from the food web.

The intercept of relationship between logTHg and $\delta^{15}N$ has previously been used as an estimate of contaminant concentrations at the base of the food web (Broman et al., 1992). However, this may not give a correct estimate, and an estimation of Hg baseline according to the following equation and $\delta^{15}N$ of the baseline species is suggested as the better way to estimate Hg at the base of the food web (Lavoie et al., 2013):

$Log_{10}Hg = \delta^{15}N\ (b) + a$

For calculation of THg concentration of the baseline species (trophic position 2, primary consumer), blue mussel collected from different areas of NEAO was used and THg baseline was calculated from average $\delta^{15}N$.

3.7 Risk benefit evaluation of seafood consumption

Seafood is a unique source of the essential LC-PUFAs of EPA and DHA and consumption of seafood is considered an important part of a healthy diet. Several studies have addressed the Adequate Intake (AI) necessary for human health. General recommendation of AI for omega-3 LC-PUFA (EPA + DHA) consumption to receive a health benefit from fish varies between 250 – 500 mg day^{-1} (Deckelbaum et al., 2008; Harris et al., 2008; Kris-Etherton et al., 2009; Mozaffarian and Rimm, 2006). It is suggested that 250 mg day^{-1} of EPA and DHA is sufficient to significantly reduce the risk of death from coronary heart disease (CHD) in adults (Mozaffarian and Rimm, 2006). Pregnant and nursing women have higher requirements and a minimum of 200 – 300 mg DHA day^{-1} is advised (Kris-Etherton et al., 2009). The EFSA panel on Dietetic Products, Nutrition and Allergies (NDA), has set 250 mg day^{-1} of EPA+DHA for adults to protect against cardiovascular disease (EFSA, 2015). An additional intake of 100 to 200 mg DHA for pregnant women is recommended by NDA. Based on these AIs, we developed two scenarios for sum EPA+DHA of 250 and 500 mg day^{-1} to obtain the health beneficial effects from seafood consumption for adults and pregnant women, respectively. The sum of EPA+DHA for different species are extracted from Sjømatdata (www.sjomatdata.nifes.no) or The Norwegian Food Composition Table

(www.matvaretabellen.no). On the other hand, seafood is the major dietary source of MeHg and MeHg is the main cause of seafood consumption advisories.

A hazard quotient (HQ) was determined as an index to evaluate risk-benefit of fish consumption considering the content of both essential fatty acids (EPA and DHA) and MeHg in each fish species and the relevant recommended daily intake for EPA+DHA and the TWI for MeHg.

Thus, the HQ was calculated for adults based on a recommended daily intake (RDI) of EPA+DHA of 250 mg day^{-1} as HQ_{250} and for pregnant and nursing women based on RDI of 500 mg day^{-1} as HQ_{500}.

HQ was calculated from a formula developed by Gladyshev et al. (2009) and later used by other scientists (Razavi et al., 2014; Strandberg et al., 2016):

$$HQ = \frac{R_{(EPA+DHA)} \times C_{MeHg}}{C_{(EPA+DHA)} \times RfD_{MeHg} \times AW}$$

$R_{(EPA+DHA)}$ = recommended daily intake of essential fatty acids (EPA+DHA).
C_{MeHg} = MeHg concentration in fish fillet (mg kg^{-1} ww); since more than 93 percent of Hg in fish fillet was in the form of MeHg (Paper I), THg concentration was used in a conservative approach.
$C_{(EPA+DHA)}$ = concentration of EPA+DHA in fish fillet (mg g^{-1} ww).
RfD_{MeHg} = reference dose for MeHg per day was calculated from the TWI established by EFSA (1.3 µg kg^{-1} body weight) at 0.186 µg kg^{-1} body weight
AW = average weight of an adult consumer (70kg)

4. Results and discussion

4.1 Mercury variation between fish species in NEAO

A large variation in Hg levels was found between the investigated species from NEAO. The mean Hg concertation varied 18-fold between species with the lowest (mackerel and blue whiting) and highest (blue ling) Hg levels (Paper I). The variation in Hg levels between and within species from NEAO is extensively discussed in Paper I and here, the main drivers causing these variations, based on the complimentary data, will be discussed.

The bioaccumulation of Hg is investigated more comprehensively in fish compared to other groups of animals and marine organisms, most probably due to fish being the major source of Hg exposure for humans (Clarkson, 1993; Selin et al., 2009; Sunderland, 2007). MeHg is the most toxic and bioaccumulative species of Hg and a very high assimilation efficiency and long half-life may thus lead to bioaccumulation and biomagnification in the food web (Van Walleghem et al., 2013). Therefore, trophic position in the marine food web may determine a large part of Hg variation between species. In continuation of the study conducted in paper I, the effects of trophic position and source of carbon were studied in the same geographical set-up using measurements of $\delta^{15}N$ (as a proxy for trophic position) and $\delta^{13}C$ (as a proxy for carbon source).

4.2 Mercury trophic transfer and the effect of carbon source in different areas of NEAO

Stable carbon and nitrogen isotopes were determined to evaluate the trophic position and source of energy in different areas of NEAO (Figure 4). In five major areas of NEAO, including Barents Sea (BS), Norwegian Sea (NO), North Sea (NS), Skagerrak (SK) and fjords and coastal areas (FC), the THg levels significantly increased along the food webs ($p<0.005$). Trophic position given as $\delta^{15}N$ explained a relatively large part of the THg variation in fillet of different fish species (r^2 between 0.32 in NO and 0.66 in FC). The $\delta^{15}N$ explained more variation of fish THg in fjords and coastal areas compared to all offshore areas except the Barents Sea, probably due to smaller and more

homogeneous sampling habitats in fjords and coastal areas. In the Barents Sea, $\delta^{15}N$ explained a relatively higher percentage of Hg variation compared to other offshore areas (NO, NS and SK), possibly due to shorter and less complex food webs (Figure 5).

Different primary sources of carbon/energy in the food webs can be discriminated from $\delta^{13}C$ values. In general, marine organisms inhabiting benthic/demersal have higher $\delta^{13}C$ compared to pelagic species and species living inshore have also higher $\delta^{13}C$ compared to offshore species. Since in this study there was a combination of species from both benthic/pelagic and inshore/offshore habitats, an increase in $\delta^{13}C$ values can be associated with both inshore and benthic habitats. In the Barents Sea capelin was separated as offshore/pelagic and tusk as demersal/inshore which is in good agreement with the ecology of these species (Figure 4). In fjords and coastal areas, tusk was the most demersal/inshore. In other areas, the patterns were less clear, probably due to a mixed effect of benthic/pelagic habitat and distance from shore.

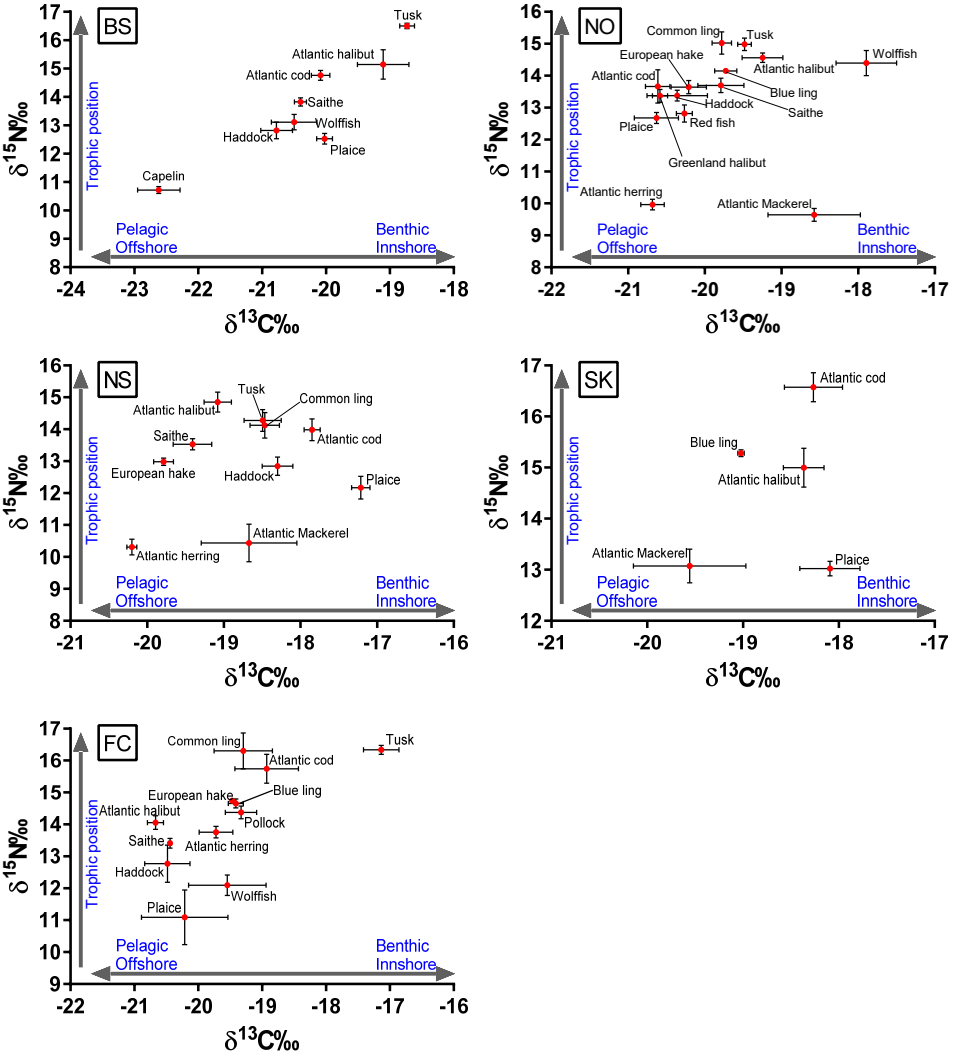

Figure 4. Mean ± SEM of $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) for fish species sampled from different areas of NEAO: BS = Barents Sea, NO = Norwegian Sea, NS = North Sea, SK = Skagerrak and FC = fjords and coastal areas.

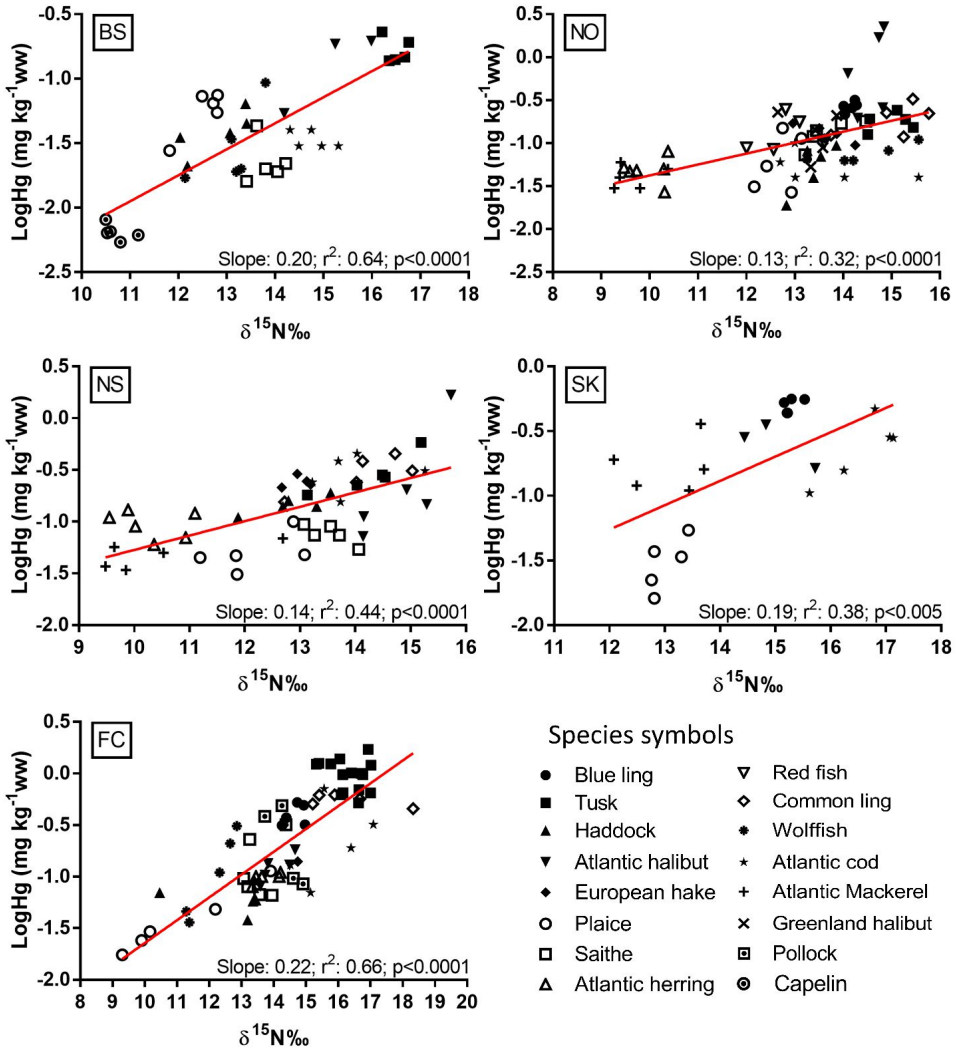

Figure 5. Relationship between log transformed THg (mg kg^{-1} ww) and $\delta^{15}N$ (‰) for fillet of fish species collected from different areas of NEAO: BS = Barents Sea, NO = Norwegian Sea, NS = North Sea, SK = Skagerrak and FC = fjords and coastal areas. Trophic magnification slope, r^2 and p value are presented. Symbols indicating different fish species are similar in different graphs.

The carbon stable isotope was significantly correlated to THg levels in the Barents Sea and fjords and coastal areas and explained a large part of the variation (r^2= 0.70 and r^2= 0.63 respectively; $p<0.0001$). In the other areas, including the Norwegian Sea, the North Sea and the Skagerrak the Hg level in fish fillet was not correlated with $\delta^{13}C$ ($p>0.05$) (Figure 6). In fjords and coastal areas, relatively high levels of terrestrial organic matter from the catchment is expected to be delivered to the fjords and coast and it can be expected that this effect will be reduced gradually when it reaches offshore areas. This can be related to the stronger water currents and generally higher mixing in offshore areas as well as larger water bodies that can dilute the terrestrial carbon source. Therefore, it is expected to have less effect from carbon source offshore where other important parameters such as trophic position or depth of forage can more important drivers of Hg level in fish and mask the effect of energy sources.

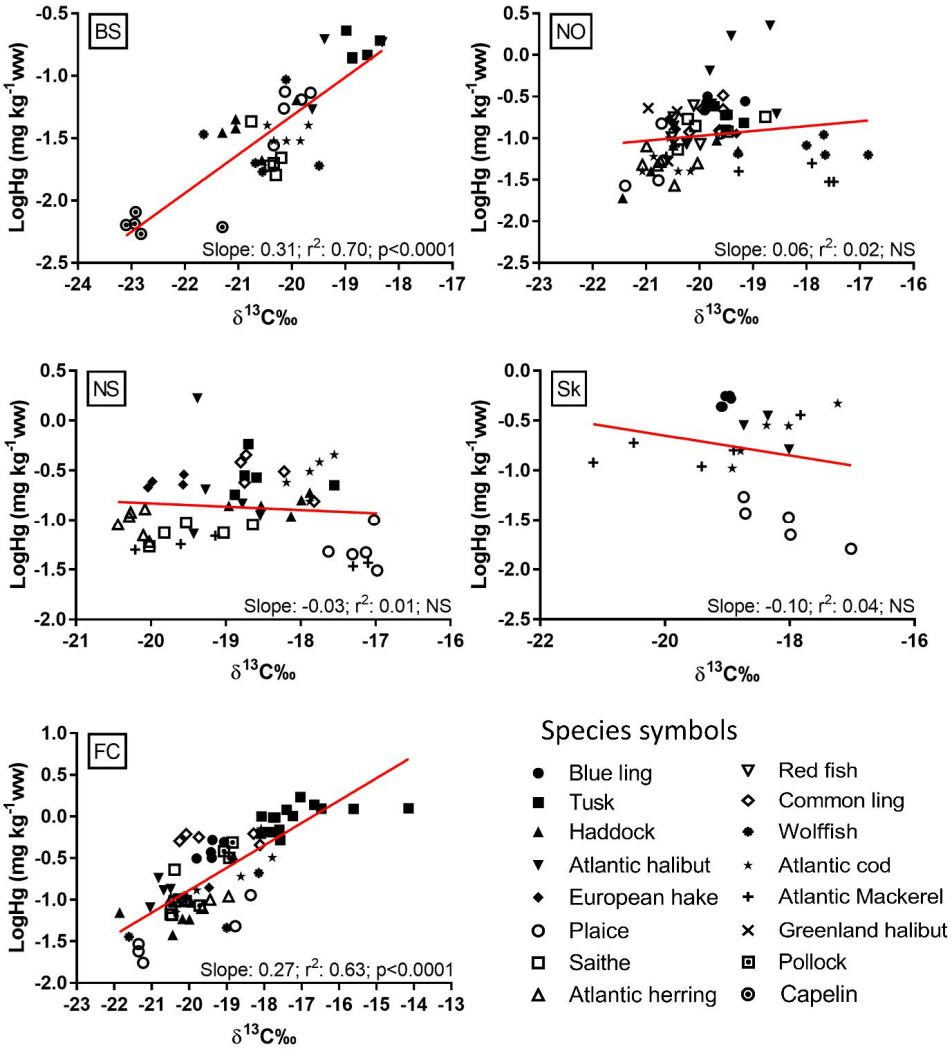

Figure 6. Relationship between log transformed THg (mg kg^{-1} ww) and $\delta^{13}C$ (‰) for fillet of fish species collected from different areas of NEAO: BS = Barents Sea, NO = Norwegian Sea, NS = North Sea, SK = Skagerrak and FC = fjords and coastal areas. Trophic magnification slope, r^2 and p value are presented. Symbols indicating different fish species are similar in different graphs.

4.3 Major drivers of geographical mercury variation within fish species

All fish species from NEAO exhibited differences in THg levels in the fillet between different offshore areas, and in nine of eleven species Hg showed a gradual increase from the north towards the south of NEAO (Paper I). For seven of nine species sampled both offshore and in fjords and coastal areas, Hg levels were significantly higher in fish sampled in fjords and coastal areas compared with those sampled offshore (Paper I).

4.3.1 Effect of eco-physiological factors

In offshore areas, Hg in fish fillet showed a gradual increase from the Barents Sea in the north towards the North Sea and the Skagerrak in the south. Considering the gradual nature of this trend in most fish species, and the fact that the samples were collected from a very large latitudinal range (approximately 25° latitude), it was speculated that variation in light regime and temperature can play important roles. In the north, a short but effective photoperiod combined with low temperatures in the north results in a short but intense primary production period. Then, according to growth bio-dilution theory, less MeHg will be incorporated to the first trophic position (phytoplankton). On the other hand, higher temperatures in the south may lead to a lower food conversion ratio and hence lower growth rates from the same amount of ingested food. This could lead to a higher Hg accumulation in organisms including fish. The details of the effect of temperature and photoperiod on the Hg accumulation are discussed in Paper I.

4.3.2 Trophodynamics and carbon flow influence on Hg accumulation

In this part the results of carbon and nitrogen stable isotope analysis in the food web of different areas of NEAO and Hg trophic transfer and source of carbon dynamics is discussed (Figure 4). Biomagnification of MeHg in marine food web is controlled by two major factors: 1) the MeHg concentration at the base of food web, phytoplankton, that is the largest step in bioconcentration of MeHg from the environment to the biota at the first trophic position. 2) the parameters controlling the biomagnification of MeHg from prey to predator along the food web of different areas.

Trophic magnification slopes (TMS) were used to compare the biomagnification rates in the different areas (Figure 5) and the effects of physiochemical and ecological differences were used to explain variation in TMS across areas. TMS is an indicator of biomagnifying potential of Hg in the food web and when TMS is larger than zero it means there is Hg biomagnification in the food web. Higher TMS values simply show more efficient biomagnification (Lavoie et al., 2013). Among all the offshore areas, the Barents Sea had the highest TMS (0.20) followed by the Skagerrak (0.19) and the North Sea (0.14) and the Norwegian Sea (0.13).

In a meta-analysis, Lavoie et al. (2013) compiled several studies investigating the TMS worldwide and they reported a mean ± SD TMS of marine food webs of different latitudinal zones as follows: polar zone 0.21 ± 0.07, temperate zone 0.22 ± 0.11, tropical zone 0.16 ± 0.08. When the study areas were divided into coastal and offshore areas, the TMS were 0.19 ± 0.08 and 0.21 ± 0.11 respectively. They also showed that TMS decreases gradually in the southern latitudes.

In this study, the Barents Sea had a TMS similar to the mean of the polar zone, but TMS in the Norwegian Sea and the North Sea were much lower than in the Barents Sea. In cold water, the excretion rates of Hg are lower than in warmer environments (Trudel and Rasmussen, 1997) and this may result in high accumulation rates in marine food web in northern areas of NEAO. Mean temperatures in the Norwegian Sea and the North Sea are approximately 7-8 °C higher than in in the Barents Sea. It has also been suggested that food webs are more complex in the southern latitudes and simpler in northern areas (Hillebrand, 2004; Kortsch et al., 2019). It has been suggested that more complex food webs consisting of more trophic steps and more prey choices for consumers can potentially reduce Hg trophic transfer efficiency (Lavoie et al., 2013). The Barents Sea, characterized with lower species diversity compared to the Norwegian Sea and North Sea, may have higher Hg biomagnification and TMS.

The amount of freshwater containing allochthonous carbon is higher in fjords and coastal areas than offshore, and this may explain the higher TMS in the fjord and coastal area compared to all other offshore areas in NEAO. Organic matter delivered to the sea

from land can enhance Hg methylation rate and it has been documented that MeHg bound to labile organic carbon is more bioavailable compared to MeHg produced in the sediment (Jonsson et al., 2014; Schartup et al., 2015). In this study the TMS in the fjord and coastal area was higher than all other offshore areas, while the meta-analysis conducted by Lavoie et al. (2013) found that coastal ecosystems have lower TMS than offshore ecosystems (0.19 vs 0.21). The difference between these two studies can be connected to the characteristics of the fjords which receive relatively large amounts of runoff and terrestrial organic carbon that deliver and produce bioavailable and highly biomagnifying MeHg. Lavoie et al. only considered data from coastal ecosystems.

According to these findings, it can be recommended that for Hg trophic transfer investigations and Hg spatial distribution, food webs from fjords and coastal areas should be categorized in separate groups and preferably for long fjords such as Hardangerfjord (Paper II) and Sognefjord (Paper III), they should be divided to inner and outer sectors.

The concentration of THg (μg kg^{-1} ww) at the base of food web (trophic position 1, phytoplankton) is another important parameter that can influence the Hg accumulation in marine fish species. Since our data set included only fish species from trophic position 3 (secondary consumers) and higher, THg of a separate baseline species occurring in all areas (blue mussel; trophic position 2, primary consumer) was used as a proxy for THg at the base of the food web and compared between areas of NEAO (for more details refer to methodological consideration). The THg concentrations of the baseline species were estimated for different areas of NEAO (in μg kg^{-1} ww) as follows: BS = 1.29, NO = 13.27, NS = 10.94, SK = 9.27 and FC = 1.83.

When the Hg level at the base of the food web is high, it is expected that transfer of Hg along the food chain may be reduced due to competitive uptake kinetics and regulation mechanisms (Phillips and Rainbow, 1989; White and Rainbow, 1982). This mechanism is in good agreement with the relatively higher TMS in the Barents Sea and the fjord and coastal area where Hg baseline was lower compared to NO, NS and SK. It is also documented that higher levels of nutrients reduce the MeHg incorporation at the base

of the food web (Pickhardt et al., 2002). This can also explain the low THg levels at base of the food web in fjord and coastal area where the runoff from large, and sometimes anthropogenically developed, catchments areas brings high levels of nutrients.

The Skagerrak food web had relatively higher TMS and lower Hg baseline compared to other offshore areas. Although Skagerrak was categorized as an offshore area in paper I, the conditions in Skagerrak has many similarities with fjords and coastal areas including a very large terrestrial catchment area and a deep basin with a relatively shallow sill, and hence limited water circulation of the bottom water that makes it different from typical offshore areas (Rodhe, 1996). Therefore, relatively high TMS can perhaps be connected to the more fjord like conditions with higher bioavailability of Hg due to allochthonous carbon sources.

In summary, based on information resulting from $\delta^{13}C$ and $\delta^{15}N$ and trophodynamics investigations, in the Barents Sea and fjord and coastal areas the Hg levels at the base of the food web are relatively low, but transfer of Hg along the food web takes place at relatively high rates. On the other hand, in the Norwegian Sea and the North Sea the Hg level at the base of the food web was higher than other areas but the rate of transfer between trophic positions was relatively low. In Skagerrak the Hg level at the base of food web and its transfer between trophic positions were both intermediate.

4.3.3 Mercury variation in fish collected from offshore versus fjords and coastal areas

In paper I, it was demonstrated that fish from fjords and coastal areas accumulate higher Hg levels than similar fish species caught offshore. This was linked to fjords and coastal areas having higher organic matter from catchment areas and higher methylation rates, less water exchange and lower oxygen compared with offshore areas. In paper II, the role of a point source of pollution (PSP) in contributing to the elevated Hg levels measured in seafood from the inner sector of Hardangerfjord was investigated. Although high levels of Hg were determined in the environment and biota close to PSP, the highest Hg in the predatory fish species, tusk was measured in Eidfjord, a branch relatively far from PSP. It was concluded that Hg contamination from PSP has a clear

role in controlling the high Hg levels in fish. However, other fjord characteristics, such as high input of organic matter and atmospherically deposited Hg from the catchment area as well as less water exchange and low oxygen levels in bottom waters, may have an additional effect. In paper III, we found high Hg levels in tusk from Sognefjord, where Hg levels in the sediment were at the background level, and Hg in fish fillet increased from open ocean via the coast and towards the inner part of the fjord. Here $\delta^{13}C$, as an indicator for source of carbon, explained a large part of the variation of Hg levels in tusk fillet collected from the different marine habitats offshore North Sea, the coastal area and outer and inner Sognefjord. We concluded that input of Hg and organic material from the catchment area probably is a major driver of spatial variation in Hg levels in fish, and this effect increases towards the inner part of the fjord. In fjords and coastal areas of NEAO, $\delta^{13}C$ also explained 63% of Hg variation indicating the importance of carbon source in Hg accumulation in the food web of these habitats.

It can be speculated that all long fjords with large catchment and stratification and restricted water exchange can have elevated Hg in the food web (Berg et al., 2000). Therefore, the inner sector of all long fjords along the western coast of Norway may possibly be considered as Hg hot spots. Accordingly, a gradual increase in Hg levels of fish from open ocean to the coast and further into the fjords may be expected as a predictable trend.

4.3.4 Hg trophic transfer in the food web of Sognefjord

In paper III, an increasing trend was found in Hg levels in tusk from offshore North Sea towards inner sector of Sognefjord. It was postulated that high levels of runoff and labile organic carbon in the inner part of the fjord, followed by methylation in the water column, drive this trend. On the other hand, it has been shown that in estuarine environments, the MeHg delivered from the catchment area and atmospheric sources has a higher accumulation rate compared to MeHg formed in the sediment (Jonsson et al., 2014). In a master project, the trophic magnification factor (TMF) of Hg in inner part of Sognefjord (Lustrafjord, Site 1 in paper III) was studied and compared to other areas (Sverrisson, 2018).

The TMF (based on trophic position) of the food web in the Lustrafjord based on 24 different species was 12 and 14 for THg and MeHg, respectively. Calculation of TMF based on $\delta^{15}N$ is independent of baseline species and is therefore not biased for baseline species when comparing with other studies. The TMF was higher for MeHg than for THg, which was expected from the higher bioavailability of MeHg compared to THg. The majority of THg in the higher trophic positions is in the form of MeHg, and the differences in TMF for these two Hg species is thus not very large. The %MeHg (ratio of MeHg to THg × 100) increased with the trophic position in Lustrafjord.

The TMF found in Lustrafjord was clearly higher than what is reported for coastal and offshore areas (Table 1). However, no similar study has been conducted in a fjord ecosystem with similar conditions. The best fjord to compare with Lustrafjord was Kongsfjord in Svalbard, which is a relatively short fjord, unlikely to have a clear gradient in freshwater input like Hardangerfjord (Paper II) or Sognefjord (Paper III). When TMF based on $\delta^{15}N$ in Lustrafjord was compared to other areas of NEAO, it was substantially higher than all other areas. Also, TMF of THg found in Lustrafjord was higher (2.2) than the average found for fjords and coastal areas in this study (1.66). This is in good agreement with the amount of terrestrial organic carbon from runoff decreasing from the inner to the outer fjord and further towards the coastal and offshore areas. Lustrafjord is the innermost site in Sognefjord (Paper III) and according to the mentioned theory, it is expected to have higher TMF (Hg biomagnification per trophic position) compared to other fjords and coastal areas.

Sognefjord is one of the longest and deepest fjords in the world with a sill close to the mouth. The high amount of runoff from a large catchment area creates a gradual trend in the organic carbon in this ecosystem. We postulate that this high level of labile organic carbon enhances the Hg methylation in the water column and that the MeHg bound to organic matter is more bioavailable to the food web. Therefore, Hg may biomagnify at a greater degree in Lustrafjord food web compared to other areas.

Table 1. Trophic Magnification Factor (TMF) of Hg in the Lustrafjord food web compared to other areas in the North Atlantic and the Mediterranean Sea.

Location	TMF calculated from trophic position		TMF calculated from $\delta^{15}N$		References
	THg	MeHg	THg	MeHg	
Gulf of St. Lawrence, Canada	3.81	6.46	-	-	(Lavoie et al., 2010)
Kongsfjord, Svalbard	3.02	-	-	-	(Jæger et al., 2009)
Lustrafjord	**12**	**14**	**2.2**	**2.5**	(Sverrisson, 2018)
Augusta Bay, Mediterranean Sea	-	-	1.22	-	(Signa et al., 2017)
Nunavut, Canada	-	-	1.2	-	(Swanson and Kidd, 2010)
Baltic Sea and Gulf of Bothnia	-	-	1.50	-	(Nfon et al., 2009)
Baffin Bay, Canada	-	-	1.57	1.67	(Campbell et al., 2005)
Lancaster sound, Canada	-	-	1.58	-	(Atwell et al., 1998)
Barents Sea	-	-	1.58	-	This PhD thesis
Norwegian Sea	-	-	1.35	-	This PhD thesis
North Sea	-	-	1.38	-	This PhD thesis
Skagerrak	-	-	1.55	-	This PhD thesis
Fjord and coastal areas	-	-	1.66	-	This PhD thesis

4.3.5 Role of habitat and depth of forage on Hg accumulation

In NEAO, the measured mean Hg concentration was lowest in pelagic species, highest in demersal species and intermediate in benthopelagic species, following a vertical trend, increasing with depth of habitat. Several studies have documented that Hg concentration increases with depth of forage (Choy et al., 2009; Cossa et al., 2012). The Hg level in the seawater column also increases with depth and several studies found a vertical trend in the water column with lower Hg levels in surface waters. The Hg concentrations are higher in sub-thermocline and low oxygen deeper waters in different oceans (Blum et al., 2013; Cossa et al., 2009; Hammerschmidt and Bowman, 2012; Sunderland et al., 2009).

The earlier recorded higher MeHg levels in deeper water support the findings of our study and may be valid also for NEAO. Of note, however, the fact that the demersal fish species sampled in the NEAO in general belong to higher trophic positions and may grow slowly to a high age. This may be as important as the seawater Hg levels in determining the MeHg levels of the fish from our study.

4.3.6 Is accumulated mercury in the food web connected to environmental mercury levels?

Measurements of Hg species in seawater, with naturally low concentrations, is technically challenging and require metal clean sampling procedure and high-resolution measurements. Thus, data on Hg speciation in seawater are scarce. On the other hand,

measurements in sediment samples are easier due to naturally higher concentrations and standard protocols applied worldwide which provide more data for geographical comparisons.

In NEAO, there is a comprehensive data set available on THg in sediment covering a large part of the study area in paper I (MAREANO project). Investigating that data (Paper I) showed that THg in sediment was slightly higher in the northern latitudes and that logTHg increased with latitude (slope= 0.009; r=0.11; p<0.0001; n=2003). Everaert et al. (2017), analyzing existing Hg data in sediment from NEAO including the MAREANO data set, showed that THg in sediment of offshore areas north of 62 ºN is higher than south of 62 ºN (median 0.02 vs 0.03 mg kg^{-1} dw). In fish from NEAO a clear negative correlation was documented between logTHg in fish fillet and latitude of sampling location in 12 of 13 species (r between -0.11 and -0.67; p<0.01) (Paper I). This trend indicates that Hg levels in fish from NEAO are independent of Hg levels in the sediment. Other parameters are influencing the geographical pattern are discussed in more detail in paper I and in this thesis (elsewhere).

In paper II, we investigated the effect of Hg point source in the Sørfjord and the Hg contamination increased in sediment, seawater and biota closer to the point source. Of note, the Hg levels in top predator fish (tusk) were as high in Eidfjord, far from the pollution source.

In order to evaluate the effect of Hg level in the sediment from different marine habitats, data from the main sites in Hardangerfjord as an example of a polluted fjord (Paper II) and Sognefjord with natural condition (Paper III) were combined with data from North Sea coast and offshore. These data and THg levels in tusk fillet were compared using ANCOVA (Figure 7). The results showed that the Hg levels clustered into three main groups where inner fjord had the highest levels, outer fjord intermediate levels and offshore the lowest levels. When THg in sediment was considered and tusk sediment accumulation factors were calculated (method in Paper I), S1 and S2 from inner Sognefjord had the highest accumulation factors and H1 from the inner Sørfjord had the lowest accumulation factor. This shows that the large amounts of Hg from the point

source in the Hardangerfjord ecosystem is accumulated far less efficiently in tusk fillet compared to the much smaller amounts of environmental Hg present in Sognefjord. In an investigation using Hg stable isotope analysis, Rua-Ibarz et al. (2019) showed that Hg accumulated in tusk from Sørfjord is probably attributed to zinc smelter. This indicates that Hg from pollution source in Sørfjord has entered the food web to some degree.

After consideration related to MeHg demethylation and storage of iHg in tusk liver, the Hg species were reinvestigated. The analyses demonstrated that THg in the tusk liver samples from Sørfjord was not higher than in samples from Eidfjord (8.39 vs 8.02 mg kg^{-1} ww).

It is important to note that from the most polluted site 1S (Paper II), only two specimens were collected, and liver was not analyzed. Unpublished data from a site close to 2S (Paper II) shows that tusk liver had elevated THg (mean= 11.42 mg kg^{-1} ww, n=25), which might indicate that close to PSP at site S1, the Hg accumulation in top of the food web is higher than in Eidfjord. Due to lack of data from 1S, it is not possible to have a complete picture for Hg bioavailability of point source of Hg pollution from zinc plant at Sørfjord.

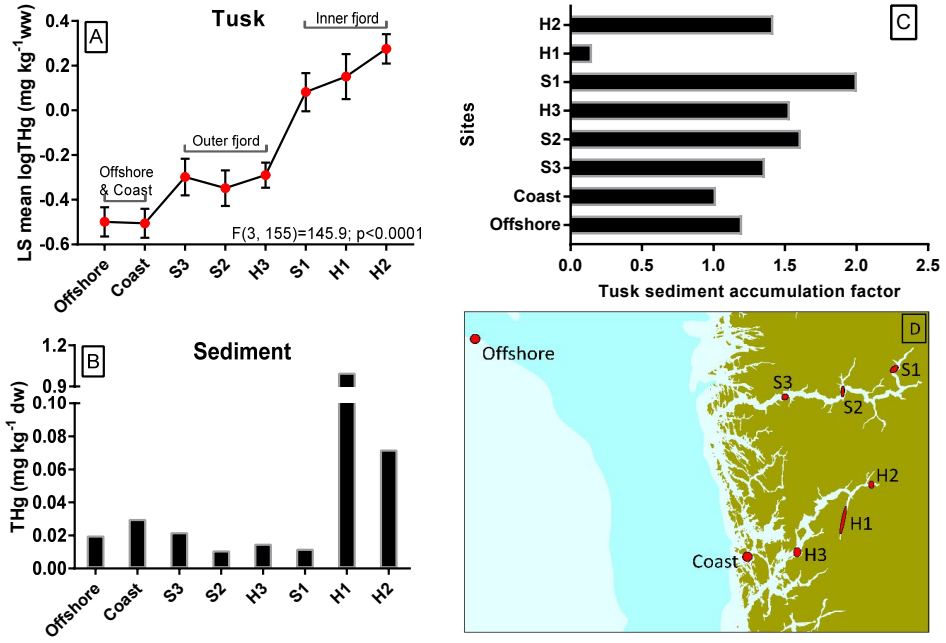

Figure 7. Least squares mean ± SEM of THg concentration in tusk fillet collected from different habitats (A), THg concentration in sediment (B), Tusk sediment accumulation factor, for methodology refer to paper II (C) and sampling location map (D).

4.4 Risk benefit evaluation and Hg exposure assessment of fish from NEAO

Fish contains healthy nutrients including LC-PUFAs that are considered as the main factor causing health benefits of seafood consumption and consumption of a certain amount of seafood is therefore advised (Kris-Etherton et al., 2009). On the other hand, seafood is the main contributor to MeHg exposure in humans (Al-Majed and Preston, 2000; Batista et al., 1996; Olivero et al., 2002) and MeHg is the main cause of the seafood consumption advisories. Therefore, the ratio of MeHg to its reference dose and LC-PUFA to its recommended daily intake in different fish species were considered for the risk benefit evaluation with comparable positive and negative human health impact.

We conducted a human exposure assessment using the TWI of MeHg for 1.3 µg/kg body weight established by the EFSA. This assessment uses a TWI of 1.3 µg kg^{-1} body weight as an acceptable risk based on different lifetime exposure scenarios considering both Hg concentrations and consumption patterns and rates (EFSA, 2012).
Hazard quotients (HQ) estimate whether the beneficial effects from essential fatty acids are higher than the potential risk from Hg exposure. A hazard quotient lower than 1 indicates that adverse effects are not likely to occur, whereas values greater than 1 indicates that additional risk management measures are required. Among the studied species in paper I, the average HQ_{500} varied from 0.03 in Atlantic mackerel to 16.6 in blue ling. In more than half of the species including haddock, Atlantic cod, wolffish, pollack, European hake, common ling, Atlantic halibut, tusk and blue ling, the HQ_{500} was greater than 1. This indicates that these fish provide more Hg relative to TWI than EPA+DHA relative to their recommended daily intake (500 mg day^{-1}).

In these calculations, Hg exposure is considered only through the consumption of seafood, while also other foods and other species of fish than those considered here will contribute to the total exposure. There is also a limitation in the data on EPA and DHA that it was not very representative for the distribution range of the species which were sampled for Hg and Se. Therefore, the variation of EPA and DHA levels for different species is not very well covered except for pelagic species with high commercial importance including Atlantic mackerel and Atlantic herring. In this study, only wild caught fish was considered. However, farmed salmon containing high levels of EPA+DHA and very low Hg levels, contributes significantly to fish consumption in European countries (Nøstbakken et al., 2015).

The HQ_{250}, which considers LC-PUFA requirements to reduce health risk of cardiovascular diseases, was above 1 in haddock, common ling, tusk and blue ling, species with high Hg levels.

Among the wild caught species, Atlantic cod is a particularly popular fish with relatively high consumption in Europe and the most valuable fisheries in Norway. This species is on average low in Hg, but also relatively low in Se and LC-PUFAs. With

regards to RDI of EPA+DHA for pregnant women (HQ_{500}) it is barely higher in risk than benefit (HQ_{500} = 1.02; Table 2), but regarding the RDI for adults to reduce the risk of CHD, Atlantic cod is well below one and provide more benefit than risk (HQ_{250} = 0.51). Cod and other lean fish are also good sources of other nutrients, such as iodine which has not been taken into consideration here.

An intake of minimum 200 mg DHA per day during pregnancy and nursery is recommended to support optimum visual and cognitive development of the baby during pregnancy and after birth as well as decreasing the risk of premature birth (Kris-Etherton et al., 2009). Among the species with HQ_{500} > 1, some have high Hg concentrations that exceed the TWI for MeHg. The main reason for the high HQ is the low levels of fat content, and hence LC-PUFAs, in these species except Atlantic halibut.

To evaluate the Hg exposure from seafood consumption, the TWI of MeHg (1.3 μg kg^{-1} body weight) set by EFSA was used and the consumption limits were calculated (for calculations refer to paper I). In this study seafood consumption of two servings of fish (as a general recommendation on seafood consumption) equal to 340 g (170 g per serving) fish per week was considered as recommendation for adults (70 kg) and four servings equal to 680 g of fish consumption for pregnant women.

For a person of 70 kg and a consumption of 340 g fish per week, TWI for MeHg will be exceeded if the Hg concentration in the fish is higher than 0.27 mg/kg ww. Thus, considering the average Hg concentration of the fish species analyzed here, two servings of Atlantic halibut, tusk or blue ling would lead to exceeded TWI for MeHg (Table 2). For pollack, Greenland halibut, European hake, common ling, Atlantic halibut, tusk and blue ling, the TWI would be exceeded if four servings per week are consumed. For blue ling, a person of 70 kg eating only one meal would exceed the MeHg TWI.

Table 2. EPA and DHA levels and their data sources, HQ_{500}, HQ_{250}, percent TWI fulfilled after 2 and 4 servings and consumption limit of different fish species from NEAO. Colors represent low risk (green), moderate risk (yellow) and high risk (red).

Species	Sum w-3 fatty acids (mg/kg)	EPA (mg/100g)	DHA (mg/100g)	Data sources	HQ_{500}	HQ_{250}	HBV_{Se}	%TWI (2 servings)	%TWI (4 servings)	consumption limit per week (g)
Blue whiting	9150	232	526	SFD	0.21	0.10	6.11	15	30	2241
Atlantic mackerel	76030	1956	2988	SFD	0.03	0.02	7.00	16	32	2114
Atlantic herring	30560	925	1168	SFD	0.08	0.04	6.60	17	34	2019
Plaice	6600	170	380	NFCT	0.42	0.21	4.76	23	45	1510
Haddock	500	20	40	NFCT	4.43	2.21	3.97	26	52	1317
Saithe	4610	111	320	SFD	0.63	0.31	3.59	26	53	1295
Atlantic cod	2990	86	198	SFD	1.02	0.51	3.44	28	56	1208
Wolffish	2497	111	100	SFD	1.69	0.84	5.57	35	69	983
European eel	81400	2200	3490	NFCT	0.07	0.04	3.73	40	80	851
Redfish	7600	310	250	NFCT	0.88	0.44	7.05	48	96	710
Pollack	3340	56	262	SFD	1.69	0.84	4.65	52	104	652
Greenland halibut	8100	320	340	NFCT	0.84	0.42	5.23	54	108	631
European hake	6020	116	384	SFD	1.49	0.75	4.12	72	145	469
Common ling	2190	39	167	SFD	4.10	2.05	5.00	82	164	415
Atlantic halibut	10200	330	480	NFCT	1.80	0.90	5.45	142	283	240
Tusk	2020	36	152	SFD	8.92	4.46	5.46	163	327	208
Blue ling	1760	26	141	SFD	16.70	8.35	2.09	270	540	126
All species	15401	414	670		0.62	0.31	5.08	65	130	521

SFD: seafood data; web page: www.sjomatdata.nifes.no
NFCT: The Norwegian Food Composition Table; web page: www.matvaretabellen.no

Most of the consumption of fish comes from commercial fisheries and catch volume of the different species provides some information about the consumption of the different species by the general population. Catch volume in 2017 of the species investigated here varied from 12 tons for European eel and 244 tons for blue ling to 526 000 tons for Atlantic herring. With a total catch volume of all the studied species of 1.9 million tons, Atlantic herring comprised 27%, whereas European eel and blue ling represented only 0.001 and 0.01 %, respectively, of the total catch. The species with the highest catch volumes, such as mackerel, herring, cod, haddock and saithe, all had relatively low concentrations of Hg, and a 70 kg person could consume more than a kilogram per week of these species without exceeding the TWI. The most contaminated species constitute a very small portion of the annual catch from NEAO. Atlantic halibut, tusk and blue ling, with mean concentrations of Hg above 0.3 mg/kg, all constituted less than one

percent of the annual catch. The catch volumes of pollack, Greenland halibut, hake and common ling, of which a 70 kg person would exceed TWI from consumption of four servings per week, were less than three percent in 2017. Therefore, they were not considered as a great risk to the general consumers on a large scale. However, local recreational fishermen and their families living by the fjords and coastal areas catching deep-water species, such as tusk and blue ling, may exceed the TWI for MeHg.

It is worth mentioning that blue ling and tusk from fjords and coastal areas were the most Hg contaminated species in this study (0.87 and 0.85 mg kg^{-1} ww, respectively). Even one serving of blue ling and tusk from this area per week would lead to exceeding the TWI for MeHg by 163 and 159 %, respectively, and the consumption limit were 107 and 105 g per week for a 70 kg adult. Considering the geographical variation in Hg level in these two species and more sensitive consumers (pregnant women and children), consumption of tusk and blue ling caught from fjords and coastal areas in the south of Norway can have a high risk of Hg intoxication. Blue ling is not subject to targeted fishery because it is an endangered species and illegal to catch, and is only caught as bycatch. On the other hand, for local recreational fishermen using long lines for fishing in fjords and coastal areas these two species may be a significant part of the catch which may pose a risk of Hg intoxication for themselves and their families.

Four servings of Atlantic mackerel, Atlantic herring, plaice, saithe, European eel and redfish will provide the requirement of DHA without exceeding the TWI of MeHg for pregnant women (Table 2). Redfish showed a very large variation in Hg concentration between areas and seven samples collected from Skagerrak contained a high Hg level and may therefore pose risk. However, most redfish are caught in the open ocean of the Norwegian Sea (www.fiskeridir.no). Blue whiting is a relatively small fish species mostly used for fish feed production (fish meal and oil) and the human consumption is very small. However, if it had been sold as food for human beings, it would have been a good source of LC-PUFAs with a very low Hg level.

Although it needs to be mentioned that other potentially associated contaminants in fish such as polybrominated diphenyl ethers, polychlorinated diphenyl ethers and other

persistent organic pollutants (POPs), particularly dioxins and dioxin like PCBs, are not considered here. Oily fish like mackerel and herring have the potential to accumulate these fat-soluble substances in polluted areas due to the high fat contents of the fillet (Knutsen et al., 2018). However, herring from the Norwegian Sea have low concentrations of these substances compared to European maximum level (EUML) (Frantzen et al. 2011), and unpublished data on herring from the North Sea and Northeast Atlantic mackerel caught in different areas also show relatively low average concentrations of POPs (www.sjomatdata.nifes.no). However according to the new TWI of dioxins and dioxin-like PCBs established by EFSA, consuming 1-2 servings of these fish per week, may exceed the TWI (Knutsen et al., 2018).

However, the above evaluations do not take into account the positive effects of Se or the interaction between Se and Hg. In order to evaluate both risk and benefits associated with consumption of fish species from NEAO, selenium health benefit value (HBV_{Se}) was calculated for each species (Table 2). HBV_{Se} varied between 2.1 in blue ling and 7.1 in redfish when overall mean levels of Hg and Se were used. Having no species with a negative value for HBV_{Se} showed that all species provided more Se than Hg in terms of molar concentrations and consumption of these species thus provides a surplus of Se.

The Recommended Dietary Allowance (RDA) for Se for adults and pregnant women is 55 and 60 µg day^{-1}, respectively, and the upper intake level for adults is set at 400 µg day-1 (Council, 2000). Two servings of fillet of fish species from NEAO per week would cover 25 – 70 % of the RDA, whereas four servings of fish with the highest Se level is still well below the upper intake considering all Se intake from fish.

Due to the high variation in Hg, Se and LC-PUFAs levels, a large variation was observed between species with regards to both risk and benefits from fish consumption. These variations will expand even further if geographical differences are taken into account.

Therefore, it would be much more accurate and beneficial for both risk managers and the consumers to have species specific fish consumption advice with regards to both contaminants and nutrients as well as the interaction between them as a complex.

4.5 Human exposure assessment for Seafood species from Hardangerfjord and Sognefjord

The consumption limit per week (maximum amount that can be consumed without exceeding TWI) for different species from Hardangerfjord was calculated for a 70kg person assuming that all Hg in fish fillet and claw and tail meat of crustaceans is in the MeHg form and excluding other sources of Hg exposure. Consumption limits were calculated for each species collected from either the inner part (Eidfjord and Sørfjord) or from outer Hardangerfjord (Table 3). Among the sampled fish species, tusk from the inner part had the lowest consumption limit (48g per week) and wolffish from the outer part had the highest limit (663g). In general, crustaceans had high consumption limits per week. Brown crab from the outer part had the highest limit (776g per week) and European lobster from the inner part had the lowest limit with 147g per week (Table 3). The average portion size for marine fish in Norway is 210g (Bergsten, 2004). Therefore, consumption of even one portion per week of blue ling, common ling and tusk from both inner and outer Hardangerfjord would exceed the TWI for MeHg. The average fish consumption rates in coastal areas in Norway are 77g and 61g per day for men and women, respectively (Bergsten, 2004). Considering these consumption rates, intake of demersal fishes from the outer Hardangerfjord would lead to men and women exceeding the TWI by a factor of 2 and 1.5 respectively. Consumption of demersal fish from inner Hardangerfjord would lead to men and women exceeding the TWI by a factor of 8 and 6, respectively. Recreational and professional fishermen who catch deep-water fish species using long line in this fjord, and their families, may risk exceeding the EFSA TWI. The Norwegian Food Safety Authority (NFSA) has issued a consumption warning not to eat deep-water fish for nearly the entire Hardangerfjord ecosystem.

Usually, crustaceans are served and consumed at smaller portion weights. Although extreme consumers and fishermen may readily reach the TWI, average consumers are not likely to reach the TWI for MeHg by intake of crustaceans. Still, intake of 147g European lobster tail from inner part of Hardangerfjord would be sufficient to reach the TWI of MeHg.

The blue ling and tusk from inner part of Hardangerfjord had HBV_{Se} with negative values of -1.7 and -0.6, respectively indicating higher molar concentration of Hg than Se with higher health risk for consumers.

In fillet of tusk from Sognefjord, mean THg at 7 of 8 sites exceeded the EUML and TWI of MeHg will be exceeded by consuming one portion of tusk fillet from 5 of the sites (Table 3). The other three sites have a consumption limit of one serving per week. Based on the findings of this study, NFSA has issued a consumption warning on tusk from Sognefjord. HBV_{Se} tusk fillet was above 3.6 in all sites showing excess Se to Hg in molar concentration which may provide some protection against MeHg toxicity.

Table 3. Consumption limit per week (g) and HBV_{Se} of demersal fish and crustacean species from Hardangerfjord sampled in 2011 (left) and tusk from Sognefjord sampled in 2013 and 2015 (right). Red color represents high risk, yellow represents medium risk and green represents no risk. For more details of sites location refer to figure 1 in paper I and III.

Species	Area	N	Consumption limit per week (g)	HBV_{Se}
Blue ling	Out.Hard.	33	85	0.4
	Inn.Hard.	8	63	-1.7
Common ling	Out.Hard.	28	186	5.0
	Inn.Hard.	2	84	4.7
Tusk	Out.Hard.	97	108	5.0
	Inn.Hard.	41	48	-0.6
Sprat	Out.Hard.	3*	9100	5.4
	Inn.Hard.	2*	3033	5.3
Wolffish	Out.Hard.	4	663	5.8
All fishes	Out.Hard.	162	260	4.1
	Inn.Hard.	51	65	0.8
Brown crab	Out.Hard.	10	776	18.6
	Inn.Hard.	10	413	9.5
European lobster	Out.Hard.	21	475	7.6
	Inn.Hard.	5	147	5.5
Norway lobster	Out.Hard.	10	445	12.4
All Crustaceans	Out.Hard.	41	565	12.9
	Inn.Hard.	15	280	7.5
All species	Out.Hard.	203	391	7.8
	Inn.Hard.	67	151	3.5

* Each sample is a composite of 25 whole specimens

Site	N	Organ	HBV_{Se}	Consumption limit per week (g)
1	14	Fillet	3.6	77
		Liver	75.2	
2	25	Fillet	6.1	112
		Liver	80.2	
3	17	Fillet	4.9	100
		Liver	52.9	
4	25	Fillet	6.3	172
		Liver	77.9	
5	16	Fillet	5.7	154
		Liver	69.5	
6	25	Fillet	6.3	284
		Liver	63.8	
7	15	Fillet	7.5	128
		Liver	76.9	
8	15	Fillet	6.3	175
		Liver	62.0	
9	25	Fillet	4.8	379
		Liver	46.1	
10	24	Fillet	4.1	303

4.6 Mercury and selenium co-exposure in fish from NEAO

The co-occurrence of THg and Se in the fillet of several commercially important fish species was investigated (Paper I). Our findings showed a clear positive correlation between these two elements in most of the species. The correlation tended to be stronger when THg levels were high and was not significant in the four species with lowest THg levels (Paper I).

Sakamoto et al. (2015) investigated the THg, MeHg and iHg in muscle of marine mammals (bottlenose dolphins, Risso's dolphins, striped dolphins, and short-finned pilot whales) and documented that %MeHg decreases from 90 – 100% to 20 – 40% in skeletal muscle with increasing THg and found that THg and Se have strong correlation. Additionally, they used X-ray absorption fine structure analysis and showed that iHg occurs as HgSe in the muscle. In paper III, we found that %MeHg decreases with increasing THg in tusk fillet. Hg may bind Se, and HgSe is more stable than HgS (Dyrssen and Wedborg, 1991; Ralston, 2018). Likewise, MeHg also has higher affinity for Se than for sulfur and therefore, MeHg may form a stable binding to selenoprotein (Carty et al., 1983; Sugiura et al., 1978). Formation of MeHg-selenocysteine complexes is documented in organisms exposed to MeHg, leading to interruption of selenoenzyme synthesis and activity (Ralston et al., 2008). This is suggested as one of the main mechanisms of MeHg toxicity (Asaduzzaman and Schreckenbach, 2011; Peterson et al., 2009a). In fish collected from NEAO, the majority of Hg exists as MeHg (Paper I) and a correlation between THg and Se in the fillet of fish from NEAO may indicate that MeHg is bound to selenoprotein. A surplus of Se in fish fillet from NEAO may reduce the toxicity of MeHg with significant influence on food safety and Se:Hg molar ratios should be considered for potential toxic effect of Hg in fish (Peterson et al., 2009a).

Negative health outcomes from consumption of seafood were revealed by epidemiological studies conducted on populations consuming species with negative HBV_{Se} values such as marine mammals (Faroe study) and sharks (New Zealand cohort study) (Paper I).

4.7 MeHg metabolism and possible demethylation

The concentration of iHg in liver of tusk from the inner Sognefjord was much higher than in the outer fjord. In liver of tusk from the Hardangerfjord ecosystem, we observed similar relatively low %MeHg in both inner fjord (20.5%) and outer fjord (21.8%), showing dramatic increase in iHg accumulation in liver compared to fillet (ca 100%) (Paper II). This high level of iHg can be explained in three ways: 1) due to high levels of iHg pollution in the ecosystem, tusk has fed on organisms containing high levels of iHg. 2) *in vivo* demethylation of MeHg and storage of iHg in the liver. 3) direct uptake of iHg from seawater or by ingestion of sediment in the polluted sector of Hardangerfjord. In paper III, we found a negative correlation between %MeHg and THg in both tusk fillet and liver from Sognefjord, and iHg levels in tusk liver were higher in sites with higher THg levels in tusk (inner fjord). In Sognefjord, the Hg concentration in the sediment samples were at the background level (0.02 mg kg^{-1} dw), and environmental Hg levels appear to be relatively low. Therefore, the direct uptake from the environment (seawater or sediment) (explanation 3) or high iHg in prey (explanation 1) seem unlikely. Thus, we suggested that demethylation of MeHg is taking place.

Demethylation of MeHg has been reported in many marine mammals, and it has been documented that high fractions of THg in liver, kidney and brain exist as iHg (Evans et al., 2000; Sakamoto et al., 2015; Wren et al., 1986). In a controlled experimental condition, guinea pig showed demethylation of MeHg and after three weeks of exposure the ratio of iHg to THg in the liver increased to 60% (Komsta-Szumska et al., 1983).

After demethylation, the inorganic form of Hg, formed *in vivo*, can be stored in specific organs such as liver and kidney bound to Se. In general, it is suggested that in the liver of marine mammals, the MeHg is the dominant form when THg concentration is less than ca. 9 mg kg^{-1} ww. When Hg concentrations are higher than this threshold, Hg usually occurs as iHg due to *in vivo* demethylation (Wiener et al., 2002). A corresponding threshold in liver of seabirds (feeding on marine organisms) has been suggested as 8.5 mg kg^{-1} dw, and above this level demethylation started in birds liver (Eagles-Smith et al., 2009). In marine mammals and birds, MeHg in liver is less than

15% when THg in liver exceeds 10 mg kg^{-1} ww (Dietz et al., 1990). It is documented that dolphins with high THg concentrations in the liver (more than 100 mg kg^{-1} ww) have %MeHg less than 10% with high concentrations of Se in the liver (Cardellicchio et al., 2002).

In contrast to marine mammals and birds, very little research on demethylation of MeHg in marine fish exists, and existence of demethylation in fish is controversial. Several studies have suggested the demethylation process in fish species based on 1) decreased %MeHg during post exposure and increased metallothionein genes to bind produced iHg in the liver (Gonzalez et al., 2005), 2) %MeHg reduction with age, from 50 to 20 %, in the liver of sardines (Joiris and Holsbeek, 1999) 3) increase of iHg in brain and liver of zebrafish (Feng et al., 2015), 4) increase in whole body iHg and demethylation of MeHg in the intestine based on feeding experiment using Hg species with different stable isotopes in sea bream (Wang et al., 2017). On the other hand, some studies had contradictory findings: 1) Transfer of MeHg from liver to muscle in wild yellow perch (Van Walleghem et al., 2007) and 2) no evidence of demethylation in freshwater fish tilapia after ingestion of isotopically labelled MeHg (Wang et al., 2013).

In the Sognefjord study (Paper III), it was found that demethylation of MeHg occurs even at very low concentrations of THg in tusk liver, but when the THg in liver reached approximately 3 mg kg^{-1} ww, the %MeHg level stabilized in a range between 10 – 30%. It can be suggested that at the 3 mg kg^{-1} ww threshold the demethylation rate increases and keep the %MeHg stable (Paper III). One issue with interpretation of tusk data for demethylation process was related to the low number of individuals that had higher THg in liver than this threshold. However, this is an indication that both marine mammals and marine fish can demethylate MeHg, but this process in fish is less obvious than in mammals due to lower accumulated levels.

Formation of HgSe after demethylation of MeHg has been documented in muscle of bottlenose dolphin (Sakamoto et al., 2015) and it is suggested that HgSe can be formed in the brain of dolphins (Nakazawa et al., 2011) as a mechanism to cope with MeHg toxicity in marine mammals that can accumulate very high levels of Hg. MeHg

demethylation has also been documented in the brain of primates (Vahter et al., 1995). Additionally, chemical demethylation of MeHg by selenoamino acids has been reported with formation of HgSe as the final product (Khan and Wang, 2010). In an experimental study using black seabream, Wang and Wang (2017) showed that Se increased the MeHg demethylation and reduced the MeHg accumulation.

In this thesis, the interaction of Hg and Se was only investigated through their co-occurrence. In Hardangerfjord, Se concentration in tusk liver was higher in the inner part (Paper II) and in the Sognefjord study, Se level showed positive correlation with THg in liver (r between 0.27 to 0.45; $p<0.05$). Selenium concentrations in tusk from the fjord were higher than tusk from the coastal area (Paper III). In tusk from Sognefjord, in the second phase of demethylation (above the threshold of 3 mg kg^{-1} ww THg in liver) the Se concentration increased concomitantly with iHg in the liver indicating the possible formation of a HgSe compound.

Many organisms can cope with high MeHg via loosing hair or molting feathers (terrestrial mammals and birds), but marine mammals and fish usually do not have such a mechanism. Additionally, it is showed that demethylation in birds with infrequent molting of the plumage, is more critical and iHg can reach up to 99% of THg in osprey liver (Hopkins et al., 2007). Therefore, marine mammals and predator fish species that are exposed to high Hg levels and may accumulate high Hg levels, will use demethylation to cope with MeHg toxicity.

It can be speculated whether the mechanisms that freshwater and marine fish use to deal with MeHg toxicity are different. It is worth mentioning that, to our knowledge, there is no study showing reduction in the %MeHg in the liver of freshwater fish.

If demethylation of MeHg does take place in tusk and high levels of THg in liver from tusk sampled in the inner sector of Hardangerfjord is the result of demethylation of MeHg, this can have a direct effect on the interpretation of Hg bioavailability in tusk from different sources. Since we mostly focused on fillet while to compare the bioaccumulated Hg in tusk from different sites and habitats it will be more accurate to calculate the Hg burden in the whole body and then conduct the comparison. This will

be valid for fjords study (Paper II and III), where we compared the industrially polluted area (Sørfjord) with less polluted (Eidfjord) or background Hg level area (inner Sognefjord).

Fish intestine has been shown as an important site for MeHg demethylation (Wang et al., 2017). According to existing knowledge, the iHg produced from demethylation of MeHg either bind to Se or metallothionein for detoxification. It also suggested that iHg can be eliminated from the fish intestine (Peng et al., 2016). If this is the case, then the eliminated part during the life span of individual fish can be considered as additional uncertainty that is out of control. This might be one explanation for why tusk from the most polluted part of Hardangerfjord does not have that much higher Hg levels than the much less polluted areas of Eidfjord and Sognefjord.

5. Conclusions

1) Geographical trends in mercury levels of fish from NEAO

A clear gradual trend of Hg increasing concentrations from north to south was identified in several fish species across a wide latitudinal range in NEAO. It was suggested that the trend is mostly driven by gradual increase in temperature and effective light period for primary production towards south and this trend was independent of Hg contamination in sediment. Generally, fish from fjords and coastal areas had higher Hg levels than fish collected from offshore areas, probably due to high runoff from catchment areas bringing organic matter and atmospherically transported Hg.

2) Mercury contamination in Hardangerfjord ecosystem (fjord with point source of mercury pollution)

Legacy Hg from a point source is still present in the Hardangerfjord ecosystem and methylated in the sediment. Mercury concentrations in seawater, sediment, fish and crustaceans were high close to the point source at the inner most part of Sørfjord, but as high or higher Hg concentration in tusk (*Brosme brosme*) was found in Eidfjord far from PSP with low Hg contamination in the sediment. This showed that atmospherically deposited Hg from the catchment in addition to the industrial point source may be an important source of Hg for biota.

3) Mercury contamination in Sognefjord (fjord with no major mercury pollution)

Investigating the fjord with a well-known point source of pollution (Hardangerfjord) and comparing that with fjord with no major source of pollution (Sognefjord), provided valuable insight into the major drivers of bioavailability and biogeochemistry of Hg. Tusk from Sognefjord had elevated THg concentrations with mean values above EUML at most sites, although Hg concentrations in sediment were very low (background level). The accumulated Hg in tusk fillet gradually increased from offshore towards the coast and outer and inner fjord, and it was independent from Hg levels in sediment, which were very low. Source of energy in the food web, investigated using $\delta^{13}C$, varied in a linear gradient from offshore towards inner Sognefjord and explained the majority of

the THg variation in tusk. This indicates that terrestrial carbon has a significant effect on mercury bioaccumulation in the food web of fjord ecosystems. The findings of this study strongly suggest a link between terrestrial organic matter and MeHg bioaccumulation in a demersal, long-lived, top predator fish. It was postulated that even in deep-water demersal fish, the MeHg accumulation levels are driven by runoff containing MeHg and THg from the atmosphere or formed terrigenously and bound to labile organic matter. These Hg species have high bioavailability and enhanced methylation rates in the inner fjord followed by outer fjord, coast and open ocean.

4) Methylmercury demethylation in tusk

The iHg levels in tusk liver increased gradually in samples collected towards the contaminated sites in Sognefjord. Increased THg levels accompanied with decreased proportion of Hg in the methylated form (%MeHg) in both fillet and liver indicate MeHg demethylation as a mechanism to cope with Hg toxicity.

5) Mercury and selenium co-variation and food safety

Hg and Se concentrations in the majority of samples collected from NEAO correlated positively and average Se:Hg molar ratio was above 1.5. All species had on average HBV_{Se} above 2.1 emphasizing an excess of Se in molar concentration. Surplus Se may reduce MeHg toxicity but the mechanisms involved in this process are not fully understood. Among fish species from NEAO, blue ling had an average Hg level above EUML of 0.5 mg kg^{-1} ww. Methylmercury TWI will be exceeded by intake of two servings of Atlantic halibut, tusk and/or blue ling.

Deep-water fish species including tusk, blue ling and common ling from the entire Hardangerfjord area and European lobster from the inner part of the Hardangerfjord are highly polluted, and the Hg levels are well above the EUML. Tusk and blue ling from inner Hardangerfjord had negative HBV_{Se}. In Sognefjord, tusk collected from seven out of eight sites had Hg levels exceeding the EUML. As a consequence of these results, human consumption advice for tusk from Sognefjord has been issued by Norwegian Food Safety Authority.

6) Mercury trophodynamics in NEAO food webs

Mercury trophodynamics study of different areas of NEAO food web showed that $\delta^{15}N$, as a proxy for trophic position, can explain most of the large variation in fillet THg between fish species from different areas. On the other hand, $\delta^{13}C$ was only correlated with THg in samples from the Barents Sea and Fjords and coastal area food webs. The source of energy to the food web appear to be important drivers of Hg in the fjord and coastal areas and the Barents Sea areas, but not in offshore areas. Trophic magnification slope (TMS), as a proxy for Hg biomagnification rate, and Hg at the base of the food web were different in different part of the NEAO. Samples from the Barents Sea and fjords and coastal area showed lower THg at the base of food web, but higher magnification rates compared with offshore areas.

6. Future perspectives

1) A positive correlation between Hg and Se concentrations was found in the majority of commercially important fish species from NEAO. Except in few highly contaminated sites (e.g. inner part of Hardangerfjord), marine fish from NEAO contained surplus Se to Hg in molar concentration. Although several studies have documented the ameliorating effects of Se against Hg toxicity (Bjerregaard and Christensen, 2012; Bjerregaard et al., 2011; Parizek and Ostadalova, 1967; Ralston et al., 2008; Ralston and Raymond, 2010), it has also been demonstrated that excess Se cannot completely prevent MeHg related toxic effects. Under certain conditions, Se may enhance the toxicity of MeHg, (Lemire et al., 2010; Penglase et al., 2014). In these studies, MeHg chloride and selenite or selenate as Se source of exposure are used. However, in seafood these compounds usually exist in other complex forms such as selenomethionine and MeHg-cysteine or MeHg-selenoprotein. Conducting an experiment using naturally accumulated MeHg and Se in different ratios using animal models and investigating the natural forms of MeHg and Se would contribute to a better understanding of how Se may influence MeHg toxicity in a more relevant food safety context. These findings would have a large effect on seafood safety and security and can adjust the seafood consumption advisories to become more accurate and realistic.
2) Norway is one of the countries having a large proportion of its electricity production from hydroelectric power plants. In many parts of the world, such as Canada and South America (Amazon River basin), the effect of hydropower reservoirs on Hg and MeHg cycle downstream of the reservoirs are well documented. In Norway this remains as an overlooked phenomenon which could play an important role in MeHg accumulation in marine food webs particularly in the fjords and coastal areas. Future research on Hg accumulation in fjords should address the effect of hydropower dams and elaborate their role.
3) In paper II and III we showed that high levels of Hg bioaccumulated in the food web of fjords are linked to fjord characteristics and particularly high amount of

runoff from catchment. Therefore, high levels of Hg in biota from fjords with high runoff, less water exchange and stratified water can be expected. Further investigation of Hg levels in high trophic organisms in other Norwegian fjords along the coast may identify other Hg hot spots. Since locals may eat a lot of fish caught recreationally from fjords, a documentation of the Hg levels and relevant consumption advisories may be needed.

4) In paper III, both THg and MeHg in tusk fillet and liver were analyzed across a gradient of Hg exposure level from offshore to costal North Sea and to outer and inner Sognefjord. We found that the iHg levels in liver increased towards the Inner part where tusk had high Hg levels. This could be explained by demethylation of MeHg in the fish and a subsequent redistribution and storage in liver. We showed that high concentration of iHg in tusk liver from Sørfjord could be due to higher portion of accumulated MeHg being demethylated. Analysis of Hg species in different organs of tusk from Hardangerfjord compared to Sognefjord may contribute to elucidate the MeHg demethylation and the mechanistic interaction of Hg and Se under different environmental contamination levels. Investigating the co-variation of Hg and Se in different organs may shed light on the possible role of Se in MeHg demethylation.
5) Generating data on seafood consumption and more data on nutrients levels in different fish species from geographical areas provides necessary information for better risk-benefit evaluation of fish consumption in Norway and in other countries importing seafood from Norway.
6) In both paper II and III, it was postulated that freshwater runoff is a main driver governing the Hg levels in food webs and top predator fish species. Future studies should emphasize on more investigations on catchment characteristics in connection with the cycle of Hg species to improve the understanding of drivers controlling the Hg levels in food webs. Using parameters such as seasonal variation in DOC and Hg species in runoff and soil, catchment areas of different basins, levels in soils from different basins with a modeling approach may provide useful tools to explain temporal and spatial Hg variations in different habitats and areas of NEAO.

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Paper I

Atabak M. Azad, Sylvia Frantzen, Michael S. Bank, Bente M. Nilsen, Arne Duinker, Lise Madsen, Amund Maage

Effects of geography and species variation on selenium and mercury molar ratios in Northeast Atlantic marine fish communities

Science of the Total Environment 652 (2019) 1482–1496

Science of the Total Environment 652 (2019) 1482–1496

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Effects of geography and species variation on selenium and mercury molar ratios in Northeast Atlantic marine fish communities

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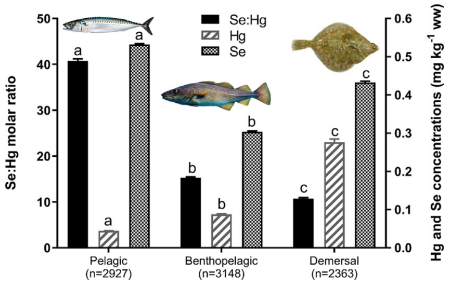
HIGHLIGHTS

- MeHg is the primary contaminant of concern for seafood consumption advisories.
- Selenium and mercury molar ratios were investigated in fish from the North East Atlantic Ocean.
- Hg concentrations in similar species were higher in coastal areas compared to offshore.
- In offshore areas mercury in fish increased from north to south.
- Two servings of tusk, blue ling, and Atlantic halibut exceeded the tolerable weekly intake of MeHg.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 21 July 2018
Received in revised form 26 October 2018
Accepted 29 October 2018
Available online 1 November 2018

Editor: Mae Sexauer Gustin

Keywords:
Mercury
Selenium
Marine
Norway
Seafood safety

ABSTRACT

Methylmercury (MeHg) is a potent neurotoxin that bioaccumulates in seafood. Co-occurrence of selenium (Se) may affect the bioavailability and toxicity of MeHg in organisms. Here we report the concentrations of total mercury (Hg) and Se in 17 teleost fish species (n = 8459) sampled during 2006–2015 from the North East Atlantic Ocean (NEAO) and evaluate species variation and effects of geography. Mean Hg concentration ranged from 0.04 mg kg^{-1} ww in Atlantic mackerel (*Scomber scombrus*) and blue whiting (*Micromesistius poutassou*) to 0.72 mg kg^{-1} ww in blue ling (*Molva dypterygia*). Se concentrations were less variable and ranged from 0.27 mg kg^{-1} ww in Atlantic cod (*Gadus morhua*) to 0.56 mg kg^{-1} ww in redfish (*Sebastes* spp.). The mean Se:Hg molar ratio ranged from 1.9 in blue ling to 43.3 in mackerel. Pelagic species had the lowest Hg concentrations and the highest Se:Hg ratios, whereas demersal species had the highest Hg concentrations and the lowest Se:Hg ratios. Se and Hg concentrations were positively correlated in 13 of the 17 species. Hg concentrations increased from the North to South in contrast to the Se:Hg molar ratio which exhibited the opposite trend. Fish from fjord and coastal areas had higher concentrations of Hg and lower Se:Hg molar ratios compared to fish sampled offshore. All species had average Se:Hg molar ratios >1 and Hg concentrations were largely below the EU maximum level of 0.5 mg kg^{-1} ww with few exceptions including the deep water species tusk (*Brosme brosme*) and blue ling sampled from fjord and coastal habitats. Our results show that two fillet servings of tusk, blue ling or Atlantic

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https://doi.org/10.1016/j.scitotenv.2018.10.405

halibut (*Hippoglossus hippoglossus*) exceeded the tolerable weekly intake of MeHg although the surplus Se may possibly ameliorate the toxic effects of MeHg. However, some individuals with selenium deficiencies may exhibit greater sensitivity to MeHg.

1. Introduction

Seafood is the main dietary source of methylmercury (MeHg) exposure for humans (Berry and Ralston, 2008; Hrenchuk et al., 2011; Rice et al., 2000) and MeHg is a primary contaminant of concern for seafood consumption advisories. During the past 150 years, human activities, mostly gold mining and coal combustion, have dramatically increased the concentrations of anthropogenic mercury (Hg) in the environment, although some recent studies have shown a decreasing trend in atmospheric Hg concentration (Zhang et al., 2016) and in Hg concentrations in fish from the North Atlantic Ocean (Cross et al., 2015; Lee et al., 2016). Hg is a natural element existing in all major compartments of the earth, and can easily be emitted to the atmosphere due to its volatility. Hence, Hg can travel long distances and be deposited from the atmosphere to remote areas (Fitzgerald et al., 1998) and therefore, all organisms are exposed to Hg to some degree (Lorey and Driscoll, 1999; Sonke et al., 2013; Streets et al., 2011).

Fish are mainly exposed to MeHg through their diet (Lindqvist et al., 1991), and factors such as trophic level, age and foraging depth may affect the MeHg concentrations in marine fish (Choy et al., 2009). Further, when species from extensive geographical areas are compared environmental factors that vary across broad spatial areas may influence the overall bioaccumulation regime of marine fish. Temperature is one of the most important environmental parameters that can directly affect MeHg bioaccumulation by increasing the rate of Hg elimination (Trudel and Rasmussen, 2006).

Compared with MeHg, inorganic Hg is assimilated less efficiently from ingested food (Dutton and Fisher, 2010) and the ratio of MeHg to total Hg typically increases with food web position (Lavoie et al., 2013). Heavy metals, as well as other contaminants present in seafood, can accumulate in the human body. High levels of seafood consumption may result in an elevated body burden of MeHg as has been reported for the Seychelles (Davidson et al., 1998), Faroe Islands (Grandjean et al., 1997) and French Guiana (Bourdineaud et al., 2008). Seafood consumption varies within and among European countries and MeHg exposure can be influenced by seafood species specific consumption rates (Agostoni et al., 2014). Hg contamination in seafood is regulated and in Europe the maximum level of Hg has been set by the European Union at 0.5 mg kg^{-1} ww for most of the marine fish species and at 1.0 mg kg^{-1} ww for large predatory species (EU Commission, 2006). The European Food Safety Authority (EFSA) has set the tolerable weekly intake (TWI) for MeHg at 1.3 μg kg^{-1} body weight.

Dietary intake of seafood, in particular fish with high MeHg concentrations may cause adverse effects in humans (Karagas et al., 2012; Oken et al., 2005). Both the Seychelles and the Faroe studies investigated the harmful effects of prenatal and postnatal MeHg exposure in 5.5 and 7 year old children. The Seychelles study found no significant negative effects of either prenatal or postnatal MeHg exposure, but the Faroe study found neurophysiological dysfunctions related to language, attention and memory at comparable MeHg exposure levels (Davidson et al., 1998; Grandjean et al., 1997). Although in Faroe Island, pilot whale is a popular seafood with Se:Hg molar ratio less than one (Julshamn et al., 1987; Ralston et al., 2016). However, the Seychelles Child Development Study was followed up by a cohort study where some delayed neurotoxic effects were found (Davidson et al., 2006). Recently the Seychelles investigators updated the ocean fish consumption effect on the same cohort at 17 years and found consistent positive nutritional effects from prenatal seafood exposure (Davidson et al., 2011). Additionally, other recent epidemiological studies, reported the beneficial effects of fish consumption on child neurodevelopmental outcomes (Avella-Garcia and Julvez, 2014; Golding et al., 2017; Hibbeln et al., 2007; Julvez et al., 2016; Llop et al., 2016).

The trade-off between beneficial nutrients and contaminants is still an issue of significant debate within the scientific community. However, several clinical studies have shown that health benefits from consuming a variety of seafood species in the recommended amounts outweigh the health risks associated with MeHg (Mozaffarian, 2009; Mozaffarian and Rimm, 2006; Mozaffarian et al., 2011). Fish is a high quality protein source and contains relatively high concentrations of long chain polyunsaturated fatty acids (LC n-3 PUFA), including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) with well documented health benefits (Mozaffarian and Rimm, 2006). These include improvement of blood lipid profiles, potential reduced risk of cardiovascular disease, lower potential for high blood pressure and stroke. A balanced seafood diet may also enhance eye and brain development (Dewailly et al., 2003; Ginsberg and Toal, 2009; Virtanen et al., 2008). Moreover, selenium (Se) and Hg co-exposure in seafood is a classic example of the trade-offs between nutrients and the bioavailability of toxic substances. The protective and antagonistic effects of Se against Hg toxicity have been addressed in several studies using Se:Hg molar ratios (Parizek and Ostadalova, 1967; Ralston et al., 2008; Siscar et al., 2014).

The protective effect of Se against Hg toxicity may be linked to different roles of Se including: 1) Hg has a higher affinity for Se than for the thiol group of amino acids (Berry and Ralston, 2008), 2) formation of stable MeHg-selenocysteine compounds may block Se bioavailability due to MeHg exposure and the antioxidant activities of selenoenzymes may be inhibited or lowered. However, available Se from the diet or body supply may compensate for the reduced Se in HgSe or MeHg-selenocysteine and preserve the Se dependent enzyme function in the central nervous system (Peterson et al., 2009; Spiller, 2018), 3) enhance demethylation of MeHg to the inorganic form and redistribution of Hg to less sensitive organs (Spiller, 2018) and 4) a reduction in the Hg uptake in the gastrointestinal tract (Spiller, 2018).

The molar ratio of Se:Hg is suggested as an important human risk factor and a ratio above 1 may provide protection against MeHg toxicity in humans and fish (Burger and Gochfeld, 2012; Peterson et al., 2009; Ralston, 2008). However, due to the biochemical interactions of Se with other components, it is difficult to determine the actual effectiveness of Se amelioration on Hg toxicity in seafood and consumers. The underlying mechanisms of Hg-Se interactions are not fully understood and practical information on the protective ratio is lacking. Still, the Se:Hg molar ratio may provide a relatively more accurate, and physiologically relevant, indicator for MeHg toxicity in the body than MeHg concentrations alone. Recently, a Health Benefit Value of Se (HBV_{Se}) has been suggested as an index to better estimate the health risk associated with Hg reflecting the biochemical mechanisms of MeHg toxicity and the interactions with Se. Thus, fish with positive HBV_{Se} values would provide surplus Se while negative values would indicate a relative deficiency in Se (Ralston et al., 2016).

Here we evaluate variation in Hg and Se concentrations and Se:Hg molar ratios across a latitudinal gradient in NEAO marine fish communities to assess species differences and the effects of geography on Se and Hg dynamics and exposure. We present Hg and Se data from several commercially important fish species in NEAO collected during 2006–2015. To our knowledge, this is the first extensive study analyzing the NEAO marine fish community for Hg and Se from a large sampling area encompassing Arctic, subarctic and temperate zones of the NEAO. Data from this investigation were used to test the following hypotheses

and a priori predictions on length normalized fish concentrations: 1) individuals of the same species inhabiting coastal areas would have greater concentrations of Hg compared to offshore environments, 2) fish species from geographical areas in the southern region of our study area would have greater concentrations of Hg compared to more northerly sampling sites, 3) demersal fish species would have greater concentrations of Hg compared to benthopelagic and pelagic species and 4) concentrations of Hg and Se in fish fillets would be positively correlated across species. We integrate these hypotheses and incorporate them into our interpretations of Se:Hg molar ratios using geography, species variation and coastal vs. offshore habitat comparisons as potential drivers. Additionally, we also conducted an exposure assessment of MeHg based on the European consumption rate of fish species from the NEAO and used TWI metrics established by EFSA.

2. Materials and methods

2.1. Study area and sample collection

Fish samples (n = 8459) comprising 17 commercially important marine teleost species including Atlantic cod (*Gadus morhua*), Atlantic halibut (*Hippoglossus hippoglossus*), Atlantic herring (*Clupea harengus*), Atlantic mackerel (*Scomber scombrus*), blue ling (*Molva dypterygia*), blue whiting (*Micromesistius poutassou*), common ling (*Molva molva*), European eel (*Anguilla anguilla*), European hake (*Merluccius merluccius*), Greenland halibut (*Reinhardtius hippoglossoides*), haddock (*Melanogrammus aeglefinus*), plaice (*Pleuronectes platessa*), pollack (*Pollachius pollachius*), redfish (*Sebastes* spp.), saithe (*Pollachius virens*), tusk (*Brosme brosme*) and wolffish (*Anarhichas* spp.) were collected from Norwegian fisheries areas in NEAO (Table S1; Fig. 1). Fish were sampled using different sampling gears including long line, gill net, purse seine and pelagic trawl between 2006 and 2015 by the authorized Norwegian reference fleet research vessels of the Institute of Marine Research (IMR), Bergen, Norway or local professional fishermen along the coastal areas of Norway. The Hg concentrations of a few fish species including cod, herring and Greenland halibut have been reported previously but without discussion of the selenium content (Frantzen et al., 2015; Julshamn et al., 2013a; Julshamn et al., 2013b; Julshamn et al., 2011; Julshamn et al., 2006). Fish were caught from different parts of NEAO covering most of the important fishing areas (from 22.9°W to 41.6°E and 50.2°N to 75.6°N). The study area is delineated by the Svalbard Islands in the north, Yuzhny Island in the east, Strait of Dover in

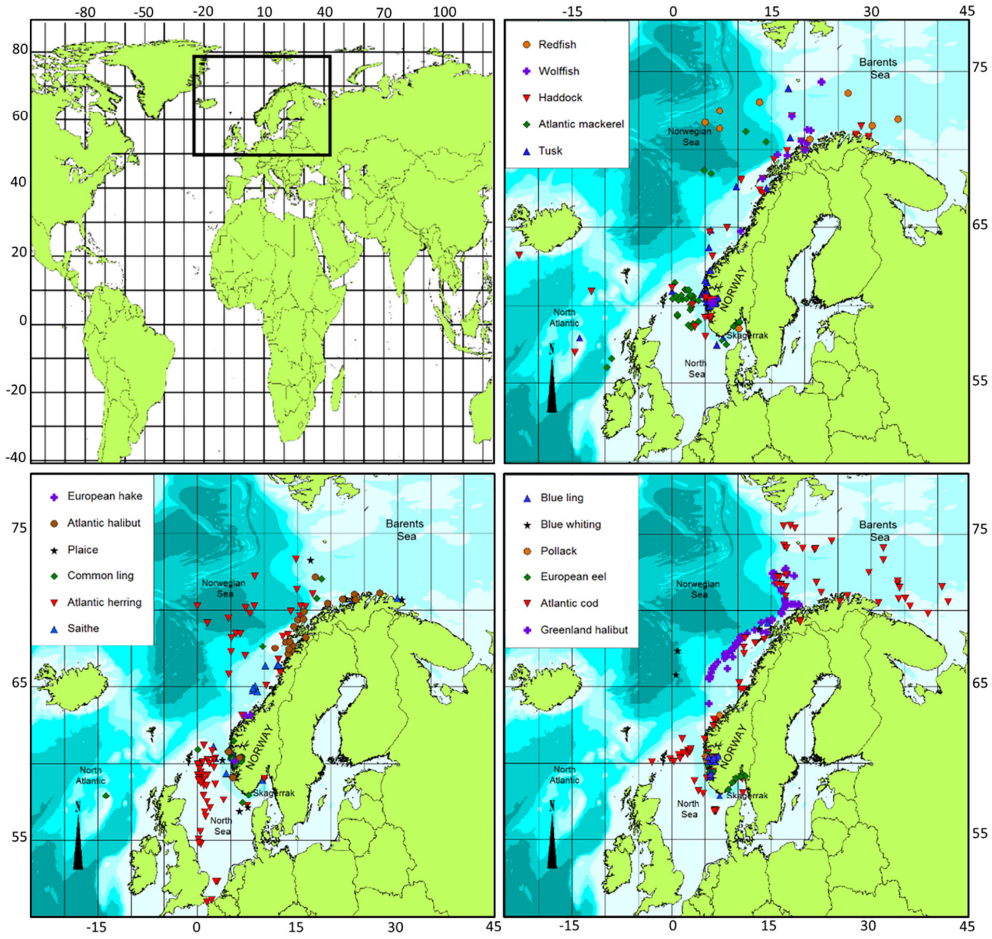

Fig. 1. Sampling sites of fish species analyzed in this study from NEAO collected during 2006–2015. The position of the study area in the world map is highlighted on the top left map in black rectangle. To avoid overlap, different species are showed in three maps.

the south and Iceland in the west, representing a major part of the NEAO (Fig. 1). This large area was divided into 2 primary habitats, 1) offshore ecosystems and 2) fjords and coastal areas. To ease the geographical comparison, the offshore area was divided into five smaller areas including the Barents Sea (BS), the Norwegian Sea (NO), the North Atlantic (NA), the North Sea (NS) and Skagerrak (SK), an arm of the NS. The borders between areas and the study area are described in more detail in the supplementary materials.

2.2. Sample preparation

All fish were shipped whole and frozen to the Institute of Marine Research where individual fish were registered in the Laboratory Information Management System (LIMS) and weight and length were recorded. Hg and Se were analyzed in fillet, since fish fillet is an important storage compartment for MeHg and the main tissue consumed by humans. One side fillet (bone and skin free) was homogenized except for 1) Greenland halibut for which the fillet sample was taken from the upper side of the fish with a cut from the middle of the fish towards the tail (Julshamn et al., 2006) and 2) Atlantic halibut for which the fillet sample was taken from a special cut of the upper part of the pectoral area (i.e., B cut area – see Nortvedt and Tuene (1998) for more details). A subsample was freeze dried and dry matter was recorded as g per 100 g and then samples were ground to a powder before analytical measurements. In the available data there were some composite samples that were excluded from the data set except for common ling, eel, Greenland halibut and tusk (composite samples were 113 of 1968) in order to increase the sampling points and cover larger geographical distribution of those species. The differences in mean and standard error of Se:Hg molar ratio, Se and Hg concentrations (with and without composite samples) for these four species are presented in the Supplementary materials section (Table S2).

2.3. Analytical methods

The concentration of elements was determined using inductively coupled plasma-mass spectrometry (ICP-MS) following microwave digestion. First, weighed samples were digested using concentrated (65%) HNO_3 and 30% H_2O_2 in a microwave oven (Milestone Microwave digestion system MLS-1200 MEGA Microwave Digestion Rotor - MDR 300/10). Hg and Se were determined using quantitative ICP-MS (Agilent 7500 with collision cell and ICP-ChemStation software). A standard curve was used to determine the concentration of Hg and Se. Germanium (Ge), thulium (Tm) and rhodium (Rh) were used either individually or in combination as an internal standard, and gold was added to stabilize the Hg signals. The method is a Nordic and European standard for these two elements (CEN, 2009; NMKL, 2007) and is described in detail by (Julshamn et al., 2007). MeHg was measured using an isotope dilution method and gas chromatography coupled with ICP-MS and details of this method are presented in (Valdersnes et al., 2012).

2.4. Quality assurance

The ICP-MS method is accredited according to ISO 17025 for Hg and Se. The accuracy and precision of the method has been tested by analyzing certified reference materials and the recoveries of both Hg and Se ranged from 80% to 120% for the whole period of analysis (2006–2015). Certified reference materials (CRM) 1566 (oyster tissue) from the National Institute of Standards and Technology (Gaithersburg, USA) and lobster hepatopancreas (TORT-2, TORT-3) from the National Research Council (Ottawa, Canada) were used for measurement quality control by including them in each sample run.

Reproducibility (% RSD) from five day analyses of reference materials showed a variation in the results <10% on analysis values above limit of quantification (LOQ) of the method. The LOQ of the method for Hg and Se were 0.03 and 0.1 mg kg^{-1} dry weight from 2006 until 2010 when the laboratory instrumentation was changed and LOQs were reduced to 0.005 and 0.01 mg kg^{-1} dry weight for Hg and Se, respectively.

The internal method reproducibility for MeHg (RSD) was between 1 and 12% and the *Z*-score for different CRM's was better than |1.5| and the method was validated in different seafood matrices (Valdersnes et al., 2012).

2.5. Mercury in sediment

Hg concentrations in sediment samples collected from NEAO between 62.3 and 76.6°N latitude and 4.3 and 37.2°E longitude have been analyzed in the MAREANO project and was included to determine the spatial distribution of seabed Hg pollution. This data set is accessible online from the MAREANO project website (www.mareano.no downloaded on 07.02.2018 for this study). The sediment samples were collected mostly with a sediment multi-corer and in some cases with Van Veen grab or box corer during 2003–2015. Hg concentrations were measured using Cold Vapor Atomic Absorption Spectrometry (CV-AAS) in freeze-dried samples.

2.6. Statistical analysis

Prior to all correlation and analysis of variance (ANOVA) or analysis of covariance (ANCOVA) tests, outliers were removed from the data using Grubbs test. Outliers were found in 8 of 17 species and in total 21 of 8459 measurements (<1%) were removed as outliers. In order to improve the assumption of normal distribution, all statistical analyses were conducted on log-transformed data (Zar, 2010).

Geographical variation within each species (different offshore areas and offshore versus fjords and coast) were investigated using ANCOVA followed by Tukey unequal sample HSD post-hoc test, with length as a covariate for each species. To show the North-South gradient, least squares means adjusted for length, derived from Generalized Linear Model (GLM) and ANCOVA models, were used. To compare the Se:Hg ratio, Hg and Se concentrations in fish from different habitats, ANOVA was conducted followed by Tukey unequal sample HSD post-hoc test to determine the binary differences between groups. Linear regression tests were used to examine the relationship between Se:Hg molar ratio, Hg and Se concentrations and fish length. Pearson correlation (r) tests were used to examine the relationship between Hg concentrations and latitude of sampling as well as sediment Hg concentration and geographical location expressed as latitude and longitude. Statistical significance was accepted at $P < 0.05$ (Zar, 2010). All statistical analyses were performed using STATISTICA 13 (Statsoft Inc., Tulsa, USA) or GraphPad Prism 7.02 (GraphPad software Inc., San Diego, CA, USA).

2.7. Se:Hg molar ratio calculation

The Se:Hg molar ratio was calculated for all fish individuals. First, the concentration of Se and Hg (mg kg^{-1} ww) were divided by the molar masses 78.96 and 200.59 g mol^{-1} respectively and then the Se:Hg molar ratio was calculated using the following formula:

$$\text{Se : Hg molar ratio} = \left(\text{mmol Se kg}^{-1}\text{ww}\right) / \left(\text{mmol Hg kg}^{-1}\text{ ww}\right)$$

All Se:Hg molar ratio means reported in this study were averaged from specimen values for each species, area and habitat.

2.8. Selenium health benefit value

Selenium health benefit value (HBV_{Se}) has been suggested as an evaluation index showing the Se amount provided in fish after sequestration of

Hg and was calculated using the following formula (Ralston et al., 2016):

$$HBV_{Se} = \frac{Se - Hg}{Se} \times (Se + Hg)$$

Se = Selenium content in molar concentration.
Hg = Mercury content in molar concentration.

3) The amount of fish that can be consumed safely per week was calculated using the following formula:

$$A = \frac{W \times I}{C}$$

A = the amount of fish that can be safely consumed per week (g).
W = average body weight of consumer (70 kg).
I = TWI of MeHg (1.3 μg kg^{-1} body weight).
C = MeHg concentration in fish fillet (mg kg^{-1} ww).

3. Results and discussion

3.1. Inter- and intraspecies variation in Se:Hg molar ratios, Hg and Se concentrations

The mean Hg concentrations ranged from 0.04 to 0.72 mg kg^{-1} ww with the lowest concentration in mackerel and blue whiting and the highest in blue ling (Table 1). Most blue ling were sampled from fjords and coastal areas (55 out of 79) where many sampled individuals had high concentrations of Hg. However, the Hg concentrations, both for arithmetic and length adjusted means, in 12 samples of blue ling from the Norwegian Sea were also higher than the other species from the same area (Table 2; Fig. 2B). Our data show that the observed high concentrations of Hg in blue ling was independent of geography and possibly driven by trophic position or energy sources. Based on average Hg concentrations, we grouped sampled fish into three categories: 1) Highly contaminated species with mean Hg concentration higher than 0.5 mg kg^{-1} ww, i.e. only blue ling. 2) moderately contaminated species with mean Hg concentration between 0.3 and 0.5 mg kg^{-1} ww including Atlantic halibut and tusk, and 3) low contaminated species with mean Hg concentration lower than 0.3 mg kg^{-1} ww, including the rest of species (Table 1).

The mean Se concentrations ranged from 0.27 mg kg^{-1} ww in cod to 0.56 mg kg^{-1} ww in redfish. Hg concentrations exhibited higher variation (~18 fold between the lowest and the highest) than Se concentrations (~2 fold). Similar patterns of variation for Hg and Se have been reported in marine fish from other areas (Burger and Gochfeld, 2012; Polak-Juszczak, 2015). The difference in variation is likely a result of Se being an essential trace element with a regulated pattern of uptake and excretion (Thiry et al., 2012). The range between essential, beneficial and toxic concentrations of Se for living organisms is narrow and in general Se concentrations often tend to show lower overall variability compared to Hg.

Blue ling, tusk and hake had the lowest mean Se:Hg molar ratios of 1.9, 5.1 and 5.4, respectively, whereas mackerel had the highest Se:Hg ratio followed by blue whiting and herring (43.3, 41.6 and 39.3 respectively, Table 1). Variation in Hg concentrations caused most of the variation in Se:Hg ratio for most species, although species such as wolffish, redfish and Atlantic halibut had higher Se:Hg molar ratios as a result of higher Se concentrations (Table 1).

All species showed significant geographical variation ($P < 0.05$). Additionally, individuals from the same species sampled from different offshore areas were also significantly different for Se:Hg molar ratio, and

Table 1
Mean Se:Hg molar ratio, Hg and Se concentrations (mg kg^{-1} ww), HBV_{Se}, Hg intake as percentage of TWI (TWI %), consumption limit per week, landed catch from Norwegian fisheries and percentage of total catch (% Catch) for fish species from NEAO. TWI % and HBV_{Se} were calculated from mean values. Species are sorted according to Hg concentrations. Data are from NEAO sampled during 2006–2015. Colors represent low risk (green), moderate risk (yellow) and high risk (red).

Species	N	Se:Hg molar ratio	Hg	Se	HBVSe	TWI % (2 servings)	TWI % (4 servings)	Consumption limit per week (g)	Landed catch from Norwegian fisheries (in tons, 2017)*	% catch
Blue whiting	75	41.6	0.04	0.48	6.11	15	30	2241	399210	20.6
Atlantic mackerel	1042	43.3	0.04	0.55	7.00	16	32	2114	221588	11.4
Atlantic herring	1810	39.3	0.05	0.52	6.60	17	34	2019	526167	27.2
Plaice	198	23.2	0.06	0.38	4.76	23	45	1510	848	0.04
Haddock	245	17.4	0.07	0.32	3.97	26	52	1317	113776	5.9
Saithe	439	16.9	0.07	0.29	3.59	26	53	1295	177196	9.2
Atlantic cod	2105	16.4	0.08	0.27	3.44	28	56	1208	412441	21.3
Wolffish	89	21.3	0.09	0.44	5.57	35	69	983	6451	0.3
European eel	185	11.2	0.11	0.30	3.73	40	80	851	12	0.001
Redfish	185	22.9	0.13	0.56	7.05	48	96	710	22582	1.2
Pollack	58	8.1	0.14	0.38	4.65	52	104	652	2028	0.1
Greenland halibut	546	10.3	0.14	0.42	5.23	54	108	631	16687	0.9
European hake	92	5.4	0.19	0.34	4.12	72	145	469	5307	0.3
Common ling	294	7.7	0.22	0.41	5.00	82	164	415	18481	1.0
Atlantic halibut	53	9.7	0.38	0.48	5.45	142	283	240	2648	0.1
Tusk	943	5.1	0.44	0.49	5.46	163	327	208	10191	0.5
Blue ling	79	1.9	0.72	0.38	2.09	270	540	126	244	0.01
All species#	8438	17.7	0.17	0.41	5.08	65	130	521	1935857	100

#Means of all species were averaged for Se:Hg molar ratio, Hg and Se and TWI % and safe consumption limit were calculated based on mean of all species.
*Numbers obtained from www.fiskeridir.no.

Table 2
Mean, standard error (SE) and quartile range for Se:Hg molar ratio, Hg and Se concentrations and length of fish species from different areas of NEAO sampled during 2006–2015. Since some species had missing length data, N is presented separately for fish with and without length data.

Species	Area	N[a]	N[b]	Se:Hg molar ratio				Hg (mg kg^{-1})				Se (mg kg^{-1})				Length (cm)			
				Mean	SE	Q25	Q75	Mean	SE	Q25	Q75	Mean	SE	Q25	Q75	Mean	SE	Q25	Q75
Atlantic cod*	BS	507	507	24.7	0.6	15.2	30.5	0.03	0.001	0.02	0.04	0.25	0.002	0.23	0.27	64.8	0.6	55	73
	NO	472	471	21.1	0.6	11.6	25.4	0.04	0.001	0.02	0.05	0.23	0.002	0.21	0.25	65.6	0.5	57	73
	NA	25	25	8.9	0.5	7.1	9.8	0.09	0.01	0.07	0.10	0.28	0.01	0.26	0.28	60.8	0.7	59	62
	NS	490	490	9.4	0.3	5.2	11.7	0.11	0.003	0.06	0.14	0.28	0.002	0.25	0.31	64.8	0.8	50	80
	SK	23	23	8.0	0.8	4.6	10.7	0.16	0.02	0.09	0.19	0.38	0.01	0.34	0.39	53.4	2.3	47	59
	FC	588	588	11.9	0.3	5.8	15.7	0.11	0.004	0.05	0.14	0.31	0.003	0.27	0.36	58.7	0.5	50	67
Atlantic halibut	NO	13	12	15.9	2.6	5.4	20.3	0.20	0.07	0.05	0.24	0.47	0.03	0.40	0.47	96.9	17.6	65	97
	FC	40	9	7.6	1.5	1.8	8.3	0.44	0.06	0.14	0.76	0.48	0.02	0.39	0.55	93.9	11.0	78	93
Atlantic herring*	NO	798	798	51.1	0.9	31.8	66.1	0.04	0.001	0.02	0.05	0.61	0.01	0.51	0.69	31.4	0.1	30	33
	NS	963	960	30.7	0.5	18.3	39.8	0.05	0.001	0.03	0.06	0.46	0.003	0.38	0.51	27.2	0.1	26	30
	FC	49	49	17.4	1.1	11.6	22.2	0.06	0.003	0.04	0.08	0.38	0.01	0.35	0.40	28.1	0.2	27	30
Atlantic mackerel*	NO	77	77	36.8	1.2	31.0	40.6	0.04	0.001	0.04	0.05	0.60	0.01	0.55	0.64	38.4	0.2	38	40
	NA	134	134	29.8	0.9	22.2	36.2	0.06	0.001	0.04	0.07	0.61	0.01	0.53	0.67	35.4	0.2	33	37
	NS	647	647	49.3	1.0	31.9	61.2	0.03	0.001	0.02	0.04	0.54	0.004	0.47	0.60	31.9	0.2	28	36
	SK	184	184	34.7	2.0	14.0	48.6	0.07	0.004	0.03	0.09	0.54	0.01	0.46	0.61	32.8	0.4	28	37
Blue ling*	NO	12	12	3.2	0.3	2.8	3.4	0.27	0.02	0.22	0.32	0.31	0.01	0.29	0.34	93.9	3.9	85	101
	SK	12	12	1.5	0.1	1.3	1.6	0.52	0.03	0.44	0.56	0.29	0.01	0.28	0.30	110.5	1.5	107	113
	FC	55	53	1.8	0.1	1.0	2.3	0.87	0.08	0.49	1.13	0.41	0.01	0.34	0.50	94.5	1.7	87	101
Blue whiting	NO	75	50	41.6	2.0	23.6	56.3	0.04	0.003	0.02	0.07	0.48	0.01	0.41	0.54	22.0	0.4	19	25
Common ling*	NO	75	75	10.1	0.5	7.0	12.1	0.12	0.01	0.08	0.15	0.38	0.01	0.36	0.42	87.9	1.2	81	94
	NA	23	22	5.9	0.4	4.2	7.1	0.25	0.02	0.16	0.35	0.50	0.02	0.44	0.54	76.4	2.1	69	82
	NS	132	106	6.5	0.3	3.8	8.4	0.20	0.01	0.11	0.26	0.39	0.01	0.34	0.42	82.2	1.8	69	93
	FC	64	59	8.0	0.8	2.5	13.1	0.37	0.05	0.08	0.50	0.47	0.01	0.41	0.51	75.8	2.0	68	84
European eel	FC	185	88	11.2	0.8	5.1	13.2	0.11	0.01	0.05	0.15	0.30	0.01	0.18	0.38	58.1	1.2	51	67
European hake	FC	92	92	5.4	0.3	3.9	6.0	0.19	0.01	0.13	0.24	0.34	0.004	0.32	0.37	75.0	1.2	67	81
Greenland halibut	NO	546	525	10.3	0.3	5.7	12.0	0.14	0.004	0.07	0.19	0.42	0.01	0.29	0.47	62.3	0.4	57	68
Haddock*	BS	12	12	17.3	1.6	13.4	19.8	0.04	0.003	0.03	0.04	0.22	0.01	0.20	0.24	56.0	0.8	54	58
	NO	65	65	19.7	1.0	14.2	23.3	0.05	0.004	0.03	0.06	0.29	0.01	0.25	0.32	55.0	0.5	53	58
	NA	24	24	14.0	1.9	6.1	22.9	0.10	0.01	0.05	0.15	0.38	0.02	0.33	0.43	54.8	2.1	49	65
	NS	24	24	6.4	0.7	4.6	6.8	0.15	0.02	0.11	0.18	0.32	0.01	0.27	0.36	53.3	1.0	51	57
	FC	120	120	19.0	1.1	10.7	23.6	0.06	0.004	0.03	0.07	0.32	0.01	0.27	0.38	50.9	0.7	46	56
Plaice*	BS	25	25	29.4	3.1	19.2	36.3	0.06	0.01	0.03	0.05	0.42	0.03	0.31	0.45	42.0	1.0	39	45
	NO	49	24	30.9	2.1	19.1	41.0	0.04	0.004	0.02	0.04	0.31	0.01	0.26	0.35	41.3	0.7	39	43
	NS	124	123	18.9	0.7	13.2	24.1	0.07	0.005	0.04	0.09	0.39	0.01	0.30	0.46	29.3	0.5	26	32
Pollack	FC	58	57	8.1	0.5	5.4	9.9	0.14	0.01	0.10	0.18	0.38	0.01	0.34	0.40	56.5	0.8	53	61
Redfish*	BS	56	56	32.2	2.7	17.2	45.2	0.06	0.01	0.03	0.08	0.54	0.01	0.45	0.62	41.2	0.5	39	44
	NO	123	100	19.7	1.6	7.6	22.5	0.13	0.01	0.06	0.18	0.57	0.01	0.51	0.64	34.4	0.2	33	36
	SK	6	6	2.5	0.6	1.4	2.6	0.67	0.12	0.47	0.94	0.54	0.05	0.46	0.62	29.2	1.2	27	30
Saithe*	BS	48	25	37.5	1.6	30.2	43.7	0.02	0.001	0.02	0.03	0.30	0.01	0.28	0.32	41.9	0.6	40	44
	NO	122	97	11.3	0.7	5.7	14.7	0.11	0.01	0.05	0.12	0.28	0.003	0.26	0.30	60.7	1.2	52	68
	NS	75	50	11.0	0.5	8.6	13.6	0.07	0.004	0.05	0.08	0.26	0.003	0.24	0.28	47.9	0.6	45	51
	FC	194	194	17.6	0.6	11.0	23.1	0.06	0.003	0.03	0.07	0.30	0.004	0.26	0.32	46.8	0.9	37	54
Tusk*	NO	124	124	9.2	0.4	6.8	10.4	0.14	0.01	0.10	0.16	0.42	0.004	0.39	0.44	49.4	0.6	45	54
	NA	25	25	6.5	0.7	3.8	8.4	0.23	0.03	0.12	0.29	0.44	0.01	0.41	0.45	57.0	1.5	50	63
	NS	465	465	5.1	0.1	3.6	6.3	0.27	0.01	0.19	0.34	0.47	0.003	0.42	0.52	49.2	0.4	43	54
	SK	45	45	3.6	0.2	2.6	4.1	0.44	0.03	0.29	0.55	0.53	0.02	0.45	0.58	61.0	1.1	56	66
	FC	284	272	3.4	0.2	1.4	4.8	0.85	0.05	0.24	1.20	0.56	0.01	0.43	0.66	63.0	0.8	54	72
Wolffish	BS	36	36	33.5	4.7	11.6	49.7	0.05	0.01	0.02	0.06	0.41	0.04	0.23	0.53	66.8	1.9	58	77
	NO	51	42	23.4	3.9	6.9	21.6	0.12	0.01	0.06	0.14	0.79	0.13	0.35	0.59	74.3	3.1	60	90
	FC	14	6	29.9	10.2	8.2	38.1	0.13	0.02	0.09	0.19	1.22	0.31	0.31	2.50	66.3	2.4	63	70
All species#	BS	684	661	26.7	0.6	15.9	31.8	0.04	0.001	0.02	0.04	0.29	0.004	0.23	0.30	61.1	0.6	49	70
	NO	2594	2473	27.6	0.5	9.4	39.4	0.08	0.002	0.03	0.10	0.45	0.004	0.28	0.57	50.5	0.4	33	64
	NA	231	231	21.0	0.9	8.3	29.5	0.10	0.01	0.05	0.11	0.52	0.01	0.42	0.61	46.7	1.0	34	60
	NS	2920	2865	24.9	0.47.3	7.3	36.1	0.10	0.002	0.03	0.13	0.44	0.002	0.34	0.52	41.0	0.3	28	49
	SK	270	270	25.0	1.6	5.1	40.3	0.17	0.01	0.03	0.24	0.52	0.01	0.44	0.59	42.6	1.2	29	51
	FC	1739	1591	10.7	0.2	4.0	14.7	0.27	0.01	0.05	0.22	0.37	0.003	0.29	0.42	59.4	0.4	48	69

N[a] number of samples with Se:Hg molar ratio, Hg and Se concentrations data.
N[b] number of samples with length data.
BS: Barents Sea; NO: Norwegian Sea; NA: North Atlantic; NS: North Sea; SK: Skagerrak; FC: fjords and coastal areas.
* Species with significant differences in length between areas (ANOVA-test; $P < 0.05$).
\# Means of individuals.

Hg and Se concentration with the exception of Se concentrations in blue ling and wolffish (ANCOVA; $P < 0.05$: Fig. 2, Table S3). The Se:Hg molar ratio varied between 51.1 in herring from the Norwegian Sea and 1.5 in blue ling from Skagerrak (~34 fold). The mean Hg concentrations varied from 0.02 mg kg^{-1} ww in saithe from the Barents Sea to 0.87 mg kg^{-1} ww in blue ling from fjords and coastal areas (~44 fold). The mean Se concentration varied between 0.22 in haddock from the Barents Sea to 1.22 in wolffish from fjords and coastal area (~6 fold: Table 2).

The highest variation for each species in terms of difference between lowest and highest Se:Hg molar ratio between areas was found in redfish (~12.9 fold) followed by saithe (~3.4 fold) and cod (~3.1 fold, Table 2). Also, Hg concentrations in redfish had the greatest differences between areas (~11.2 fold), followed by tusk (~6.1 fold), saithe (~5.5 fold) and cod (~5.3 fold, Table 2). Redfish also had the highest Hg concentrations among all species from offshore areas (0.67 mg kg^{-1} ww from Skagerrak, Table 2).

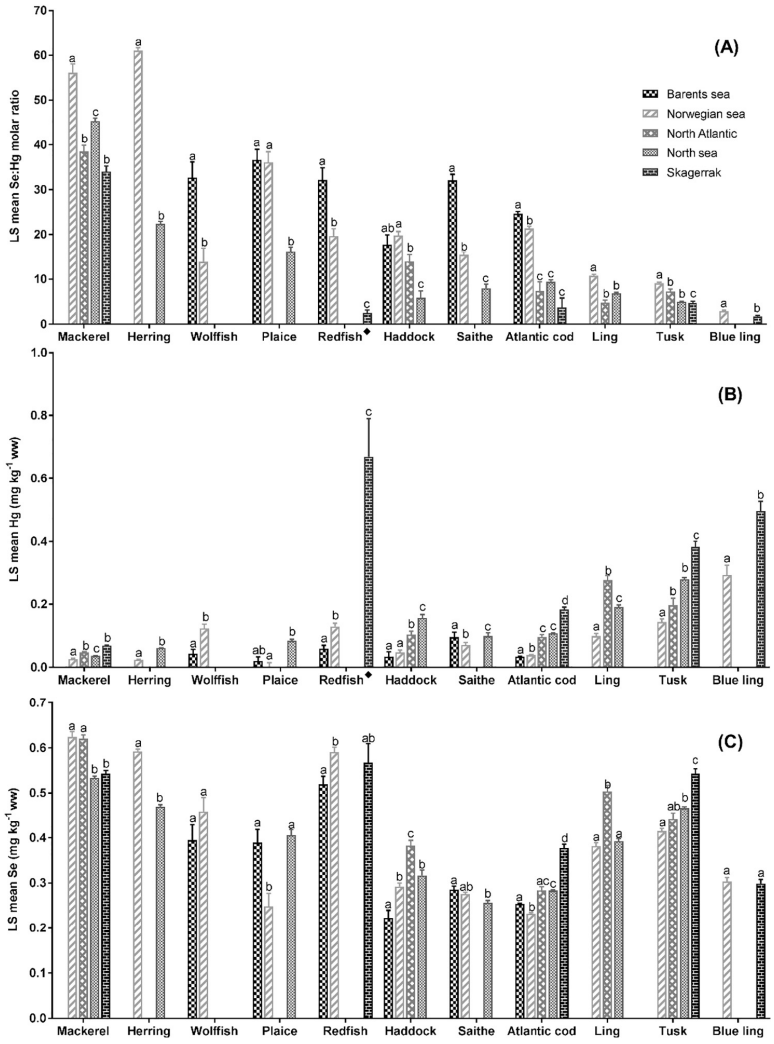

Fig. 2. Least squares mean (length adjusted) of Se:Hg molar ratio, Hg and Se concentrations in fish species from different offshore areas of NEAO sampled between 2006 and 2015. Areas are sorted from north to south. Error bars represent +1 standard error. Post hoc comparison (ANCOVA; $P < 0.05$) between areas are shown by letters above error bars. For redfish, Se:Hg molar ratio and Hg results (♦) are presented as arithmetic means for better graphical illustration since LS means was negative for some areas due to large variation in length of fish between areas and the area with largest fish was lowest in Hg.

Burger and Gochfeld (2012) studied saltwater teleost fish species from the North West Atlantic Ocean (NWAO) and found mean Hg concentrations ranging from 0.01 to 0.52 mg kg^{-1} (Fig. 3), whereas the measured Hg concentrations in species from NEAO, in this study, varied between 0.04 and 0.72 mg kg^{-1}. The Se concentrations in fish from NWAO (0.18–0.48 mg kg^{-1} ww) were lower compared with NEAO (0.27 to 0.56 mg kg^{-1} ww). Burger and Gochfeld (2012) found a mean Se:Hg molar ratio < 5 in fish from NWAO for 11 of 19 species, whereas blue ling was the sole species with a mean Se:Hg molar ratio < 5 in our study, demonstrating that fish with similar Hg concentrations from NWAO had a lower Se:Hg molar ratio (Fig. 3). These results and comparisons suggest that for fish at the same Hg concentration, variations in the Se:Hg molar ratio may also become pronounced when widespread species distributions are considered and evaluated.

3.2. Se and Hg in fish from different NEAO habitats

In order to assess the impact of habitat on Hg concentration, different species were grouped into three major habitat use categories as either pelagic (3 species), benthopelagic (4 species) or demersal (10 species, Table 3). The mean Se:Hg molar ratio, Hg and Se concentrations were significantly different between habitats in all binary comparisons (Se:Hg molar ratio: $F(2, 8435) = 3243.2$, $P < 0.0001$; Hg concentration: $F(2, 8435) = 1846.5$, $P < 0.0001$; Se concentration: $F(2, 8435) = 3083.7$, $P < 0.0001$).

Hg concentrations were observed in the following order for each habitat category: demersal > benthopelagic > pelagic, and demersal fish species on average (0.28 mg kg^{-1} ww) had about three times higher Hg concentrations than benthopelagic species (0.09 mg kg^{-1}

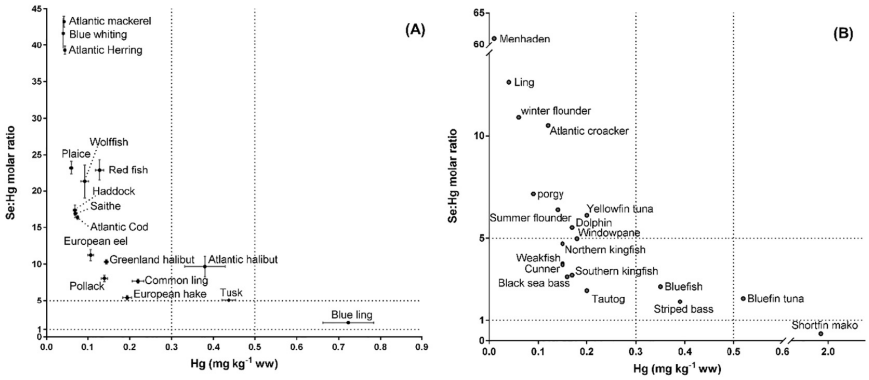

Fig. 3. Relationship between mean Se:Hg and mean Hg in fish from NEAO sampled during 2006–2015 (A) and in fish from NWAO redrawn from Burger and Gochfeld, 2012 (B). The vertical lines are placed at 0.5 and 0.3 mg kg^{-1} ww, the EU and the US maximum levels for Hg in muscle meat of most fish species. The horizontal lines are placed at 1, where below this value Hg exceeds the Se in mole and the suggested safe ratio, and 5 for comparative purposes. Error bars represent ±1SE for both axes.

ww) and more than six times higher than pelagic species (0.04 mg kg^{-1} ww). The Se:Hg molar ratio followed the opposite order of Hg concentration. Pelagic species had the highest ratio (40.8), >2.5 times higher than benthopelagic (15.3) and >3.5 times higher than demersal species (10.7). The Se concentration was highest in the pelagic group (0.53 mg kg^{-1} ww), followed by the demersal (0.43 mg kg^{-1} ww) and benthopelagic group (0.30 mg kg^{-1} ww) and the difference between the highest and lowest groups was <2-fold (Fig. 4). Saei-Dehkordi et al. (2010) measured Hg concentrations in 15 fish species from the Persian Gulf and reported the highest concentrations in demersal species (similar to this study) and lowest in benthopelagic, while pelagic species were intermediate. The pelagic group in the Persian Gulf included high trophic level and predatory species such as Spanish mackerel (*Scomberomorus commerson*), barracuda (*Sphyraena jello*), cobia (*Rachycentron canadum*) and long tail tuna (*Thunnus tonggol*), whereas pelagic species in this study mostly comprised low trophic level species such as mackerel, blue whiting and herring. Thus, variation observed between different habitats may likely be more related to the differences in life histories and trophic position of fish from different habitats than from a habitat effect alone. In general, food sources, and hence contaminant concentrations, vary in different marine habitats and geographical areas. In NEAO, pelagic species are mostly zooplankton feeders and at the lowest trophic level among fish species (Bachiller et al., 2016), while demersal species mostly include more long lived and deep water dwelling predatory species that feed on other fish species with some degree of cannibalism (Jaworski and Ragnarsson, 2006). Although some demersal species like plaice feeds on benthic invertebrates and thus belongs to a lower trophic position (McMeans et al., 2010).

The effect of forage depth was not investigated in this study, but species such as tusk, common ling, blue ling and Greenland halibut, having some of the highest Hg concentrations (Table 1) all inhabit deep sea environments (>150 m). The effect of forage depth on Hg accumulation in marine fish from different ecosystems has been reported in previous studies (Choy et al., 2009; Madigan et al., 2018; Magalhães et al., 2007). These studies showed that Hg concentrations were higher in species and individuals feeding at greater depths.

3.3. Se and Hg antagonism in fish species from NEAO

Mean Se and Hg concentrations showed weak to moderate positive correlation (Pearson r range = 0.24 to 0.70) in most species (13 of 17 species), while no significant correlation was observed in mackerel, herring, saithe or pollack (Table S4, Fig. S1). The strongest correlation was found in blue whiting, caused by two separate batches of samples

Table 3
Percent of specimens with Se:Hg molar ratio of 0–1, 1–5 or > 5 and Hg concentration (mg kg^{-1} ww) ≥0.3 or ≥0.5. Habitat data are collected from www.imr.no and www.fishbase.com. The species are sorted based on Hg concentration. Data are from NEAO sampled during 2006–2015.

Species	N	Habitat	Se:Hg molar ratio			Hg concentrations (mg kg^{-1} ww)	
			0–1	1–5	>5	Hg ≥ 0.3	Hg ≥ 0.5
Blue whiting	75	Pelagic	0.0	0.0	100.0	0.0	0.0
Atlantic mackerel	1042	Pelagic	0.0	0.6	99.4	0.0	0.0
Atlantic herring	1810	Pelagic	0.0	0.1	99.9	0.1	0.0
Plaice	198	Demersal	0.0	2.0	98.0	0.5	0.0
Haddock	245	Demersal	0.0	6.5	93.5	1.2	0.0
Saithe	439	Benthopelagic	0.2	9.8	90.0	0.9	0.7
Atlantic cod	2105	Benthopelagic	0.0	11.4	88.6	1.8	0.1
Wolffish	89	Demersal	0.0	4.5	95.5	3.4	1.1
European eel	185	Demersal	0.5	22.7	76.8	4.3	0.5
Redfish	185	Demersal	0.0	7.0	93.0	7.6	2.2
Pollack	58	Benthopelagic	0.0	19.0	81.0	1.7	0.0
Greenland halibut	546	Benthopelagic	0.0	17.6	82.4	8.4	1.1
European hake	92	Demersal	0.0	46.7	53.3	9.8	2.2
Common ling	294	Demersal	1.0	31.6	67.3	19.4	7.5
Atlantic halibut	53	Demersal	3.8	49.1	47.2	45.3	34.0
Tusk	943	Demersal	3.9	52.8	43.3	42.1	20.1
Blue ling	79	Demersal	17.7	81.0	1.3	81.0	59.5
All species	8438		0.7	14.2	85.1	8.0	3.5

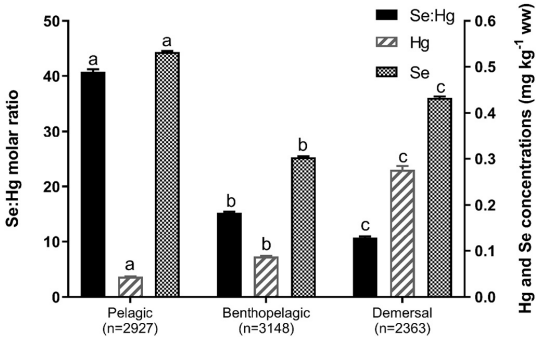

Fig. 4. Mean +1SE of Se:Hg molar ratio (left Y axis), Hg and Se concentrations (right Y axis) in fish species from different habitats of NEAO sampled between 2006 and 2015. Different letters above the columns denote significant differences between habitats (ANOVA; $P < 0.05$).

with different sizes (22 vs 30.9 cm) and Hg concentrations driving this correlation. The correlation in each size class, when analyzed separately, was not significant. Excluding blue whiting, the best correlation was found in plaice, tusk and blue ling (Pearson r range = 0.63 to 0.66).

The protective effect of Se against Hg toxicity has been reported in a variety of organisms and is most commonly linked to the antagonistic effect of Hg and Se (Khan and Wang, 2009). If Se plays an important role in ameliorating Hg toxicity due to antagonism between these two elements, a correlation between Hg and Se in the wild species can be expected. This may be due to upregulation of Se to ameliorate the Hg toxicity and to replace the reduced Se body burden after formation of Hg-Se. The other possible reason is that fish receive a significant part of Hg as Hg-Se compounds (methylmercuric selenide and MeHg selenocysteinate, selenoprotein P-bound HgSe clusters) in their diet from consuming lower trophic marine organisms (Khan and Wang, 2009). In species with low concentrations of Hg, particularly the pelagic species, no correlation was observed between Hg and Se concentrations, but a tendency towards stronger correlation was observed when the concentration of Hg was higher. These findings support a possible antagonistic effect of Se against Hg in wild fish species collected from our large study area, indicating a potential interaction between Se and Hg.

As fish and seafood contain both nutrients and contaminants, potential health benefits from the nutrients should be considered simultaneously along with the contaminants. A correlation between Hg and Se at higher concentrations of Hg may have implications for human risk assessment, food security and environmental management. Since Se may ameliorate MeHg toxicity, it is conceivable that the Se:Hg molar ratio may be used as a better indicator when assessing seafood safety that may be more informative than evaluating fish MeHg concentration alone.

3.4. Effects of geography

Nine of the 17 species investigated in this study were sampled from both offshore and fjord and coastal areas of the NEAO, whereas 11 of 17 species were sampled from different offshore areas (Fig. 2, Fig. 5). In most species, fish from fjord and coastal areas had higher Hg concentrations than fish sampled from offshore areas. When offshore areas were compared, fish from the south, i.e. the Skagerrak and the North Sea had higher Hg concentrations than fish from Norwegian Sea and the Barents Sea located in the northerly sector of our study area (Table 2, Fig. 1).

Fish length also varied in 10 of 12 species between geographical areas. The exceptions were Atlantic halibut and wolffish (ANOVA; $P < 0.05$, Table 2). Fish size (length) is a well-established covariate of Hg concentration and the high assimilation efficiency of MeHg (>95%) combined with a very long half-life of MeHg (3.3 years) lead to bioaccumulation of MeHg over time (Van Walleghem et al., 2013). Therefore, MeHg concentrations are expected to be higher in older and larger individuals compared with younger, smaller individuals of the same species.

Hg concentrations increased with length in most species sampled during the investigation (Table S5) while no significant correlations were found for blue whiting, wolffish, plaice and blue ling. When all individuals from all areas were considered, Hg concentration was not correlated with length in plaice and redfish and Hg concentrations decreased with length for these species. However, when linear regression was conducted for different areas separately, Hg concentrations showed an increasing trend with length in all areas for both species (Fig. S2). The Se:Hg molar ratio decreased significantly with length in most species (R^2 between 0.05 and 0.76; $P < 0.05$) except blue whiting, wolffish and eel (no relationship observed). Similarly, when all individuals from all areas were considered, no correlation between the Se:Hg ratio and length was found in plaice and in redfish, the Se:Hg ratio increased with length. However, when areas were analyzed separately, the Se:Hg ratio in both plaice and redfish decreased with length in all areas (Fig. S3). Selenium concentrations increased with length in some species including blue whiting, herring, Greenland halibut and tusk and decreased with length in mackerel, wolffish, haddock, cod, pollack and blue ling. Thus, when comparing Hg and Se concentrations and the Se:Hg molar ratio between areas, fish size was taken into account. In order to remove the effect of size when evaluating geographical trends, least squares means adjusted for mean length of each species were compared using ANCOVA. When comparing fillet Hg concentrations after adjusting for length, there was still a clear gradual increasing trend from north towards south in offshore areas, and Hg concentrations were higher in most species from fjords and coastal areas compared with offshore areas (Figs. 2, 5).

Pearson correlation showed a significant weak to moderate negative correlation (Pearson r range = −0.11 to −0.67) between logHg concentration in fish fillets and sampling latitude in 12 of 13 species (Table S6). The only exception was Greenland halibut, where no correlation was found. In cod and haddock we observed a strong correlation ($r = -0.67$, $P < 0.0001$ and $r = -0.60$, $P < 0.0001$) across a latitudinal gradient of 19.1 and 15.2°, respectively, covering a large range of the study area (Table S6). The slopes of the regression equations were between −0.005 in herring and -0.12 in Atlantic halibut. Se:Hg molar ratio varied significantly in all 11 species when samples from different offshore areas were compared (Fig. 2A), demonstrating a northward gradual increase in Se:Hg molar ratio for all species from NEAO. Se concentrations also varied significantly, but not with a clear latitudinal trend for most species (Fig. 2C) and variations in Se:Hg molar ratios were driven by variation in Hg concentrations rather than Se concentrations.

Se concentrations varied between areas in three different ways. In pelagic species including mackerel and herring, Se concentration varied in the opposite direction of Hg concentration, decreasing from north to south areas. In saithe and blue ling, Se concentrations were unrelated to the Hg concentrations, and in the rest of the species such as wolffish, cod and tusk, Se concentrations followed the Hg concentrations, increasing from north towards south (Fig. 2).

It is important to note that samples investigated in this study were collected over an extensive time period spanning 10 years during 2006–2015. Some studies showed a decline (−2.5% per year) in atmospheric Hg from the North Atlantic during 1990–2009 (Mason et al., 2001; Soerensen et al., 2012). Additionally, a decreasing trend of Hg concentrations is reported in Atlantic bluefin tuna (*Thunnus thynnus*) at −2.4% per year during 2004–2012 (Lee et al., 2016) and in coastal bluefish (*Pomatomus saltatrix*) at approximately −1% per year from 1972 to 2011 (Cross et al., 2015). A large part of the data set presented in this investigation were derived from different baseline studies. However, when samples of each species from different areas were compared, the sampling time overlapped in most cases or the maximum difference in sampling time between areas was only three years. Therefore, sampling in different years was shown to have a negligible effect on Hg variation when fish from different areas were compared.

3.4.1. Mercury in the NEAO environment

In most of the sampled species from NEAO we observed a gradual increasing trend in Hg concentrations from north to south and this may be driven by an increase in effects of populated and industrialized areas in the southern region of our study area (Fig. 1). The Skagerrak and the North Sea are more impacted by industrialization and terrestrial run off in comparison to the more northerly areas such as Barents Sea and the northern Norwegian Sea, which are considered to be more pristine. Thus, the correlation between Hg concentrations in sediment and latitude of sampling location was used as a proxy to evaluate the influence of anthropogenic contamination on Hg concentrations in fish. A very weak correlation (slope = 0.009; $r = 0.11$; $P < 0.0001$; n = 2003) was found between sediment Hg concentrations and latitude (Fig. S4), showing a very small increase towards the north, the opposite trend as found in fish, however this analysis had poor explanatory power

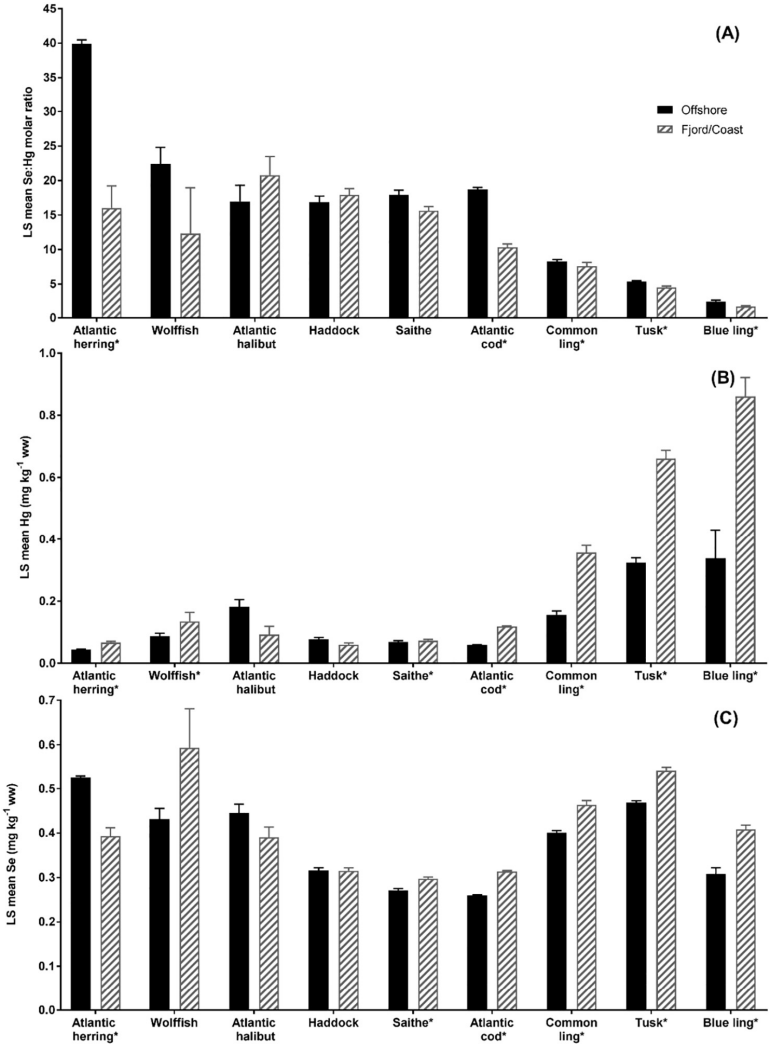

Fig. 5. Least squares mean (length adjusted) of Se:Hg molar ratio, Hg and Se concentrations in fish species from offshore and fjord and coastal areas of NEAO sampled between 2006 and 2015. Error bars represent +1SE. Asterisks (*) indicate species with significant difference between the areas (ANCOVA test; $P < 0.05$).

with only 11% variance explained. Hg concentrations in sediment and longitude were not correlated (slope = 0.0002; $r = 0.008$; $P > 0.05$; n = 2003) (Fig. S4).

Air sea exchange of Hg is considered an important component of the global Hg cycle. It is estimated that the open ocean receives the majority of total Hg input from the atmosphere (Mason and Sheu, 2002; Soerensen et al., 2010). In NWAO, Fitzgerald et al. (1974) investigated the Hg concentration in seawater between Halifax and Bermuda and reported no latitudinal trend in this area. Hg concentration in sediment may reflect Hg concentration in seawater (Gworek et al., 2016) however this relationship is highly variable and inconsistent. No comprehensive study on Hg and MeHg concentrations in NEAO seawater has been undertaken. It is possible that other abiotic and biotic factors rather than environmental Hg concentrations are the main drivers for the observed geographical trends in Hg concentrations in fish species from NEAO.

3.4.2. Latitudinal changes in light and temperature and their effects on Hg concentrations in biota

Photoperiod, sea temperature and photosynthesis dynamics are important environmental parameters that vary across broad latitudinal ranges. In the southern part of the NEAO the planktonic bloom starts earlier in spring than in the northern part. There is a negative correlation between bloom timing and its duration and the blooming period in the northern sector of our study area starts later and is shorter, compared with the southern areas (Friedland et al., 2016).

Thirty-one years of data on seawater surface temperature measurements in the North Atlantic showed a decreasing gradient on both sides

of the Atlantic Ocean (Baumann and Doherty, 2013). In NWAO, the temperature decreased 0.91 °C per degree latitude (in the range of 26–60°N) while in NEAO this decreased only 0.34 °C per degree latitude in the range of 37–70°N on average. Lower temperatures as well as shorter periods of effective light in the northern areas will shorten the period of primary production in which carbon from the environment is pumped into the biomass at the base of the food web (phytoplankton) and may influence MeHg and Hg cycling and biomagnification dynamics. According to the growth bio-dilution theory (Trudel and Rasmussen, 2006), MeHg incorporation from seawater to the first trophic level biomass is higher in southern areas where the production period is relatively prolonged. In northern areas, where the planktonic primary production takes place over a shorter time period but at a higher rate, MeHg incorporation to phytoplankton and biomagnification at higher trophic levels are reduced. Additionally, experimental mesocosm studies on freshwater taxa have shown that increased algal bloom intensity will reduce the MeHg bioaccumulation at higher trophic levels through a bio-dilution effect of MeHg in algae and lead to a two- to three fold reduction in zooplankton MeHg concentration (Pickhardt et al., 2002). During the shorter algal bloom period at the northern latitudes, the primary productivity may be particularly high due to the longer photoperiod. However, MeHg assimilation efficiency will increase in lower temperatures mainly due to lower elimination and longer half-life of MeHg (Lavoie et al., 2013; Trudel and Rasmussen, 2006). In contrast to the findings of this study, this could lead to increasing Hg concentrations northwards, but in NEAO this effect may be confounded by other biological/ecological changes.

In estuarine fish higher temperature has been reported to increase the Hg accumulation in mummichogs (*Fundulus heteroclitus*) in both experimental (12 °C temperature range) and in situ (2.6 °C temperature range) sampling approaches, potentially as a result of increased metabolic rates and energy budgets (Dijkstra et al., 2013). Considering the large variability in seawater temperature between the north and south regions of our study area (NEAO, approximately 8.5 °C; 25° latitude and 0.34 °C change per latitude degree), temperature may be an important driver of increased Hg bioaccumulation in fish samples from the southern region of our study area (Fig. 1).

Fish growth efficiency may also affect Hg concentrations in fish fillets. Higher growth rates and food conversion efficiency have been reported in the Atlantic halibut populations in northern parts of their range compared to southern regions of the NEAO (Jonassen et al., 2000). Counter gradient growth capacity has also been reported in striped bass (*Morone saxatilis*) and Atlantic silverside (*Menidia menidia*) from NWAO as a compensatory mechanism for the short growth period in northern latitudes (Conover et al., 1997; Conover and Present, 1990). Higher growth efficiency in fish from northern areas would result in an increase in body mass from the same amount of ingested food compared with southern areas, which may result in lower MeHg accumulation due to potential bio-dilution effects (Trudel and Rasmussen, 2006; Ward et al., 2010). Considering that most of the Hg is assimilated from food, a higher growth efficiency in the northern areas may lead to lower Hg concentrations for the second trophic level (zooplankton) potentially resulting in lower Hg exposure and bioaccumulation in higher trophic positioned fish from northern areas.

Methylation of inorganic Hg into MeHg is the mechanism that makes it more bioavailable to biota and this process takes place in both sediment and in the open water column (Ullrich et al., 2001). It is also reported from field studies that higher temperatures, as a result of seasonal changes, can increase methylation rates and elevate the concentration of the more labile MeHg (Hammerschmidt and Fitzgerald, 2004; Korthals and Winfrey, 1987; Wright and Hamilton, 1982). Other studies have also shown latitudinal trends with Hg concentration in wild fish populations. Cutshall and Pearcy (1978) reported an increasing trend with latitude, in Pacific hake (*Merluccius productus*) from the North Pacific Ocean, whereas Hall et al. (1976) reported that Hg concentrations decreased towards the north in Pacific halibut (*Hippoglossus stenolepis*) from Washington State towards the Bering Sea in the North Pacific Ocean. Baumann et al. (2017) performed a comprehensive study on bioaccumulation of Hg in Atlantic silverside populations and showed a latitudinal increase in Hg concentration along NWAO coast between 38.4 and 45.2°N, contrary to the findings in this study. The authors suggested that higher ingestion and higher MeHg assimilation are the main reasons for higher Hg in the northern populations. The main difference between this study and Baumann et al. (2017) was different latitudinal ranges. We studied different marine fish species (oceanic) between 50.2°N and 75.6°N whereas they analyzed a low trophic level fish from more southern latitudinal range from 38.4°N to 45.2°N in estuarine habitats, which are dramatically different compared to offshore ecosystems and fjord and coastal areas. We postulate that it is likely that the difference in temperature and light regimes between the extreme north and south sampling were less pronounced in their study area compared with this study and thus higher Hg assimilation efficiency in lower temperature outweighs the other driving parameters in NWAO.

3.4.3. Offshore versus fjord and coastal areas

Nine of the species investigated in this study were sampled in both offshore areas and in fjord and coastal ecosystems. After adjusting for fish length, the Se:Hg molar ratio was significantly higher in the samples of all species from offshore areas than in the same species sampled from fjords and coastal areas except for wolffish, Atlantic halibut, haddock and saithe (Table S7; Fig. 5A). The largest difference between these two areas was found for herring and cod. The samples from offshore areas contained significantly lower Hg concentrations in seven of nine species, except for Atlantic halibut and haddock which were not significantly different. Blue ling, tusk, common ling and cod showed the largest variation in Hg concentrations between offshore and fjord and coastal areas. Se concentrations were higher in fish from fjord and coastal areas in most species (6 of 9 species) and varied mainly in accordance with Hg concentrations. The exceptions were for herring from offshore areas which contained higher Se and Atlantic halibut and haddock where no significant differences were found (Table S5; Fig. 5).

In general species such as herring, with low Hg concentrations, had large differences in their Se:Hg ratios as Se concentrations varied in the opposite direction as the Hg concentrations. In species with higher Hg contamination, such as blue ling, tusk and common ling, Se concentrations varied in the same direction as Hg and were higher in samples from fjord and coastal areas. Thus the Se:Hg molar ratio values did not exhibit considerable variation between offshore and fjord and coastal areas (Fig. 5).

Fjord and coastal areas are more affected by anthropogenic activities than the open ocean due to centralization of industries and households and the fact that in Norway, >80% of population lives <20 km from the coast (NMFA, 2017). Hence, these areas are expected to be more contaminated by Hg than offshore areas. Fjord and coast also receive more runoff from terrestrial catchments and likely deliver more organic matter and atmospherically deposited Hg compared with offshore areas (Everaert et al., 2017; Grigal, 2002). Therefore, more Hg is bound to organic matter in fjord and coastal areas (Jonsson et al., 2014). Fjord and coastal areas have relatively limited water exchange than offshore areas with higher water circulation due to oceanic currents. Furthermore, the addition of organic matter from terrestrial environments and Hg-organic matter compounds may lead to an enhancement of higher Hg methylation (Jonsson et al., 2014). MeHg originating from atmospheric and terrestrial sources has greater bioavailability compared with MeHg produced in marine sediments (Jonsson et al., 2014).

Salinity is an important factor determining Hg methylation in sediment. Within the natural salinity range (0.03–2.4%), Hg methylation may be reduced by more than half in high salinity sediments (Compeau and Bartha, 1987). Additionally, freshwater inputs from terrestrial catchments lead to fjord and coastal areas generally having lower salinity than offshore areas, although the water in deep parts

(below the halocline) may not be lower in salinity but may be influenced by water residence times. Considering all factors, Hg methylation is possibly occurring at a higher rate and may therefore exist in more bioavailable and labile forms in fjords and coastal areas potentially leading to higher MeHg accumulation in fish inhabiting these environments. Additionally, in the inner sectors of fjord ecosystems dissolved oxygen may also be lower compared to the open ocean which may also contribute to enhanced methylation efficiency.

3.5. MeHg to THg ratio

We measured MeHg concentrations in 278 samples comprising five species. The percent mean ± SD of Hg present as MeHg was >93% for all measured species (Greenland halibut 104 ± 12; n = 71, tusk 97 ± 10; n = 118, saithe 93 ± 5; n = 44, cod 104 ± 12; n = 30 and blue ling 100 ± 5; n = 15. These five species represent benthopelagic (cod, Greenland halibut and saithe) and demersal (tusk and blue ling) as well as both lean (cod, tusk and saithe) and oily fish (Greenland halibut). These findings are in good agreement with the general assumption that the MeHg fraction in marine fish fillets is approximately 95% of the measured total Hg (Bank et al., 2007; Bloom, 1992; Razavi et al., 2014). It is well established that the MeHg to THg ratio varies according to the trophic position of marine organisms and that this ratio increases along the food web due to higher assimilation efficiency of MeHg and consumption of more contaminated prey and higher MeHg ratio in higher trophic position organisms has been reported by others (Lavoie et al., 2010; Lavoie et al., 2013). Therefore, THg serves as a good proxy for MeHg in fish fillets from species that inhabit higher trophic positions in marine ecosystems. For exposure assessment, a conservative assumption was made and 100% of THg in fish fillets was assumed to be in the MeHg form.

3.6. Comparison with reference levels

Different reference values for Hg in seafood and fish are set by guidelines authorized by different countries in the world including 0.3 mg kg^{-1} ww in USA (EPA, 2001) and 0.4 mg kg^{-1} ww in Japan (Marumoto and Imai, 2015; Ministry of Health and Welfare, 1973). In the EU the maximum level for Hg in muscle meat from fish for human consumption is 0.5 mg kg^{-1} ww for most fish species including most of those investigated in this study. The exceptions are wolffish, eel, Atlantic halibut and redfish, where the EU maximum level is 1.0 mg kg^{-1} ww (EU Commission, 2006). Among all individual fish investigated, 8.0% and 3.5% contained Hg concentrations equal or above 0.3 and 0.5 mg kg^{-1} ww, respectively (Table 3). None of the samples from herring, plaice, haddock, blue whiting or mackerel had Hg concentrations above 0.5 mg kg^{-1} ww and none of the samples of blue whiting and mackerel had Hg concentrations above 0.3 mg kg^{-1} ww. Blue ling and tusk had the highest portion of specimens with Hg concentrations above the 0.3 and 0.5 mg kg^{-1} ww reference values. For blue ling, 81% and 60% of the fish were above the two reference values, while 42% and 20% of the tusk were above these values respectively.

Se:Hg molar ratio and Se have no regulated reference levels. However, it has been suggested that fish with a molar ratio above 1.0 may be protective, although considerable uncertainties regarding the level of protectiveness still exist (Burger, 2012; Peterson et al., 2009; Ralston, 2008; Ralston et al., 2016). In this study all species had a mean Se:Hg molar ratio above one and considering all individual fish, 0.7% had ratios below one. Only common ling, Atlantic halibut, tusk and blue ling had equal or >1% of samples with a molar ratio below one.

3.7. Hg exposure assessment from NEAO fish consumption

Fish and other types of seafood provide healthy nutrients including essential fatty acids (EFA), and consumption of seafood therefore is advised (Kris-Etherton et al., 2009). The US Environmental Protection Agency (EPA), have issued a recommendation of 340 g seafood consumption per week for pregnant women (EPA, 2004). An EFSA panel on contaminants concluded that consumption of one to two servings of seafood per week in general for adults and three to four servings per week during pregnancy are associated with better health outcomes (Agostoni et al., 2014). Most of the European countries recommend two servings of at least 150 g per week, although the recommended amount varies from 100 g per week up to 200 g per day (Agostoni et al., 2014). Hg exposure was calculated based on two servings of fish (as a general recommendation) equal to 340 g (170 g per serving) fish per week for adults (70 kg) and four servings equal to 680 g of fish consumption for pregnant women (Table 1).

For a person of 70 kg and a consumption of 340 g fish per week, TWI for Hg will be exceeded if the Hg concentration in the fish is higher than 0.27 mg kg^{-1} ww. Thus, considering the average Hg concentration of the fish species analyzed here, two servings of Atlantic halibut, tusk or blue ling and even only one serving of blue ling would lead to a dietary intake of Hg exceeding the TWI (Table 1). Four servings of pollack, Greenland halibut, hake, common ling, Atlantic halibut, tusk and blue ling would lead to Hg intake exceeding the TWI if other sources of MeHg exposure are excluded.

Blue ling and tusk from fjord and coastal areas were the most Hg contaminated species in this study (0.87 and 0.85 mg kg^{-1} ww, respectively, Table 2). One serving of blue ling and tusk from this area per week would lead to Hg intake of 163% and 159% of TWI, respectively. Excluding other factors for MeHg exposure, intake of these species (from fjord and coastal areas) should not exceed 107 and 105 g per week for a 70 kg adult. Considering the geographical variation in Hg concentration in these two species and more sensitive consumers (pregnant women and children) consumption of tusk and blue ling caught from fjords and coastal areas in the south of Norway may lead to high levels of MeHg exposure. However, the Norwegian Food Safety Authority has issued warnings against consumption of deep water species including tusk and blue ling from some of the large fjords in western Norway.

Most of the consumption of fish comes from commercial fisheries, and catch volume of the different species gives some information about the consumption of the different species by the general population. The species with the highest catch volumes, such as mackerel, herring, cod, haddock and saithe, all had relatively low concentrations of Hg, and a 70 kg person could consume more than a kilogram per week of these species without exceeding the TWI (Table 1). The most highly contaminated species constitute a very small portion of the annual catch from NEAO. Atlantic halibut, tusk and blue ling, having mean concentrations of Hg above 0.3 mg kg^{-1}, all constituted <1% of the annual catch. The catch volumes of the species with a risk after four servings per week (pollack, Greenland halibut, hake and common ling) were below 3% in 2017. Therefore, these species were not considered as a great risk to the general consumers at a large scale. However, local recreational fishermen, and their families, living in the fjord and coastal areas catching deep water species such as tusk and blue ling may well exceed the TWI for Hg, and may be considered at risk of greater MeHg exposure if they consume these species regularly.

We next calculated the weekly consumption limits (i.e. the amount that can be consumed without exceeding the TWI, for a 70 kg adult) using the mean Hg values for each species. The consumption limits of blue whiting, mackerel and herring were high (2241 g, 2114 g and 2019 g respectively) whereas the limits for consumption of Atlantic halibut, tusk and blue ling were low (240 g, 208 g and 126 g respectively). These calculations are based on TWI for Hg exposure only. Hence, the concomitant exposure of other potentially associated contaminants in fish such as dioxins, polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers and other persistent organic pollutants (POPs), are not considered here.

Further, as these calculations do not take the interaction between Se and Hg into account we calculated the health benefit value (HBV_{Se}) for each species (Table 1). HBV_{Se} varied between 2.1 in blue ling and 7.1 in

redfish when overall mean concentrations of Hg and Se were used. No negative HBV_{Se} values were found. Hence, all species provided more Se than Hg at the molar concentrations and consumption of these species thus provides a surplus of Se, potentially ameliorating the adverse effects of MeHg. The majority of the epidemiological studies that reported adverse health effects of MeHg due to high levels of seafood consumption, were conducted in populations consuming species with negative HBV_{Se} values. In the Faroes study, pilot whale was a significant part of the diet with a negative HBV_{Se} (−18.6 to −82.3) (Julshamn et al., 1987; Ralston et al., 2016), but in the Seychelles study, where only oceanic fishes were consumed (no marine mammals), with the similar Hg exposure level as the Faroes study, no clear health effect of Hg was found. In a New Zealand study, another cohort study showing negative health effect of maternal Hg exposure in children, shark species with negative HBV_{Se} (−120) was consumed frequently (Ralston et al., 2016). However, several studies have demonstrated positive HBV_{Se} in oceanic fish corresponding to more Se than Hg in molar concentration. Negative values for HBV_{Se} are only reported in pilot whale, mako shark, other shark species and swordfish (Ralston et al., 2016) and it seems there is a connection between consumption of species with negative HBV_{Se} and potential health effects from MeHg exposure.

The Recommended Dietary Allowance (RDA) for Se for adults and pregnant women is 55 and 60 μg day^{-1} respectively and the upper intake level for adults is set at 400 μg day^{-1} (IOM, 2000). Two servings of fish species from NEAO per week would cover 24–49% of the RDA (adults) while four servings of fish with the highest Se concentration is still well below the upper intake assuming all Se intake is from fish.

4. Conclusions

The large variation in Hg concentrations is the main driving factor for the observed level of Se:Hg ratio variability. A gradual increasing trend of Hg concentrations from north to south was observed, where fish from southern areas had higher concentrations of Hg and lower Se:Hg molar ratios compared to fish from northern sectors of the study area. Generally, fish from fjord and coastal areas had higher Hg and therefore a lower Se:Hg molar ratio compared with fish collected from offshore areas. The majority of species sampled in this investigation showed a positive correlation between Hg and Se concentrations and this relationship was strongest for species with higher Hg concentrations. Surplus Se may reduce MeHg toxicity although substantial uncertainty still exists in understanding the relationships between Se and Hg interactions and human health. All species had on average Se:Hg molar ratios above 1.9 and HBV_{Se} above 2.1 emphasizing the excess Se after sequestration of Hg. Generally, fish from NEAO can be considered safe regarding Hg contamination except for some deep water species including Atlantic halibut, Greenland halibut, tusk and blue ling especially fish from southern sections of our study area and fjord and coastal ecosystems. Two servings of Atlantic halibut, tusk and blue ling exceed the Hg TWI and therefore this is an important consideration for children, pregnant women and women of child bearing age. Further research is required to address the detailed mechanisms causing the protective effect of Se on MeHg toxicity in different Se:Hg molar ratios and to achieve more fine scale risk-benefit information from Se:Hg molar ratios with regard to human health risk assessments. Providing more data on fish nutrients and elaborating on the interaction between contaminants and nutrients will improve risk communication and enable authorities to provide more specific and meaningful advisories.

Acknowledgements

The authors wish to thank the technical staffs in sample reception and laboratory departments at the Institute of Marine Research (previously NIFES) for sample collection, preparation and metal analysis. Data presented in this article originated from several biomonitoring projects and several organizations contributed funding including: Norwegian Ministry of Trade, Industry and Fisheries, Norwegian Food Safety Authority, Institute of Marine Research, The Norwegian Seafood Research Fund and the Norwegian Herring Sales Organization. Photos used in the graphical abstract were reprinted with permission Atlantic mackerel (photo credit: Lorentzen E. A.), plaice (photo credit: Kvalsund M.), Atlantic cod (photo credit: Paulsen O.).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.10.405.

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Supplementary materials

Effects of geography and species variation on selenium and mercury molar ratios in Northeast Atlantic marine fish communities

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Table S1. Number of fish samples collected from different geographical areas of NEAO, 2006-2015.

Common Name	Scientific Name	Family	Number of fish samples from different areas						Sampling Date	N
			Barents Sea	Norwegian Sea	North Atlantic	North Sea	Skagerrak	Fjords and Coast		
Atlantic cod	*Gadus morhua*	Gadidae	507	472	25	490	23	588	2009-2011	2105
Atlantic halibut	*Hippoglossus hippoglossus*	Pleuronectidae	---	13	---	---	---	40	2007-2013	53
Atlantic herring	*Clupea harengus*	Clupeidae	---	798	---	963	---	49	2006-2015	1810
Atlantic mackerel	*Scomber scombrus*	Scombridae	---	77	134	647	184	---	2006-2014	1042
Blue ling	*Molva dipterygia*	Lotidae	---	12	---	---	12	55	2011-2015	79
Blue whiting	*Micromesistius poutassou*	Gadidae	---	75	---	---	---	---	2013-2014	75
Common ling	*Molva molva*	Lotidae	---	75	23	132	---	64	2006-2011	294
European eel	*Anguilla anguilla*	Anguillidae	---	---	---	---	---	185	2007-2011	185
European hake	*Merluccius merluccius*	Merlucciidae	---	---	---	---	---	92	2014	92
Greenland halibut	*Reinhardtius hippoglossoides*	Pleuronectidae	---	546	---	---	---	---	2006-2014	546
Haddock	*Melanogrammus aeglefinus*	Gadidae	12	65	24	24	---	120	2009-2015	245
Plaice	*Pleuronectes platessa*	Pleuronectidae	25	49	---	124	---	---	2007-2014	198
Pollack	*Pollachius pollachius*	Gadidae	---	---	---	---	---	58	2009-2014	58
Redfish	*Sebastes* spp.	Sebastidae	56	123	---	---	6	---	2007	185
Saithe	*Pollachius virens*	Gadidae	48	122	---	75	---	194	2006-2015	439
Tusk	*Brosme brosme*	Lotidae	---	124	25	465	45	284	2007-2012	943
Wolffish	*Anarhichas* spp.	Anarhichadidae	36	43	---	---	---	10	2011-2014	89

Border definition of the study areas

Areas are defined according to limits of oceans and seas (1953) with some changes as follows:

Barents Sea is a relatively shallow area that is restricted by Norway and the Kola Peninsula to the south, Novaya Zemlya to the east, Franz Josef Land to the north and Norwegian Sea to the west. The border between the Barents Sea and the Norwegian Sea is a line between North Cape on the Norwegian mainland- Bear Island - South Cape of Spitzbergen.

Norwegian Sea is mainly the deep part of the northeast Atlantic Ocean which is restricted by the North Sea to the south at 62°N, Norway mainland to the east, Barents Sea and Svalbard to the northeast and a line connecting the northern tip of Shetland to the east extreme of Gerpir in Iceland to Jan Mayen island to the South Cape of Spitsbergen from the west and north west.

North Sea is restricted by Germany, Netherland, Belgium and France to the south, the line between Calais (France) – Dover (Britain) to the southwest, Britain to the west, the line between the northern tip of Scotland – Shetland to the northwest, Norwegian Sea to the north and Norway and Denmark to the east. The border between Skagerrak and the North Sea is a line between Lindesnes (Norway) – Hanstholm (Denmark).

Skagerrak is the extension of the North Sea towards the east which is located between Norway, Denmark and Sweden. Skagerrak is restricted by Denmark to the south, the line between Skagen (Denmark) and Paternoster Skær – Tjörn Island (Sweden) to the south east, Sweden to the east, Norway to the north and North Sea to the west.

In this study, we had a few sampling points from the North Atlantic that were located west of the Norwegian Sea and northwest of the North Sea and which bordered Iceland to the north and Britain to the south.

Fjord and Coasts: This area is delineated by a contractual line around the coastal area of Norway defined as straight line segments drawn between points on the outermost headlands and rocks emerged over the ocean at low tide (www.fiskeridir.no).

Table S2. Mean and standard error (SE) of Se:Hg molar ratio, Se and Hg concentrations in species with composite samples. Data are from NEAO, 2006-2015.

Species	Sample Type		Se:Hg molar ratio		Hg		Se	
		N	Mean	SE	Mean	SE	Mean	SE
Common ling	Individual	292	7.7	0.3	0.22	0.01	0.41	0.005
	Individual+composite	294	7.7	0.3	0.22	0.01	0.41	0.005
European eel	Individual	161	11.6	0.9	0.10	0.01	0.28	0.01
	Individual+composite	185	11.2	0.8	0.11	0.01	0.30	0.01
Greenland halibut	Individual	471	9.81	0.3	0.15	0.005	0.42	0.01
	Individual+composite	546	10.32	0.3	0.14	0.004	0.42	0.01
Tusk	Individual	931	5.1	0.1	0.44	0.02	0.49	0.004
	Individual+composite	943	5.1	0.1	0.44	0.02	0.49	0.004

Table S3. ANCOVA analysis output used for comparison of log Se:Hg molar ratio, log Hg and log Se concentrations in fish species from different offshore areas of NEAO sampled between 2006 – 2015.

Species	LogSe:Hg		LogHg		LogSe	
	F (df)	*P*	F (df)	*P*	F (df)	*P*
Atlantic mackerel	112.1 (3, 1037)	<0.0001	135.7 (3, 1037)	<0.0001	38.4 (3, 1037)	<0.0001
Atlantic herring	1979.7 (1, 1755)	<0.0001	1468.6 (1, 1755)	<0.0001	307.2 (1, 1755)	<0.0001
Wolffish	22.3 (1, 76)	<0.0001	45.1 (1, 76)	<0.0001	4.9 (1, 76)	<0.04
Plaice	30.4 (2, 168)	<0.0001	28.2 (2, 168)	<0.0001	14.9 (2, 168)	<0.0001
Redfish	112.5 (2, 158)	<0.0001	115.9 (2, 158)	<0.0001	6.4 (2, 158)	<0.003
Haddock	31.1 (3, 120)	<0.0001	43.7 (3, 120)	<0.0001	25.1 (3, 120)	<0.0001
Saithe	40.2 (2, 168)	<0.0001	29.9 (2, 168)	<0.0001	7.9 (2, 168)	<0.0007
Atlantic cod	425.7 (4, 1510)	<0.0001	522.9 (4, 1510)	<0.0001	146.6 (4, 1510)	<0.0001
Common ling	77.3 (2, 200)	<0.0001	95.6 (2, 200)	<0.0001	32.2 (2, 200)	<0.0001
Tusk	83.6 (3, 654)	<0.0001	114.8 (3, 654)	<0.0001	35.7 (3, 654)	<0.0001
Blue ling	23.0 (1, 21)	<0.0002	15.2 (1, 21)	<0.001	0.08 (1, 21)	NS

Table S4. Pearson correlation between log Se and log Hg concentrations in fish species from NEAO sampled during 2006 - 2015.

Species	N	Slope	r	*P*
Atlantic mackerel	1042	-0.05	-0.02	NS
Blue whiting	75	2.41	0.70	<0.0001
Atlantic herring	1810	0.08	0.04	NS
Wolffish	89	0.54	0.31	<0.01
Plaice	198	1.57	0.66	<0.0001
Redfish	185	1.68	0.32	<0.0001
Haddock	245	0.67	0.24	<0.001
Saithe	439	-0.03	-0.008	NS
Atlantic cod	2105	1.93	0.50	<0.0001
Greenland halibut	546	0.85	0.50	<0.0001
European eel	185	0.87	0.60	<0.0001
Atlantic halibut	53	2.02	0.42	<0.01
Pollack	58	0.45	-0.14	NS
Common ling	294	1.85	0.46	<0.0001
European hake	92	1.66	0.43	<0.0001
Tusk	943	2.44	0.65	<0.0001
Blue ling	79	1.97	0.63	<0.0001

Table S5. Linear regression between length and log Se:Hg molar ratio, log Hg and log Se concentrations in fish species from NEAO sampled between 2006-2015.

Species	N	Log Se:Hg		Log Hg		Log Se	
		R^2	*P*	R^2	*P*	R^2	*P*
Atlantic mackerel	1042	0.18	<0.0001	0.46	<0.0001	0.01	<0.01
Blue whiting	50	0.04	NS	0.03	NS	0.36	<0.0001
Atlantic herring	1807	0.05	<0.0001	0.21	<0.0001	0.19	<0.0001
Wolffish	89	0.02	NS	0.0001	NS	0.05	<0.05
Plaice	172	0.0003	NS	0.0001	NS	0.002	NS
Redfish	162	0.07	<0.001	0.08	<0.001	0.01	NS
Haddock	245	0.10	<0.0001	0.03	<0.01	0.15	<0.0001
Saithe	366	0.54	<0.0001	0.55	<0.0001	0.002	NS
Atlantic cod	2104	0.16	<0.0001	0.11	<0.0001	0.02	<0.0001
Greenland halibut	525	0.09	<0.0001	0.11	<0.0001	0.01	<0.05
European eel	88	0.03	NS	0.06	<0.05	0.01	NS
Atlantic halibut	21	0.76	<0.0001	0.75	<0.0001	0.03	NS
Pollack	57	0.36	<0.0001	0.19	<0.001	0.47	<0.0001
Common ling	263	0.25	<0.0001	0.21	<0.0001	0.001	NS
European hake	92	0.22	<0.0001	0.20	<0.0001	0.01	NS
Tusk	931	0.39	<0.0001	0.38	<0.0001	0.10	<0.0001
Blue ling	77	0.06	<0.05	0.01	NS	0.12	<0.01

Table S6. Pearson correlation between log Hg and latitude in fish species from NEAO sampled during 2006-2015. Species with less than three sampling points or with a sampling area spanning less than 3 degrees were excluded.

Species	N	Slope	r	*P*
Atlantic cod	2105	- 0.039	-0.67	<0.0001
Atlantic mackerel	1042	- 0.008	-0.11	<0.001
Greenland halibut	546	- 0.019	-0.04	NS
Blue ling	79	- 0.035	-0.40	<0.001
Tusk	943	- 0.049	-0.45	<0.0001
Haddock	240	- 0.034	-0.60	<0.0001
Atlantic halibut	53	- 0.118	-0.49	<0.001
Plaice	198	- 0.012	-0.25	<0.001
Saithe	439	- 0.011	-0.13	<0.01
Atlantic herring	1807	- 0.005	-0.12	<0.0001
Redfish	185	- 0.042	-0.27	<0.001
Common ling	294	- 0.016	-0.25	<0.0001
Wolffish	89	- 0.054	-0.48	<0.0001

Table S7. ANCOVA analysis output used for comparison of log Se:Hg molar ratio, log Hg and log Se concentrations in fish species from offshore area versus fjords and coastal area of NEAO sampled during 2006 – 2015.

Species	Log Se:Hg		Log Hg		Log Se	
	F (df)	*P*	F (df)	*P*	F (df)	*P*
Atlantic herring	93.2 (1, 1804)	<0.0001	56.8 (1, 1804)	<0.0001	64.8 (1, 1804)	<0.0001
Wolffish	2.0 (1, 86)	NS	4.7 (1, 86)	<0.05	1.8 (1, 86)	NS
Atlantic halibut	2.1 (1, 18)	NS	4.3 (1, 18)	NS	3.3 (1, 18)	NS
Haddock	1.7 (1, 242)	NS	1.5 (1, 242)	NS	0.005 (1, 242)	NS
Saithe	0.7 (1, 363)	NS	6.4 (1, 363)	<0.02	22.1 (1, 363)	<0.0001
Atlantic cod	345.5 (1, 2101)	<0.0001	481.3 (1, 2101)	<0.0001	367.9 (1, 2101)	<0.0001
Common ling	22.5 (1, 260)	<0.0001	33.4 (1, 260)	<0.0001	36.8 (1, 260)	<0.0001
Tusk	53.9 (1, 928)	<0.0001	78.8 (1, 928)	<0.0001	61.9 (1, 928)	<0.0001
Blue ling	10.8 (1, 74)	<0.01	20.6 (1, 74)	<0.0001	38.4 (1, 74)	<0.0001

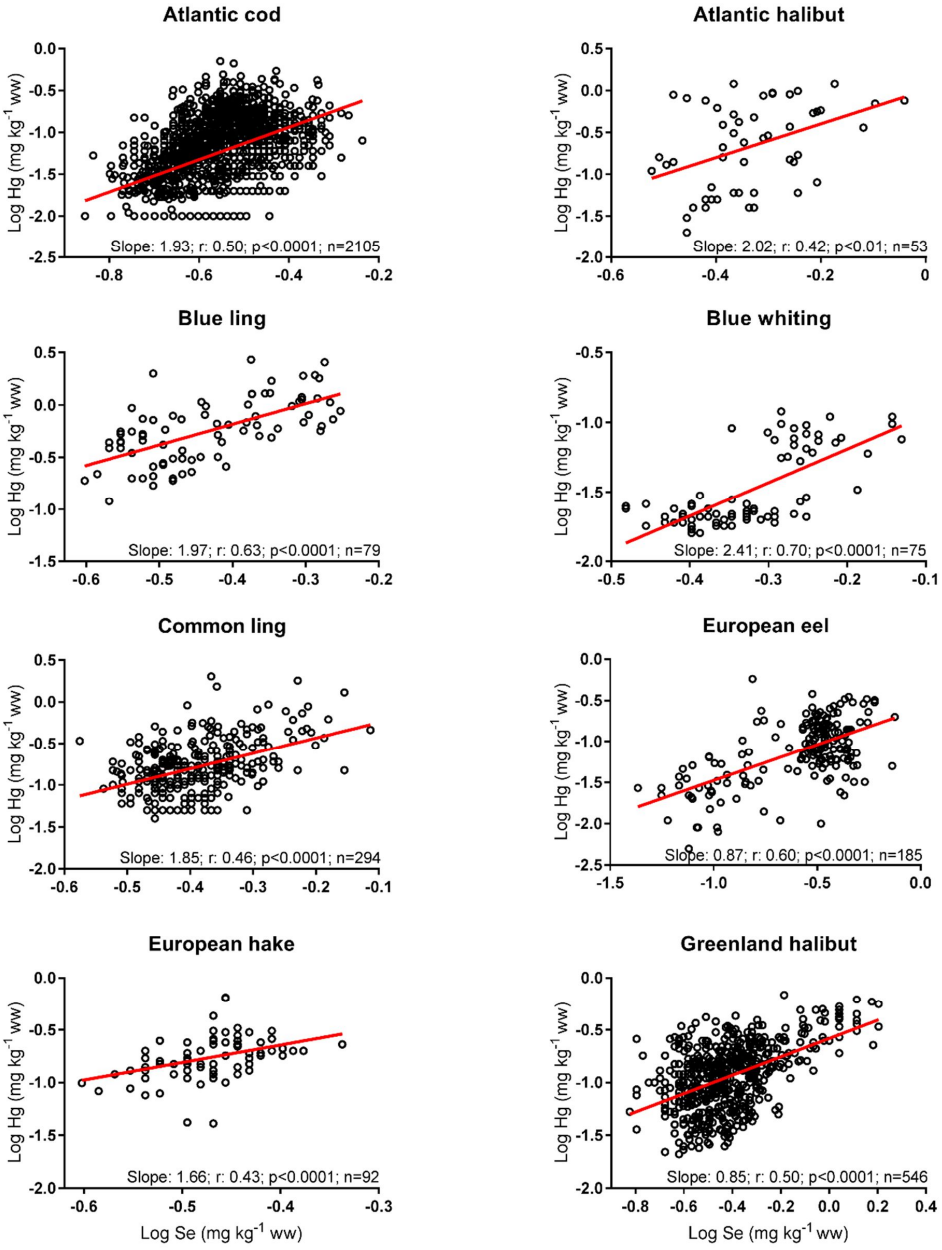

Figure S1. Correlation between log Hg and log Se in different fish species from NEAO sampled during 2006-2015. Each circle represent Hg and Se concentrations (log transformed) of individual fish and the solid red line is the linear fit. Slope, r, *P* and n are presented for each species separately.

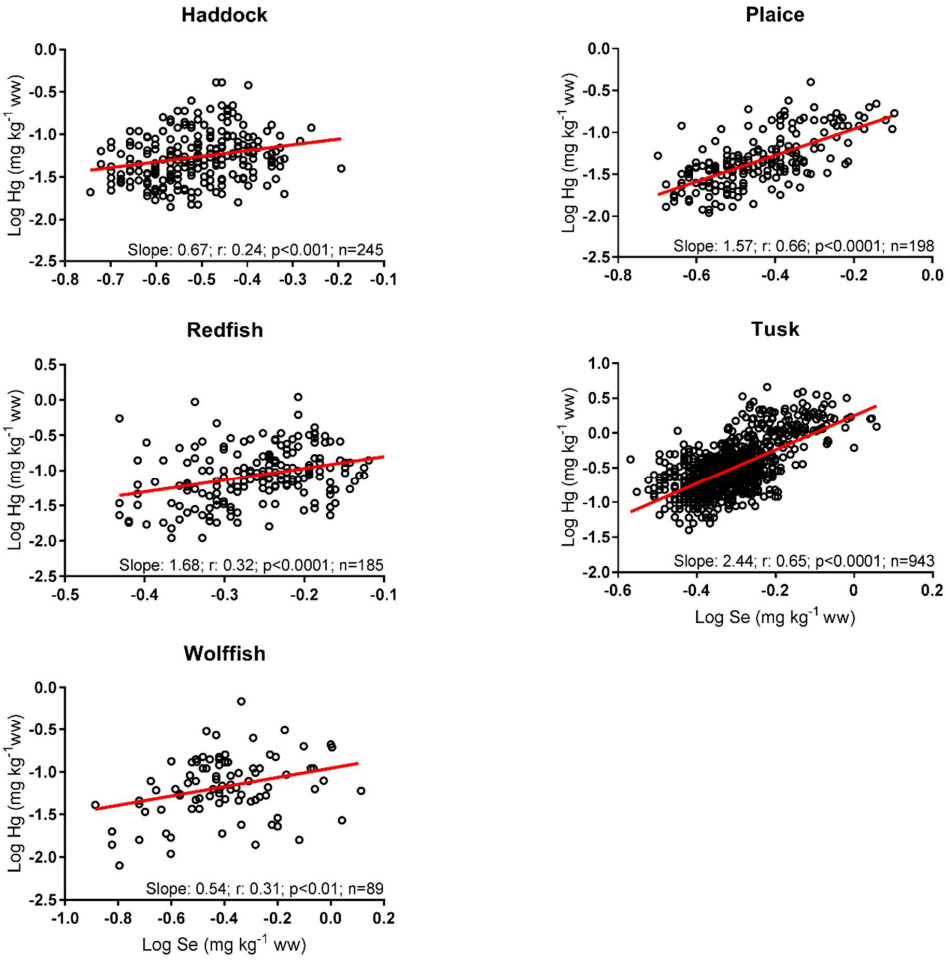

Figure S1. Continued.

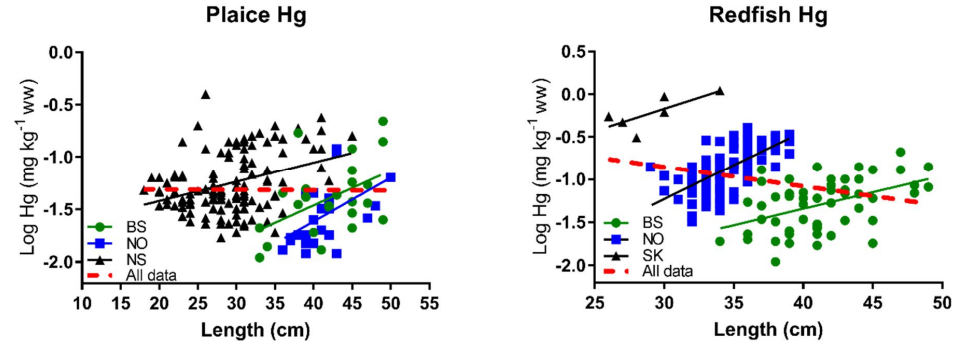

Figure S2. Correlation between log Hg and length in plaice (BS: Slope: 0.03; R^2: 0.23; P<0.05 NO: Slope: 0.04; R^2: 0.26; P<0.05 NS: Slope: 0.02; R^2: 0.12; P<0.0001 All data: Slope: -0.0003; R^2: 0.0001; NS) and redfish (BS: Slope: 0.04; R^2: 0.21; P<0.001 NO: Slope: 0.08; R^2: 0.44; P<0.0001 SK: Slope: 0.05 R^2: 0.55; NS All data: Slope: -0.02; R^2: 0.08; P<0.001). The linear regression line for each area is presented by solid lines of respective color, the regression line for all data together is presented as a dashed red line (BS: Barents Sea, NO: Norwegian Sea, SK: Skagerrak) . Data are from NEAO, 2006-2015.

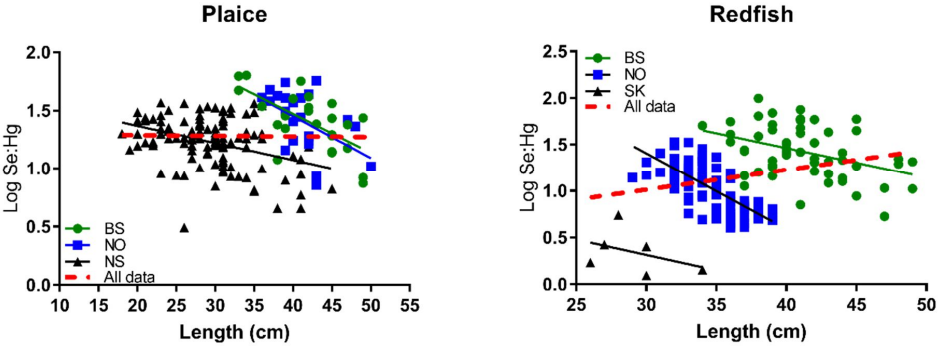

Figure S3. Correlation between Log Se:Hg and length in plaice (BS: Slope: -0.03; R2: 0.43; p<0.001 NO: Slope: -0.04; R^2: 0.24; P<0.05 NS: Slope: -0.01; R^2: 0.14; P<0.0001 All data: Slope: -0.001; R^2: 0.0003; NS) and redfish (BS: Slope: -0.03; R^2: 0.16; P<0.01 NO: Slope: -0.08; R^2: 0.51; P<0.0001 SK: Slope: -0.03 R^2: 0.16; NS All data: Slope: 0.02; R^2: 0.07; P<0.001). The linear regression line for each area is presented by solid lines of respective color, the regression line for all data together is presented as a dashed red line (BS: Barents Sea, NO: Norwegian Sea, SK: Skagerrak) . Data are from NEAO, 2006-2015.

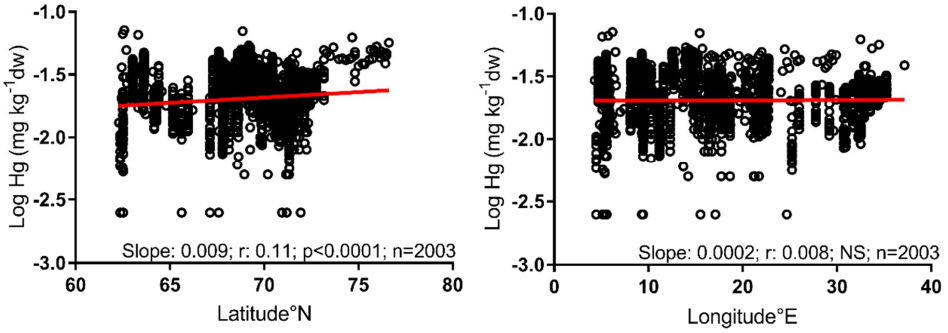

Figure S4. Correlation between latitude (left) and longitude (right) with log Hg concentration in sediment samples from NEAO sampled during 2003-2015. Data was downloaded from www.mareano.no on 07 Feb. 2018. NS = not significant.

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Limits of Oceans and Seas, 3rd edition. International Hydrographic Organization. 1953.

Paper II

Atabak M. Azad, Sylvia Frantzen, Michael S. Bank, Ingrid A. Johnsen, Emmanuel Tessier, David Amouroux, Lise Madsen, Amund Maage

Spatial distribution of mercury in seawater, sediment, and seafood from the Hardangerfjord ecosystem, Norway

Science of the Total Environment 667 (2019) 622–637

Science of the Total Environment 667 (2019) 622–637

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Spatial distribution of mercury in seawater, sediment, and seafood from the Hardangerfjord ecosystem, Norway

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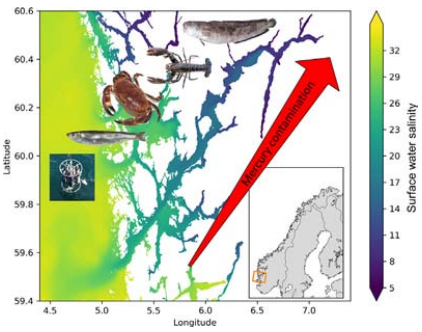
HIGHLIGHTS

- Hardangerfjord is a mercury (Hg) contaminated ecosystem with a legacy point source.
- Hg species were analyzed in seawater, sediment and seafood.
- Hg concentrations in seawater, sediment and biota increased towards the inner fjord.
- Demersal fish from the entire fjord exceeded acceptable Hg limits for human consumption.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 12 February 2019
Received in revised form 22 February 2019
Accepted 22 February 2019
Available online 23 February 2019

Editor: Mae Sexauer Gustin

Keywords:
Mercury
Bioaccumulation
Fjords
Seafood safety
Speciation
Norway

ABSTRACT

Hardangerfjord is one of the longest fjords in the world and has historical mercury (Hg) contamination from a zinc plant in its inner sector. In order to investigate the extent of Hg transferred to abiotic and biotic ecosystem compartments, Hg and monomethylmercury (MeHg) concentrations were measured in seawater, sediment, and seafood commonly consumed by humans. Although total mercury in seawater has been described previously, this investigation reports novel MeHg data for seawater from Norwegian fjords. Total Hg and MeHg concentrations in seawater, sediment, and biota increased towards the point source of pollution (PSP) and multiple lines of evidence show a clear PSP effect in seawater and sediment concentrations. In fish, however, similar high concentrations were found in the inner part of another branch adjacent to the PSP. We postulate that, in addition to PSP, atmospheric Hg, terrestrial run-off and hydroelectric power stations are also important sources of Hg in this fjord ecosystem. Hg contamination gradually increased towards the inner part of the fjord for most fish species and crustaceans. Since the PSP and the atmospheric Hg pools were greater towards the inner part of the fjord, it is not entirely possible to discriminate the full extent of the PSP and the atmospheric Hg contribution to the fjord food web. The European Union (EU) Hg maximum level for consumption was exceeded in demersal fish species including tusk (*Brosme brosme*), blue ling (*Molva dypterygia*) and common ling (*Molva molva*) from

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https://doi.org/10.1016/j.scitotenv.2019.02.352

the inner fjord (1.08 to 1.89 mg kg^{-1} ww) and from the outer fjord (0.49 to 1.07 mg kg^{-1} ww). Crustaceans were less contaminated and only European lobster (*Homarus gammarus*) from inner fjord exceeded the EU limit (0.62 mg kg^{-1} ww). Selenium (Se) concentrations were also measured in seafood species and Se-Hg co-exposure dynamics are also discussed.

1. Introduction

Mercury (Hg) is a widespread global pollutant with significant implications for environmental and public health. Anthropogenic activities, such as emissions from coal-fired plants and mining have significantly increased the concentrations of Hg and monomethylmercury (MeHg) in the environment, including marine ecosystems and their inhabitants (Lamborg et al., 2014). Increased MeHg concentrations in some Arctic marine biota have been reported in comparison to pre-industrial times (Braune et al., 2005), however, the ocean is not uniformly polluted (Lamborg et al., 2014). For example, Vo et al. (2011) reported an increase in MeHg concentrations during a 120-year period in black-footed albatross museum specimens sampled from the Pacific Ocean, but recent studies have reported small-scale temporal declines in MeHg concentrations in coastal and pelagic fish species from the Atlantic Ocean (Cross et al., 2015; Lee et al., 2016). Although air-sea exchange, terrestrial inputs and atmospheric processes are recognized as important drivers of the Hg cycle, numerous important processes governing marine Hg biogeochemical cycling and bioaccumulation have a high degree of uncertainty and remain poorly understood (Strode et al., 2007; Black et al., 2012).

Inorganic Hg may exist in different forms such as elemental Hg, Hg^{2+} inorganic complexes, Hg^{2+} organic complexes, and Hg^{2+} with different degrees of bioavailability. However, inorganic Hg can be methylated by anaerobic, mainly sulfate reducing, bacteria in marine sediments (Compeau and Bartha, 1987) and also in the open water column (Topping and Davies, 1981). MeHg is highly neurotoxic and the most bioavailable form of mercury (Hong et al., 2012). Methylation dynamics and trophic transfer are critical processes involved in MeHg bioaccumulation in coastal and open ocean food webs (Bank et al., 2007; Senn et al., 2010). MeHg easily biomagnifies in the marine food web, and in top predator marine organisms 70 to 100% of the total Hg may be present in the MeHg form (Bloom, 1992; Magalhães et al., 2007; Hong et al., 2012). Fish may bioconcentrate MeHg as much as 10^6-fold compared to low seawater concentrations (Watras and Bloom, 1992).

Atmospheric deposition is considered an important source of Hg to the marine environment (Driscoll et al., 2013). Hg precipitated in terrestrial catchments and transported via run-off can be substantial for aquatic ecosystems including streams, rivers, ponds, lakes, and coastal zones. Although biotic methylation of inorganic Hg in the sediment and in the water column is the primary process governing MeHg, abiotic methylation may also occur, but at a far lower rate (Weber, 1993; Celo et al., 2006). Hg methylation in marine sediments has been shown to be enhanced by anaerobic conditions, increased temperature, decreased pH, and intermediate concentrations of organic carbon (Ullrich et al., 2001). Additionally, organic carbon composition and overall quality (i.e., humic substances content), sulfur availability, and fraction of Hg available for methylation have been shown to have important roles in controlling Hg methylation (Avramescu et al., 2011; Bełdowska et al., 2014; Schartup et al., 2014).

Seafood is the main contributor to MeHg exposure in humans (Batista et al., 1996; Al-Majed and Preston, 2000; Olivero et al., 2002) and the EU maximum level (EUML) of Hg (0.5 mg kg^{-1} ww) applies to most fish and fishery products for legal trade (EC, 2006). The interaction between MeHg and seafood nutrients, particularly selenium (Se), may influence the bioavailability and toxicity of MeHg (Ralston et al., 2008), and it is advantageous to measure and evaluate these elements simultaneously, across fish species, to make accurate decisions pertaining to food safety and human exposure.

The Hardangerfjord ecosystem is one of the longest fjords in western Norway (Fig. 1). The fjord is polluted by industry and other anthropogenic Hg pollution sources, including a zinc plant, hydroelectric power stations, and local mining and aquaculture facilities (deBruyn et al., 2006). The zinc plant has existed for ~100 years and produces zinc and aluminum fluoride at a site located 4 km north of Odda in the inner sector of Sørfjord, an arm of the Hardangerfjord (Fig. 1). Zinc ores typically contain Hg and zinc plants may emit high amounts of Hg to the atmosphere. For instance, it is estimated that approximately 107.7 tons of Hg was emitted to the atmosphere from zinc smelting activities in 2006 in China (Yin et al., 2012). Industrial wastes associated with zinc production with high concentrations of toxic metals were released to Sørfjord until 1986 (Julshamn and Grahl-Nielsen, 1996) even though a mercury removal system was introduced early in the 1970's. In the 1970's, it was estimated that an average of 1–3 kg of solid phase Hg per day was released into the local environment (Skei et al., 1972; Melhuus et al., 1978), most likely as metacinnabar (HgS). In 1986 the company initiated a waste treatment and processing program storing the main tailings and effluents from the zinc plant on land in mountain tunnels. However, the sediments in the inner part of the Sørfjord were already highly polluted with toxic trace metals including Hg, and today the Hardangerfjord ecosystem is still widely considered to be one of the most trace metal polluted fjords in the world (Skei et al., 1972; Everaert et al., 2017).

Early investigations on toxic trace metal contamination in the area focused on zinc (Zn), arsenic (As), cadmium (Cd), lead (Pb), and Hg in marine organisms such as brown algae (*Ascophyllum nodosum*), blue mussel (*Mytilus edulis*), flounder (*Platichthys flesus*) and saithe (*Gadus virens*). Hg received relatively little attention because Hg concentrations were not very high in the investigated species which were from low positions in the food web (Haug et al., 1974; Stenner and Nickless, 1974; Melhuus et al., 1978; Julshamn and Grahl-Nielsen, 1996). Julshamn et al. (2001) reported a significant decrease in toxic trace metals in Sørfjord following the termination of jarosite discharge in 1986, however, the degree of Hg contamination in demersal fish species was unknown. More recent investigations have reported Hg concentrations in fillets of tusk (*Brosme brosme*), inhabiting the demersal habitats of Sørfjord ~3 times greater than the EUML (Ruus and Green, 2007), and additional data (Kvangarsnes et al., 2012) led the Norwegian Food Safety Authority (NFSA) to issue extended consumption advisories for deep-water fish caught in the entire Hardangerfjord ecosystem, as well as for shellfish from the Sørfjord sector.

In this investigation, we focused on evaluating the spatial extent of Hg and MeHg concentrations in several Hardangerfjord ecosystem compartments including marine organisms consumed by humans, seawater, and sediment. We hypothesized that the zinc plant and surrounding highly polluted sediments, as a point source of pollution (PSP), would be an important driver of Hg contamination and spatial distribution in seawater, sediment, and biota. This would result in higher Hg and MeHg levels in the different ecosystem compartments sampled from the inner sector of the fjord compared to the outer sectors. Additionally, we compared our measurements in seafood to the EUML and discuss Se-Hg co-exposure dynamics.

2. Materials and methods

2.1. Study area

The Hardangerfjord ecosystem is the second longest fjord system in Norway, located in the western coastal region (59.4–60.6°N, 4.5–7.3°E;

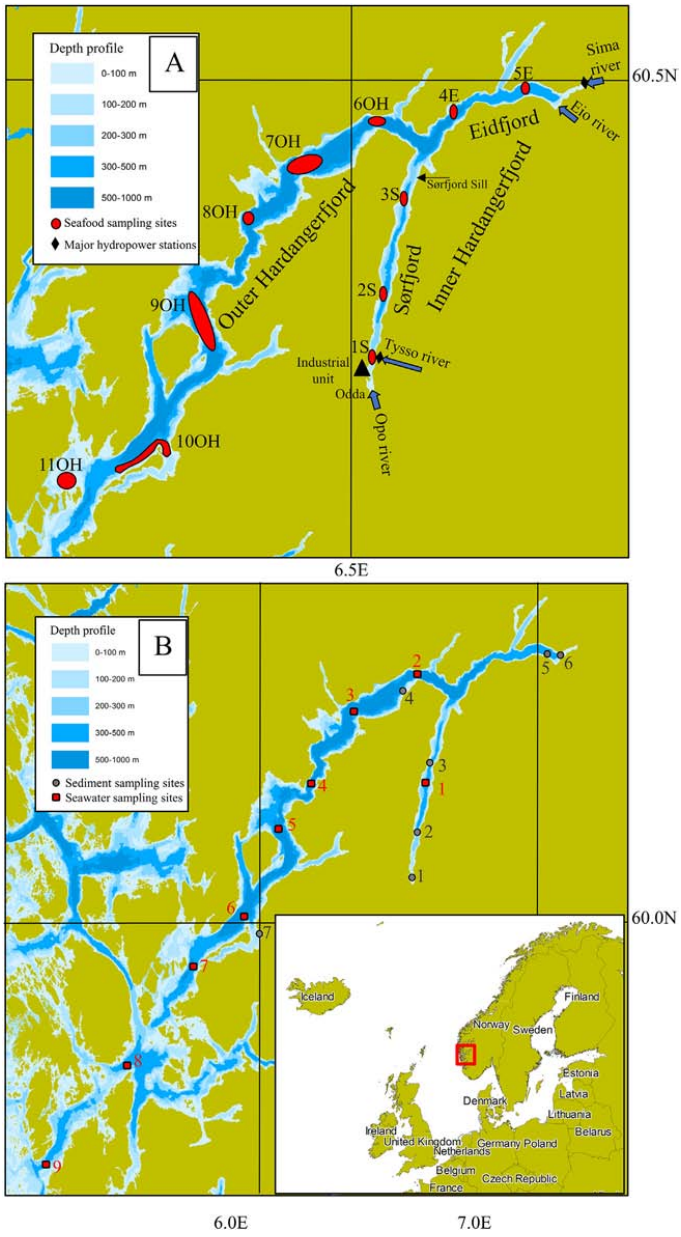

Fig. 1. Location of different sampling sites in Hardangerfjord. (A) Fish and crustacean species sampled in 2011 and (B) sediment and seawater sampled in 2015 and 2018. The letters after site numbers in map A represent the names of the fjords (S: Sørfjord; E: Eidfjord; OH: Outer Hardangerfjord). Details of biotic samples collected from each site are described in Table 1.

Fig. 1). The water depth ranges from 120 to 800 m and the fjord has several basins separated by shallower sills. The fjord ecosystem is connected to the ocean through one main fjord mouth and three narrower channels to the north. At the inner part, the fjord branches into Sørfjord to the south and Eidfjord to the northeast (Fig. 1). The Sørfjord is ~40 km long and up to 1 to 2 km wide and is substantially shallower than the main fjord, with depths of ~100 to 350 m and only ~50 m at the head of the fjord (Fig. 1). The Opo River is the main source of freshwater for Sørfjord. The Opo flows north at the head of the fjord within the Odda municipality (Fig. 1), and has a catchment area of 483 km^2 (Pettersson, 2008). River Tysso, with a catchment area of 390 km^2, is another large river which flows into the southern part of Sørfjord close to the PSP at Tyssedal that also houses a power station (Fig. 1). Eidfjord is the northwards fork extension of Hardangerfjord and is ~29 km long with depths reaching ~400–600 m. The Eio and Sima Rivers are both main sources of freshwater to the Eidfjord sector

of the Hardangerfjord ecosystem (Fig. 1), with catchment areas of 1173 km^2 and 146 km^2 respectively (Pettersson, 2008). Additionally, there is another hydroelectric power station located on the Sima River (Fig. 1). Apart from these four major rivers, the Glacier Folgefonna, consisting of three sub-glaciers with a total area of 200 km^2, is an important source of freshwater along with several other low-order and headwater streams within the catchment area of the fjord.

2.2. Sediment, seawater and seafood sampling and preparation

Fish were caught during cruises organized by the Institute of Marine Research (IMR) as part of a larger Hardangerfjord study. The demersal deep-water fishes blue ling (*Molva dypterygia*) (4 sites) and tusk (*Brosme brosme*) (8 sites) were caught using long line fishing. Common ling (*Molva molva*) (7 sites) and Atlantic wolffish (*Anarhichas lupus*) (2 sites) were sampled using a trammel net and European sprat (*Sprattus sprattus*) (5 sites) were sampled using purse seine nets. Crustacean species including brown crab (*Cancer pagurus*) (2 sites), European lobster (*Homarus gammarus*) (3 sites) and Norway lobster (*Nephrops norvegicus*) (1 site) were caught using lobster trap and trammel nets. All seafood sampling was conducted during 2011 (Table 1 and Fig. 1A). Due to a low number of samples, data for wolffish and Norway lobster were not included in the spatial distribution analyses.

All fish and crustacean specimens were shipped whole and frozen to the Institute of Marine Research, Bergen, Norway. Individual weights (g) and lengths (cm) of fish and crustaceans were measured and registered in the Laboratory Information Management System (LIMS). For all fish species except sprat, skin and bone free fish fillets were dissected. For tusk, we also analyzed liver tissue. For sprat, 25 whole fish were composited and homogenized. For European and Norway lobster, the tail meat was dissected, while for the brown crab, both claw meat (both claws) and brown meat (mixture of hepatopancreas, gonads and internal white meat), were sampled and analyzed. All biota samples were homogenized using a food processor, and all samples, except liver of tusk and brown meat of crab were subsequently lyophilized. After lyophilization to a constant mass, the water content (% moisture) of each sample was calculated and recorded prior to Hg and Se analyses.

Sediment samples (7 sites) were collected from the top 15 ± 2 cm of the bottom sediment using a van Veen grab or by diving. The sediment sampling was conducted during April – July 2015. The samples were frozen (−30 °C) before being sent to the laboratory for analyses.

Seawater samples (9 sites) were collected during May 28–31, 2018 on the *RV* Hans Brattstrøm (Fig. 1B). Seawater was collected using acid-washed Niskin-Type oceanographic general purpose, plastic water samplers (2.5 L model; Hydro-Bios Inc.) at depths of 15, 50, and 300 m. Trace metal clean sampling techniques (Bravo et al., 2018) were employed using acid-washed 120 mL and 250 mL Teflon bottles (Nalgene FEP). Teflon bottles and silicon tubing were acid washed in a Milestone acid-washer using 37% ultra-pure, trace metal grade HNO_3 and were rinsed five times using Milli-Q deionized water. The Niskin type plastic water samples were acid washed using two consecutive overnight, acid baths (1 HNO_3 and 1 HCL at 10% volume:volume prepared with milli-Q water). Teflon bottles and Niskin type bottles were dried in an EPA clean 100 room under a laminar flow hood. Bottles were stored in double plastic bags before and immediately after sampling seawater. Seawater was collected using a standard oceanographic rosette (Hydro-Bios, Inc.) and samples were transferred to individually labeled Teflon bottles using acid-washed silicon tubing that was rinsed between samples with deionized water and stored in a sterile and clean plastic bag. Seawater was then acidified using 0.5% ultrapure HCl (volume:volume) and placed in a dark refrigerator (4 °C) prior to laboratory analyses.

2.3. Total mercury and selenium measurements in biota

The concentrations of Hg and Se were determined using inductively coupled plasma-mass spectrometry (ICP-MS) after microwave digestion. First, weighed samples were digested using concentrated (65%) HNO_3 and 30% H_2O_2 in a microwave oven (Milestone Microwave digestion system: MLS-1200 MEGA Microwave Digestion Rotor - MDR 300/10). Hg and Se concentrations were determined using quantitative ICP-MS (Agilent 7500 with collision cell and ICP-ChemStation software). A standard curve was used to determine the concentration of Hg and Se. Germanium (Ge), thulium (Tm) and rhodium (Rh) were used either individually or in combination as internal standards, and gold (Au) was added to stabilize the Hg signals. The method is a CEN standard and Norway accredited laboratory method (ISO 17025) for these two elements (NMKL, 2007; CEN, 2009) and is described in detail elsewhere (Julshamn et al., 2007). Accuracy and precision of these methods have been tested by analyzing certified reference materials and the recoveries of both Hg and Se ranged from 80% to 120%. Certified reference material (CRM) 1566 (oyster tissue) from the National Institute of Standards and Technology (NIST, Gaithersburg, USA) and lobster hepatopancreas (TORT-2, TORT-3) from the National Research Council of Canada (Ottawa, Canada) were used for measurement quality control by including them in each sample run. The limits of quantification (LOQ) of this method were 0.005 and 0.01 mg kg^{-1} dry weight (dw) for Hg and Se, respectively.

2.4. Mercury speciation in sediment samples

Methylmercury concentrations in sediment samples were measured using EPA method 1630 (USEPA, 1998). Samples were prepared by leaching potassium bromide and copper sulfate solution to release the organic Hg species from inorganic complexes. MeHg was subsequently extracted by dichloromethane. An aliquot of the dichloromethane was then back-extracted into ultrapure deionized water by purging with argon. Samples were treated with sodium tetraethyl borate to form MeHg. Inorganic Hg was simultaneously converted to diethyl Hg. The ethylated Hg species are volatile and are stripped of the solution by purging with N_2 and then adsorbed onto Tenax traps. Hg-species were then thermally desorbed from the Tenax traps in a stream of helium

Table 1
Number of fish and crustacean samples collected from different sites in Hardangerfjord in 2011 (S: Sørfjord; E: Eidfjord; OH: Outer Hardangerfjord). Locations are shown on the map (Fig. 1).

Species	Scientific name	N	Sampling stations (N)									
			1S	2S	3S	4E	5E	6OH	7OH	8OH	9OH	10OH	11OH
Blue ling	*Molva dypterygia*	41			5		2		20	6	7		
Common ling	*Molva molva*	30	1		1			6	3	4	13		2
European Sprat[a]	*Sprattus sprattus*	5				1	1				1	2	
Tusk	*Brosme brosme*	138	2		8	7	24		30		13	32	22
Wolffish	*Anarhichas lupus*	4								3	1		
Brown crab	*Cancer pagurus*	20		10								10	
European lobster	*Homarus gammarus*	26		5						11		10	
Norway lobster	*Nephrops norvegicus*	10										10	

[a] Each sample is a composite of 25 whole specimens.

and separated by means of isothermal gas chromatography. Finally, the methyl/ethylated Hg species are decomposed to elemental Hg and detected using Cold Vapor-Atomic Fluorescence Spectroscopy (CV-AFS) by heating a pyrolysis column to 700–800 °C. The LOQ was 0.05 μg kg^{-1} dw. Total Hg in sediment was measured using laboratory accredited methods (EN ISO12846) and Cold Vapor-Atomic Absorption Spectrometry (CV-AAS) technique (ISO12846, 2012). The LOQ was 0.001 mg kg^{-1} dw and the measurement uncertainty was 20%. The sediment analyses were conducted by Eurofins Environment Testing Norway AS, Moss, Norway.

2.5. Mercury speciation in seawater

Inorganic Hg and MeHg concentrations in unfiltered seawater samples were simultaneously measured using the species-specific isotope dilution, and a GC-ICP-MS method developed for Hg speciation at ultra-trace levels in seawater (Monperrus et al., 2005; Cavalheiro et al., 2016; Bravo et al., 2018). The analyses were operated by a capillary gas chromatograph (Trace GC Ultra, Thermo Fisher, equipped with a TriPlus RSH auto-sampler) hyphenated to an inductively coupled plasma mass spectrometer (ICP-MS Thermo X Series 2). Briefly, an aliquot of 100 mL of unfiltered water sample was accurately weighed and spiked with known amounts and of isotopically enriched standards solutions Me(201)Hg and (199)inorganic Hg (ISC Science, Spain). Spiked samples were left overnight for equilibration in a laminar flow hood. The pH of the solution was then adjusted to 3.9 by adding 5 mL of sodium acetate-acetic acid 0.1 M buffer solution and about 1 mL of ultrapure ammonium hydroxide solution. At last, 250 μL of isooctane (HPLC grade) and 80 μL of sodium tetra-propyl borate solution (5% w/v, Merseburger Spezial Chemikalien, Germany) were added to achieve the derivatization of the Hg species and its subsequent extraction into the GC solvent. The vials were capped and shaken for 20 min at 400 rpm (orbital shaker); then the isooctane was recovered and analyzed in triplicate by GC-ICP-MS.

All materials were cleaned prior to use according to ultra-trace standard operating protocols (Bravo et al., 2018). In absence of any Certified Reference Material available for organomercury species, quality assurance and quality control (QA/QC) was based on reagent blank analyses, replicated assays and an extensive QA/QC procedure described elsewhere (Cavalheiro et al., 2016). Additionally, repeated participations in international inter-laboratory comparison exercises (GEOTRACES intercalibration cruises for Hg species in seawater) complement the QA/QC effort.

Inorganic Hg concentrations measured in the blanks averaged 0.016 ± 0.003 ng L^{-1}, whereas no MeHg was observed in the blanks. The MeHg blank equivalent concentration for the GC-ICP-MS instrument was estimated at 0.002 ± 0.001 ng L^{-1}. The detection limits of this method were 0.03 ng L^{-1} for inorganic Hg and 0.008 ng L^{-1} for MeHg, respectively. The measurement error (calculated by analyzing each sample three times) was <2.9% and 4.9% for inorganic Hg and MeHg concentrations, respectively. All seawater samples were analyzed at the IPREM laboratory (CNRS/University of Pau, France) within 28 days after sampling.

2.6. Salinity measurements and modeling

The salinity was observed in situ using a portable instrument (SAIV A/S SD 208) measuring the conductivity, temperature, and depth (CTD). The instrument was used in STD mode, and calculations of salinity from the conductivity were done automatically using the instrument's software. The accuracy of the salinity is ±0.003 with a range from 0 to 50. The instrument also measured dissolved oxygen (range: 0–20 mg L^{-1} accuracy: ±0.2 mg L^{-1}) supplied by SAIV A/S. The instrument was sampled with a time interval of 1 s and lowered with a speed of 0.2 ms^{-1}. Data was downloaded from the instrument for every 0.1 m in the upper 10 m and for every meter under 10 m depth. In addition to measuring the salinity in situ water was sampled using a multi water sampler slim line 6 with mounted plastic Niskin bottles supplied by Hydro-Bios. Water samples for salinity analyses were taken at a depth of 300 m at every site. The water samples were bottled and analyzed at the in-house salinity lab using a Guildline 8410A portasal (range: 0.004–76, Accuracy: ±0.003). By comparing the in-situ measurements to the salinity data from the seawater samples it became evident that the SAIV SD 208 instrument showed a deviation in its calibration (−0.12) and therefore we used a correction value of +0.12.

The salinity distribution of the fjord was modeled using the Regional Ocean Model System (ROMS) solving the hydrodynamic equations (Haidvogel et al., 2000; Shchepetkin and McWilliams, 2005). The model was set up with a horizontal resolution of 160 m × 160 m, with 35 terrain following coordinates in the vertical. 170 rivers were included with daily run-off from the Norwegian Water Resources and Energy Directorate (NVE) and atmospheric conditions were provided by 2.5 km resolved AROME model provided by the Norwegian Meteorological Institute (http://thredds.met.no). The model was run with an internal time-step of 6 s, writing environmental data as temperature, salinity and currents every hour. Further details of the model setup are described in Albretsen (2011). The model simulation was started 1st of April 2018, where the first month is considered spin-up time. The salinity distribution at the time of the cruise is illustrated as the mean salinity from May 28th to May 31st in 2018, for the sea surface.

2.7. Statistical analyses

Data were log transformed to meet the assumption of normal distribution and homogeneity of variances prior to statistical analyses. Analysis of covariance (ANCOVA) was used for comparison of Hg concentrations across sampling sites for seafood species with length as a covariate to remove the possible effect of length across sites. One-way analysis of variance (ANOVA) was used for crustacean species since length measurements and Hg concentrations were not correlated. In European lobster, the Hg concentrations increased with increasing weights, however since weight was not significantly different between sites, ANOVA was used for comparison across sampling sites. Independent Student's *t*-tests were used to compare length and Hg concentration between the inner and outer sections of Hardangerfjord. For post-hoc comparisons, unequal sample Tukey-HSD tests were used to evaluate the effects of unequal sampling efforts and unbalanced design. Only sites with two or more individuals were considered for spatial comparisons. Distance from PSP was calculated as distance from the industrial unit close to Odda and distance from the open ocean was calculated from the mouth of the Hardangerfjord at Kvinnsvika (Fig. 1). Statistical significance was accepted at $P < 0.05$ (Zar, 2010). All statistical analyses were performed using STATISTICA 13 (Statsoft Inc., Tulsa, USA) or GraphPad Prism 7.02 (GraphPad software Inc., San Diego, CA, USA).

2.8. Selenium health benefit value

Selenium health benefit value (HBV_{Se}) has been suggested as an evaluation index showing the Se amount provided in fish after sequestration of Hg and was calculated using the following formula (Ralston et al., 2016):

$$HBV_{Se} = \frac{Se - Hg}{Se} \times (Se + Hg)$$

Se = Selenium content in molar concentration.
Hg = Mercury content in molar concentration.

2.9. Bioconcentration Factors and Biota-Sediment Accumulation Factors

Bioconcentration Factors (BCF) and Biota-Sediment Accumulation Factors (BSAF) for tusk were calculated for total Hg and MeHg using the following formulas:

$$BCF = Log\left(\frac{Hg\ concentration\ in\ fillet}{Hg\ concentration\ in\ water}\right)$$

$$BSAF = Log\left(\frac{Hg\ concentration\ in\ fillet}{Hg\ concentration\ in\ sediment}\right)$$

BCF was calculated using average seawater concentration from 15, 50 and 300 m depths closest to the tusk sampling location and 100% of Hg in tusk fillet was assumed to be MeHg.

3. Results and discussion

3.1. Hg and Se concentrations in seafood

Tusk and blue ling fillet samples collected from the inner sector of Hardangerfjord had the highest mean Hg concentrations (1.87 and 1.44 mg kg^{-1} ww, respectively) and all individual fish were above the EUML of 0.5 mg kg^{-1} ww (Table 2). In comparison tusk and blue ling samples from outer Hardangerfjord had lower Hg concentrations, but the mean levels were still higher than EUML (mean = 0.84 and 1.07 mg kg^{-1} ww, respectively). Wolffish (0.14 mg kg^{-1} ww) and sprat (0.01 in outer and 0.03 mg kg^{-1} ww in the inner Hardangerfjord) had the lowest Hg concentrations. In a previous study, Azad et al. (2019) showed that Hg concentrations in blue ling and tusk from the Northeast Atlantic Ocean were similarly high, whereas common ling had lower concentrations and wolffish had the lowest of all demersal fish species analyzed in this study. The high concentrations of Hg in tusk, blue ling, and common ling were likely influenced by their high trophic position, and preference for deep-water, demersal habitats (Bergstad, 1991; Husebø et al., 2002; McMeans et al., 2010). Atlantic wolffish feed on molluscs, echinoderms, and other low trophic level prey species (Falk-Petersen et al., 2010), and this may explain their lower Hg concentrations.

Crustaceans had lower concentrations of Hg than demersal fish species (Table 2) likely as a result of their considerably lower trophic position and similar observations have been reported from Spain (Olmedo et al., 2013). European lobster tail meat sampled from inner Hardangerfjord had the highest mean Hg concentration of all sampled crustaceans (0.62 mg kg^{-1} ww). These values are higher than those previously reported in commercially caught European lobster from Scotland (Barrento et al., 2008; Noël et al., 2011). European lobster from outer Hardangerfjord had a mean Hg concentration of

Table 2
Mean, first and third quartiles, standard deviation and standard error of Hg and Se levels (mg kg^{-1} ww) in muscle tissue and length (cm) of demersal fish and crustacean species from the Hardangerfjord ecosystem, 2011. HBV_{Se} are calculated from mean values.

Species	Scientific name	Area	N	Hg (mg kg^{-1} ww)					Se (mg kg^{-1} ww)					Length (cm)					Percent with Hg ≥ 0.5 (mg kg^{-1} ww)	HBVSe
				Mean	Q25	Q75	SD	SE	Mean	Q25	Q75	SD	SE	Mean	Q25	Q75	SD	SE		
Blue ling	*Molva dypterygia*	Out. Hard.	33	1.07	0.64	1.19	0.83	0.15	0.43	0.38	0.49	0.08	0.01	90.73	80.00	97.00	13.65	2.38	93.9	0.4
		Inn. Hard.	8	1.44	1.07	1.85	0.66	0.23	0.50	0.49	0.53	0.04	0.01	92.33	86.00	100.00	8.41	3.43	100	−1.7
Common ling	*Molva molva*	Out. Hard.	28	0.49	0.19	0.59	0.44	0.08	0.47	0.42	0.51	0.08	0.01	73.93	61.50	83.50	18.63	3.52	35.7	5.0
		Inn. Hard.	2	1.08	0.40	1.76	0.97	0.68	0.65	0.43	0.87	0.32	0.22	78.00	72.00	84.00	8.49	6.00	50	4.7
Tusk	*Brosme brosme*	Out. Hard.	97	0.84	0.42	1.11	0.52	0.05	0.59	0.51	0.64	0.11	0.01	64.26	55.00	75.00	13.68	1.39	64.9	5.0
		Inn. Hard.	41	1.89	1.26	2.19	0.89	0.14	0.72	0.57	0.83	0.23	0.04	62.95	55.00	69.00	9.85	1.54	100	−0.6
Sprat[a]	*Sprattus sprattus*	Out. Hard.	3[a]	0.01					0.43					7.27						
		Inn. Hard.	2[a]	0.03					0.42					8.20						
Wolffish	*Anarhichas Lupus*	Out. Hard.	4	0.14	0.11	0.16	0.04	0.02	0.47	0.27	0.66	0.36	0.18	79.00	74.00	84.00	6.32	3.16	0	5.8
All fishes		Out. Hard.	162	0.63					0.49					76.98					48.6	4.1
		Inn. Hard.	51	1.47					0.62					77.76					83.3	0.8
Brown crab	*Cancer pagurus*	Out. Hard.	10	0.12	0.05	0.20	0.08	0.02	1.47	0.92	1.83	0.80	0.25	14.85	14.40	15.40	1.49	0.47	0	18.6
		Inn. Hard.	10	0.22	0.13	0.30	0.14	0.05	0.76	0.60	0.74	0.33	0.10	15.37	13.40	17.60	2.45	0.77	10	9.5
European lobster	*Homarus gammarus*	Out. Hard.	21	0.19	0.16	0.23	0.07	0.01	0.61	0.49	0.65	0.19	0.04	26.55	25.50	27.00	1.48	0.32	0	7.6
		Inn. Hard.	5	0.62	0.63	0.70	0.13	0.06	0.55	0.49	0.65	0.14	0.06	27.80	27.00	30.00	2.77	1.24	80	5.5
Norway lobster	*Nephrops Norvegicus*	Out. Hard.	10	0.20	0.19	0.22	0.03	0.01	0.99	0.87	1.05	0.21	0.07	18.27	16.90	19.30	1.63	0.51	0	12.4
All Crustaceans		Out. Hard.	41	0.17					1.02					19.89					0	12.9
		Inn. Hard.	15	0.42					0.65					21.59					45	7.5
All species		Out. Hard.	203	0.44					0.72					52.51					27.8	7.8
		Inn. Hard.	67	1.05					0.64					55.29					68	3.5

[a] Each sample is a composite of 25 whole specimens and thus, percent exceeding EUML and HBV_{Se} are not calculated.

0.19 mg kg^{-1} ww that is consistent with their reported range. Claw meat of brown crab had lower Hg concentrations than European lobster with mean values of 0.22 and 0.12 mg kg^{-1} ww in samples from inner and outer Hardangerfjord, respectively (Table 2). The Hg concentrations in brown crab samples from outer Hardangerfjord were similar to the mean reported for this species from the Norwegian coast (0.1 mg kg^{-1} ww) (IMR, 2018), whereas Hg concentrations in samples from the inner fjord were ~2-fold higher. Norway lobster was only sampled from outer Hardangerfjord. The mean Hg concentration in tail meat of Norway lobster was 0.20 mg kg^{-1} ww, similar to European lobster and within the range reported for Norway lobsters caught in other regions of Norway (IMR, 2018). The Hg levels in Norway lobster measured in this investigation were lower than the reported levels in samples from the Mediterranean (Cresson et al., 2014). All crustaceans in this study are benthic carnivores (Cristo and Cartes, 1998; Meeren, 2007; IMR, 2008), and the observed variation in Hg concentrations is likely driven by several factors including body size, toxicokinetics, growth dilution, prey type, ecosystem methylation potential, and species migration patterns.

Overall, Se concentrations in all sampled taxa were less variable than Hg concentrations. Se concentrations in fish species from outer Hardangerfjord were ~50% lower compared to crustaceans analyzed from the same area (mean = 0.49 vs 1.02 mg kg^{-1} ww; Table 2). However, Se concentrations in fish and crustaceans sampled from the inner part of Hardangerfjord, where Hg contamination in sediment and seawater was substantially higher, were similar (mean = 0.62 in fishes vs mean = 0.65 mg kg^{-1} ww in crustaceans). Fish Se concentrations were greater in the inner sector of Hardangerfjord compared to the less contaminated areas of the fjord, whereas crustacean Se concentrations were lower in the inner sector (Table 2; Fig. 1).

The liver in fishes and the hepatopancreas in crustaceans both play significant roles in the distribution of toxic trace metals and high concentrations have often been reported (Engel, 1983; Romeo et al., 1999). Tusk liver contained higher concentrations of both Hg (6.39 vs 1.37 mg kg^{-1} ww) and Se (9.95 vs 0.66 mg kg^{-1} ww) in comparison to fillet tissue (Fig. S1; Table 2). Tusk sampled from the inner sector of Hardangerfjord had greater Hg and Se concentrations in liver compared to the outer sector (Hg: 8.14 vs 4.63 mg kg^{-1} ww; Se: 10.42 vs 9.48 mg kg^{-1} ww). However, brown meat of crab (a mixture of hepatopancreas, gonad and internal connective tissue) sampled from the inner sector had higher Hg concentrations (0.16 vs 0.06 mg kg^{-1} ww), whereas Se concentrations were 42% lower compared to the outer fjord area (0.83 vs 1.42 mg kg^{-1} ww).

Se concentrations increased concomitantly with Hg concentrations in all fish species and Pearson's correlation coefficient ranged from $r = 0.36$ in common ling to $r = 0.49$ in tusk. Similar findings have been reported in several fish species from the Northeast Atlantic Ocean (Azad et al., 2019). Crustacean Se concentrations in muscle varied in the opposite direction of Hg and decreased slightly with increasing Hg concentrations and no significant correlation was observed (Fig. 3). Similarly, hepatic Hg and Se concentrations in tusk increased concomitantly ($r = 0.73$; $P < 0.0001$). However, no correlation was found between Hg and Se concentrations for brown crab hepatopancreas (Fig. S2). Collectively, these findings suggest an organ specific distribution pattern in fish and crustacean species that may be driven by differential uptake mechanisms and toxicokinetics of Hg and Se.

3.2. Seafood Hg concentrations and body size

Hg concentrations increased with both length and weight in all sampled fish species (Fig. 2; Table S1). Length explained a larger part of the variation in fillet Hg concentrations (r^2 between 0.18 and 0.60; $P < 0.01$) than weight (r^2 between 0.20 and 0.40; $P < 0.01$). Over time, Hg bioaccumulation leads to increasing concentration with fish age (Power et al., 2002). Our data shows that fish length is a better proxy for age than weight, as weight can be affected by seasonal variation and food availability, body condition and rates of gonad maturation (Table S1). Hg concentrations were not correlated to length in crustaceans except for Norway lobster, where a negative linear relationship was observed ($r^2 = 0.42$; $P < 0.05$). However, Hg concentrations increased with weight in both European lobster ($r^2 = 0.19$; $P < 0.05$) and Norway lobster ($r^2 = 0.68$; $P < 0.01$), but not in brown crab. Since crustaceans molt, their length increases incrementally and during the periods in between molting steps, the weight may be a better predictor of growth than length (Cameron, 1989).

In many crustaceans, clear differences in Hg concentrations between sexes and interactions with length have been reported. For example, female Norway lobster from the Ligurian Sea (Minganti et al., 1990) and outside Scotland (Canli and Furness, 1993) showed steeper increases in Hg concentrations with length than males. This is likely due to slower growth rates of females in comparison to males and as a result of more energy investment related to reproduction. In this study, crustaceans were not sexed and consequently it was not possible to make comparisons among sexes. Analyzing individuals without information on sex could also mask effects of size on Hg concentrations in crustaceans.

3.3. Spatial variation of Hg in seafood and sediments

In most of the studied species, Hg concentrations were higher in samples of marine organisms collected towards the inner fjord and PSP at Odda than in samples taken in the outer fjord (Figs. 1; 4). This spatial variation was consistent across crustacean and fish species including European lobster, crab, tusk and sprat, but not for blue ling or common ling.

The highest mean concentration of Hg in tusk were observed in the two Eidfjord sites 4E and 5E (2.88 and 1.78 mg kg^{-1} ww), and not in the inner part of Sørfjord where sites 1S and 2S also showed high Hg values (1.90 and 1.36 mg kg^{-1} ww). The differences between sites 4E, 5E and 1S were, however, not significant (Fig. 4) and considering the limited number of tusk collected from 4E (n = 7) and 1S (n = 2), tusk from both branches appear to be contaminated at similar levels. However, the observed high Hg concentrations in tusk from Eidfjord, 47 and 59 km from the PSP, indicate that PSP may not be the only source of Hg to the biota in Hardangerfjord. The tusk from Eidfjord may hence have been influenced by the freshwater inputs from two large rivers and the hydroelectric power station located upstream on the Sima River (Fig. 1). Moreover, substantial transport of Hg from PSP in Odda to Eidfjord does not seem very likely, based on the sediment concentrations of Hg in Sørfjord. Measured concentrations decreased rapidly from 2.26 mg kg^{-1} dw at site 1 to 0.72 mg kg^{-1} dw at site 2 and 0.03 mg kg^{-1} dw at site 3. At site 6 in Eidfjord the sediment concentration was again a bit higher, with 0.17 mg kg^{-1} dw, but still more than an order of magnitude lower than at site 1. The combination of depth (350 m) and the Sørfjord sill may also prevent the movement of contaminants. However, the run-off from Opo River and fjord/estuarine water circulation driven by local tidal conditions may also redistribute and resuspend the contaminants to outside Sørfjord, but the majority of Hg from PSP stays within the Sørfjord sector. If transport of Hg should take place from PSP to Eidfjord, resuspended Hg would have to be transported in higher water layers, over the Sørfjord sill and to the right-hand side due to the Coriolis force effect before being deposited in Eidfjord. Tusk from site 7OH close to Steinstø, had significantly lower Hg concentrations than tusk from the Eidfjord sites, but significantly higher Hg concentrations than tusk from the three outermost sites.

Hg concentrations in sediment increased from the outer Hardangerfjord towards the inner fjord and PSP at Odda (Fig. 5; Table S3) and were in good accordance with the tusk Hg data. Sediments were sampled from the top and intermediate layers (15 ± 2 cm) resulting in an integrated sample which limits our resolution of the interpretation. However, our spatial results are consistent with other studies and show an increasing gradient of mercury from offshore

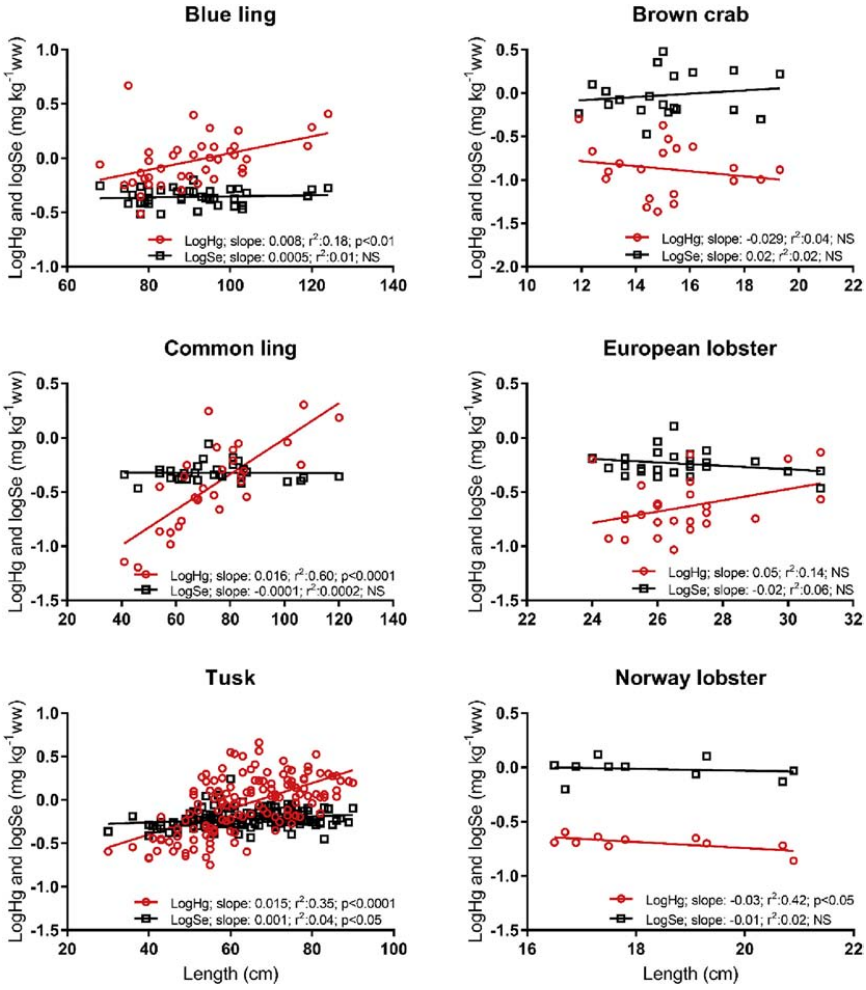

Fig. 2. Linear regression between length and log Hg (red circles) and length and log Se (black squares) in fish and crustacean species from Hardangerfjord sampled in 2011. Slope, r^2 and P are presented. NS = not significant.

to the interior of the fjord. Using a meta-analysis, Everaert et al. (2017) reported Hg concentrations in sediment samples of 0.13 mg kg^{-1} dw in Norwegian inner fjord areas and 0.02–0.03 mg kg^{-1} dw in offshore areas. Their reported levels from inner fjord areas were comparable to Hg concentrations measured at sites 5 and 6 in Eidfjord (0.072 and 0.173 mg kg^{-1}dw), whereas the reported levels in offshore areas were comparable to the Hg concentrations in sediment from the less impacted areas of outer Hardangerfjord (0.015–0.050 mg kg^{-1} dw, sites 3, 4 and 7).

Concentrations of Hg in sediments were not significantly correlated with distance from PSP (Fig. 5). On the other hand, distance from open ocean that takes into account both input from catchment and PSP in the same direction, showed a significant correlation with Hg concentrations in sediment (Kendall tau 0.90; $P < 0.05$) (Table S4). For tusk, there was a significant correlation between Hg concentration and distances from both ocean and PSP, but the correlation with distance from open ocean was stronger (Fig. 5; $r^2 = 0.59$ and $r^2 = 0.76$, respectively) and overall Hg concentrations in both tusk and sediment were in good accordance.

A recently published study, which included tusk specimens from sites 1S and 7OH as well as tusk from other areas on the Norwegian coast, showed that the Hg stable isotope values were different in Hardangerfjord, particularly Sørfjord, compared to the open coast of Norway (Rua-Ibarz et al., 2019). The isotopic composition changed somewhat from Sørfjord to the outer Hardangerfjord, to a profile more similar to that of the open coast. This indicated that in the outer Hardangerfjord there was an influence from the zinc plant in Sørfjord, but also from atmospheric sources.

In areas not impacted by specific sources of pollution, atmospheric deposition of Hg is considered a major source of Hg to the ecosystems (Mason et al., 1994), and in coastal ecosystems Hg mostly originates from freshwater input, organic matter decomposition and erosion (Bełdowska et al., 2014). Fjords naturally have large river inputs often at the ends and these often drain large catchment areas. This freshwater run-off contains Hg deposited over the entire catchment area, including throughfall (Kahl et al., 2007). In the Hardangerfjord, there are two large rivers at the end of Sørfjord located close to PSP and two large rivers at the end of Eidfjord (Fig. 1). The River Eio at the end of Eidfjord has the

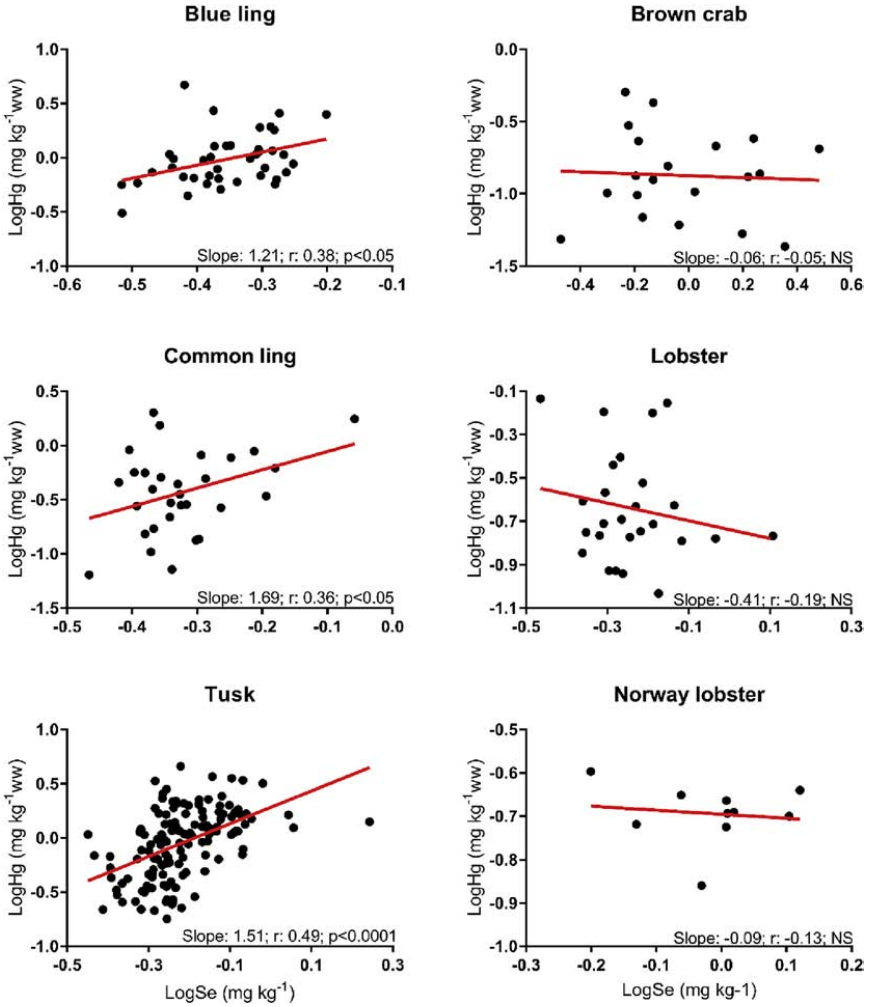

Fig. 3. Relationship between log Hg and log Se (mg kg^{-1} ww) in fish and crustacean species from Hardangerfjord, 2011. Slope, r and *P* are presented. NS = not significant.

largest catchment area in the inner part of Hardangerfjord and is likely to transport larger amounts of atmospherically deposited Hg than the other rivers. Sima, the other main river in Eidfjord sector, also has a hydroelectric power plant that may impact the Hg load as well as methylation (Schartup et al., 2015). In hydroelectric stations, water usually comes from the hypolimnion layer of the reservoir which often has favorable conditions for Hg methylation. Additionally, wetting and drying from periodic flooding of the adjacent soils can also increase MeHg production and bioavailability. These increases in MeHg are largely driven by the timing, frequency and severity of the reservoir flooding. Water released from the reservoir to the fjord is often enriched in MeHg (Pestana et al., 2018) and several studies have reported increased Hg levels in water, plankton and fish from downstream of hydroelectric dams (Hylander et al., 2006; Kasper et al., 2014). Also, in Sørfjord there are several hydroelectric power plants. Freshwater inputs from the rivers is reflected in the salinity measurements and modeling that showed a decreasing trend in surface water salinity from the outer part of Hardangerfjord towards both Sørfjord and Eidfjord (Fig. 6; Fig. S3). The rivers also deliver significant amounts of terrestrial organic matter (Jassby and Cloern, 2000) that may influence Hg methylation and bioavailability dynamics (Lambertsson and Nilsson, 2006).

3.4. Mercury methylation in sediments

Concentrations of MeHg in sediment varied from 0.12 μg kg^{-1} dw at site 4 to 8.4 μg kg^{-1} dw at site 1, closest to PSP (Fig. 5). Atmospheric deposition and terrestrial run-off have been suggested as significant sources of MeHg and inorganic Hg that can be methylated, particularly in estuarine and coastal areas (Mason et al., 2012; Schartup et al., 2015). However, close to the PSP, a relatively high concentration of MeHg indicates that methylation of inorganic Hg originating from the zinc plant is taking place to some degree. High concentrations of both total Hg and MeHg were found close to the PSP (site 1). Comparing the Hg concentrations in sediment at the end of Sørfjord, close to PSP, with Eidfjord (2.26 vs 0.17 mg kg^{-1} dw) and the MeHg concentrations in these sites (8.4 vs 0.82 μg kg^{-1} dw) shows that methylation efficiency (i.e., % MeHg) from PSP is similar (0.37% vs 0.47%) (Table S3). In general, MeHg concentrations in sediment increased towards the inner part of the fjord (Figs. 1; 5) and

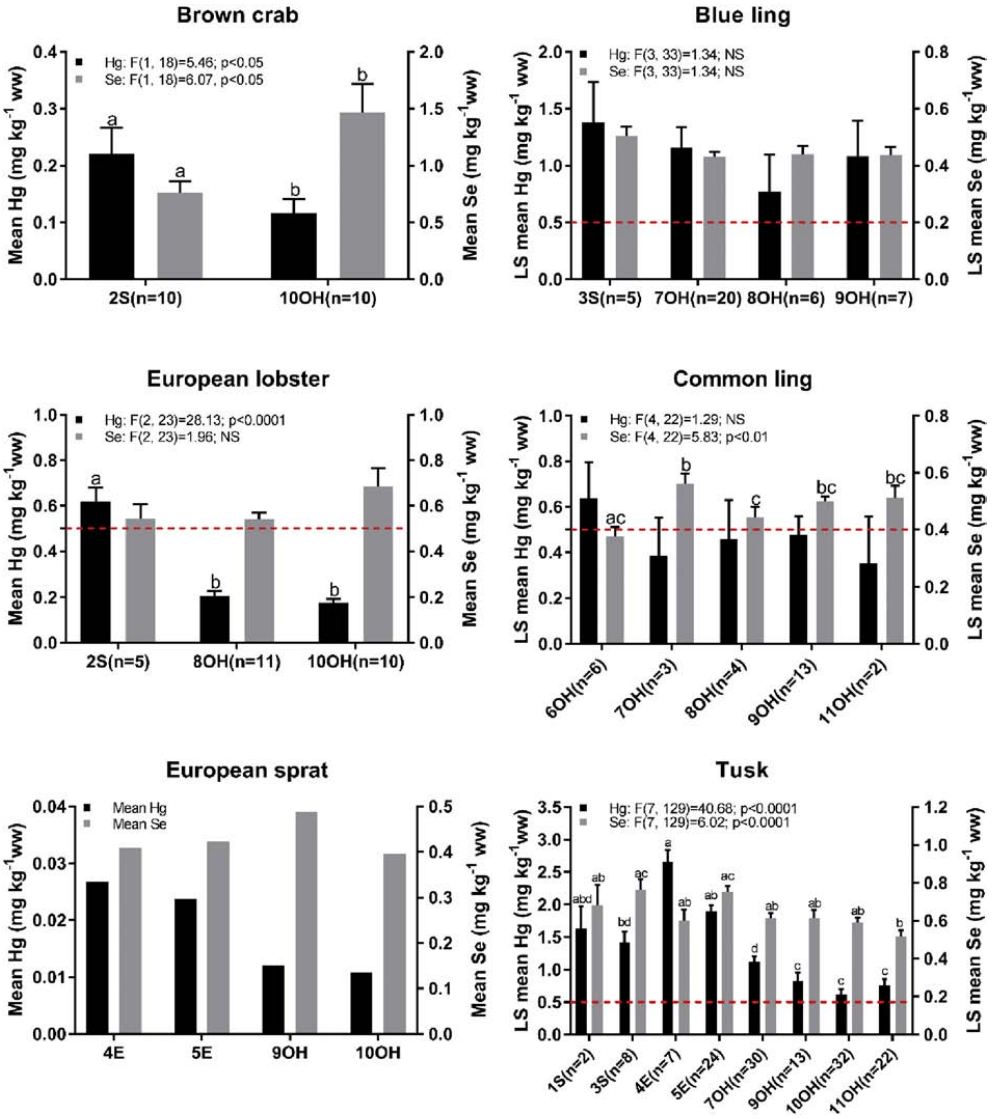

Fig. 4. Least squares means (adjusted for mean length) + standard error of Hg and Se concentrations in fish and crustacean species collected from different sites in Hardangerfjord, 2011. Hg and Se concentrations are presented on the left and right Y axes, respectively. ANCOVA/ANOVA test results are presented and letters were used to show significant differences when applicable. For lobster and brown crab ANOVA was used for comparisons between areas and arithmetic means are presented. Only composite samples of sprat and their arithmetic means are shown. Stations are sorted according to the distance from point source of pollution (PSP) at Odda. Letters after each station number represent the location in detail; S=Sørfjord, E = Eidfjord and OH=Outer Hardangerfjord. The dashed red lines show the EU maximum level of Hg (0.5 mg kg^{-1} ww).

were well correlated with total Hg concentrations (Kendall tau 0.71; $P < 0.05$), and we can conclude that Hg concentrations likely had an important influence on MeHg production in sediments in Hardangerfjord. In a study from Öre River estuary in Sweden, organic matter was shown to be a primary factor controlling MeHg formation in estuarine sediments, while total Hg had little or no effect on net MeHg production (Lambertsson and Nilsson, 2006). The main difference between the Hardangerfjord system and the Öre River Estuary studied by Lambertsson and Nilsson (2006) was the absence of local anthropogenic pollution in the Öre River Estuary, and consequently much lower concentrations of THg (ca. 18 times) in sediment samples than what we observed in inner Sørfjord. In another study, from the estuarine environment of the Penobscot River, Maine, USA, with high concentrations of Hg in sediment originating from industrial sources, a clear positive linear relationship was observed between Hg and MeHg concentrations (Rudd et al., 2018).

Distance from the open ocean was the best predictor for MeHg variation between the sites (Kendall tau 0.81; $P < 0.05$), while no correlation between MeHg and distance from PSP was detected since MeHg levels were relatively high in the inner sectors of both Sørfjord and Eidfjord (Fig. 1). Methylmercury concentrations in the sediments are

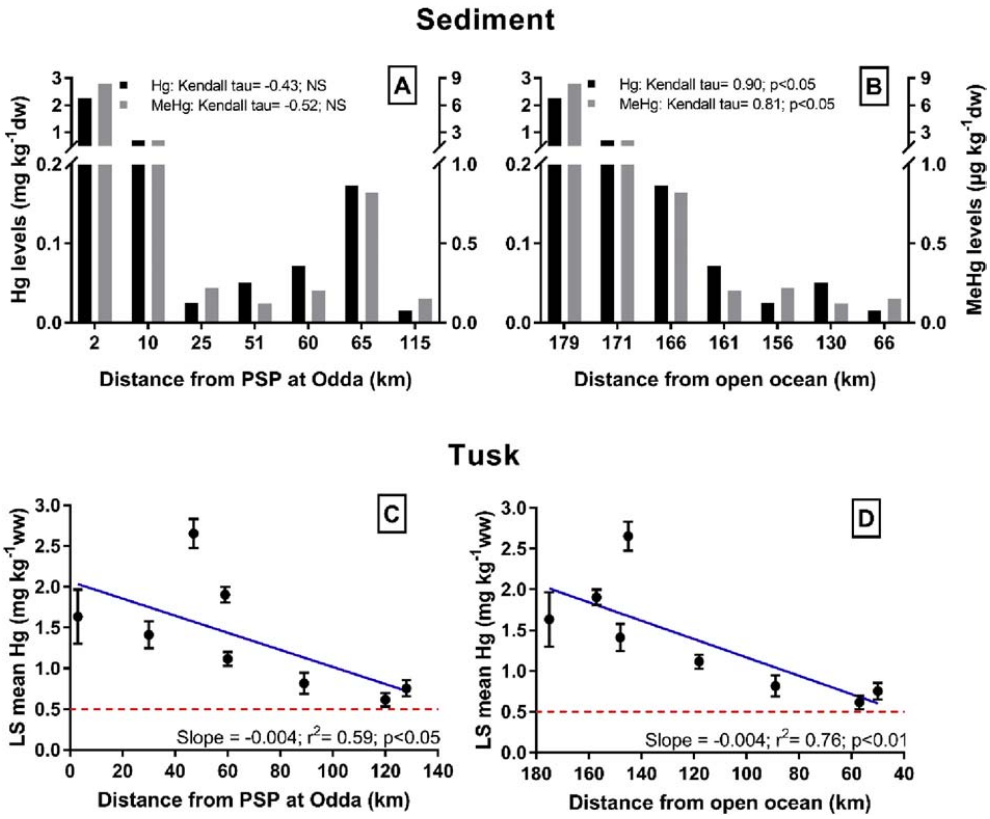

Fig. 5. Mercury pollution in sediment and tusk fillet sampled from different sites in the Hardangerfjord ecosystem. A and B: Total Hg and MeHg concentrations in sediment samples collected from different sites sorted by distance from point source of pollution (PSP) at Odda and distance from the open ocean. Nonparametric Kendall tau correlation coefficients are presented. NS = not significant. C and D: Least squares means Hg ± standard error of tusk fillet (adjusted for mean length) collected from different sites with varying distance from the point source of pollution (PSP) at Odda and distance from the open ocean. Dashed red lines show the EU maximum level of Hg (0.5 mg kg^{-1} ww).

likely governed by Hg concentrations, anaerobic microbial activity mainly driven by sulfate reducing bacteria in the inner sector of the fjord, and/or by organic matter quantity and composition.

3.5. Mercury speciation in seawater

Hg species and physiochemical parameters were measured in seawater samples taken from nine sites and three depths including 15, 50, and 300 m (Table 3). Salinity and temperature measurements showed that the three sampling depths belong to different hydrographic layers. Brackish layers were restricted to the upper 7 m of the fjord at the time of measurement. Water samples taken at 15 and 50 m depths were both within the intermediate layer while samples from 300 m depths were under the sill level in the fjord basin water. Total Hg concentrations increased with depth (mean of all sites 0.25 ng L^{-1} at 15 m; 0.43 ng L^{-1} at 50 m and 0.52 ng L^{-1} at 300 m; Fig. S4), whereas the MeHg concentrations were highest at 50 m and lowest at 15 m depth (0.02 ng L^{-1} at 15 m; 0.09 ng L^{-1} at 50 m and 0.04 ng L^{-1} at 300 m; Table 3; Fig. S4). The lower total Hg concentrations observed in the shallower layers may be related to the physical properties involved with water residence time in fjord ecosystems. Internal waves generated by wind conditions creating up- and down-welling at the coast are an important forcing mechanism for the renewal of the fjord water above the sill (Asplin et al., 1999). These internal waves are shown to occur irregularly 1 to 2 times a month and are restricted to the upper 30 m in May and June in the Hardangerfjord ecosystem (Asplin et al., 2014). Therefore, the water at 15 m depth will be exchanged more frequently than the water at 50 m depth, despite both depths being intermediate layers. The lower concentration of Hg found at 15 m depth compared to 50 m depth can be explained as a mixed effect of both different water residence times and that the deeper layers receive Hg deposited from the upper layers. The overall highest concentration of Hg was found at the 300 m depth level in sites 4 and 1 (1.65 and 1.55 ng L^{-1}) and also at 50 m at site 9 (1.2 ng L^{-1}). MeHg concentrations at all depths were highest at site 1 close to the PSP (0.04, 0.25 and 0.11 ng L^{-1} at 15, 50 and 300 m depths, respectively).

MeHg concentration at 50 and 300 m depths in seawater, as well as total Hg and MeHg concentrations in sediment, increased gradually towards the PSP indicating a possible interaction between Hg pools in surface sediments and deep layers of seawater. At deep parts of the Hardangerfjord ecosystem, below the sill, water exchange and mixing are very limited. MeHg produced in sediments as well as biological production of MeHg under the mixed layer that sinks as particles to deeper water are probably the main sources of MeHg in deep-water environments (Blum et al., 2013). MeHg concentrations in seawater at 50 and 300 m depths increased from the outer fjord towards PSP and the inner part of Hardangerfjord (Kendall tau −0.94 and −0.93 respectively; Table S5). In the inner part, a higher effect of the PSP, anaerobic conditions (i.e. lower oxygen conditions at the fjord's interior) and

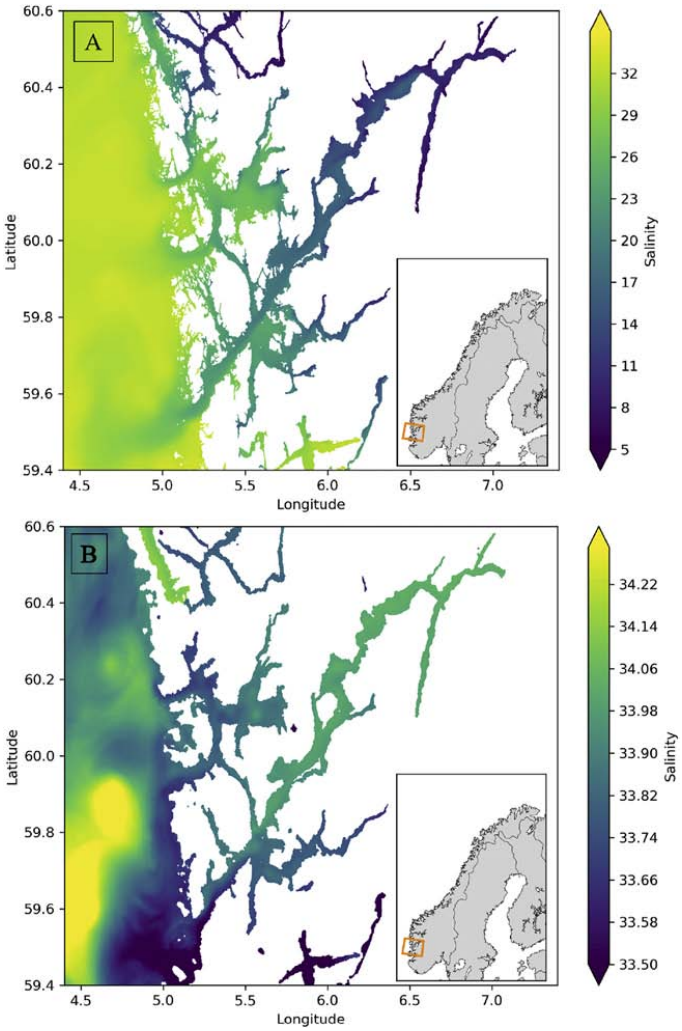

Fig. 6. Salinity level modeled for surface (A) and 50 m depth (B) in Hardangerfjord seawater sampled in May 2018. Note the different salinity scales in each map.

terrestrial run-off are expected. There was no such significant trend at 15 m depth (Table S5) where MeHg concentrations were generally low at all sites (up to 0.04 ng L^{-1}).

Percent MeHg increased significantly towards PSP for the 50 m depth samples but not at 15 m nor 300 m (Table S5). Oxygen concentrations at both 50 and 300 m depths decreased towards the inner part of the fjord, in the opposite trend of MeHg concentration and percent MeHg at 50 m depths. Lower oxygen concentrations in deep layers are typical of fjords due to lower rates of water exchange inside fjord sills. A combination of low oxygen concentrations and higher organic matter bound Hg^{2+} within fjords likely provided ideal conditions for biotic methylation and higher MeHg concentrations (Soerensen et al., 2018).

3.6. Bioconcentration Factors and Biota Sediment Accumulation Factors

For each site in the Hardangerfjord, Bioconcentration Factor (BCF) and Biota Sediment Accumulation Factor (BSAF) were calculated for total Hg and MeHg in tusk fillet tissue (Fig. S5). These are indicators of how much THg and MeHg are transferred to tusk fillet from water and sediment, respectively. Tusk was chosen for this purpose as it is a benthic feeder and a deep-water fish species with low vagility (Cohen et al., 1990). Tusk samples were collected across a broad area and in both the inner and outer sectors of the Hardangerfjord ecosystem. BCF values varied from 6.2 to 7.0 for total Hg and from 7.0 to 7.5 for MeHg (Fig. S5). Tusk BSAF values for total Hg was 0.2 at site 1S, and between 1.4 and 1.8 for the other sites and BSAF values for MeHg varied between 2.6 and 4.2. Site 1S closest to PSP had lower BSAF than the other sites due to very high Hg concentration in the sediment close to the PSP that was not reflected in the tusk fillets. Both BCF and BSAF were higher for MeHg than total Hg at all sites due to lower MeHg concentration in seawater and sediment compared to total Hg and the more efficient trophic transfer and bioavailability of MeHg. Lower BCF values close to the PSP at sites 1S and 3S and much lower BSAF values for both MeHg and Hg at site 1S compared to other parts of Hardangerfjord (Fig. 5S) may indicate that Hg and MeHg originating from PSP is less bioavailable compared to the Hg pool in other parts of the Hardangerfjord ecosystem.

Table 3
Mercury speciation and physiochemical properties of seawater from the different sampling sites in Hardangerfjord, May 2018.

Site	Sampling depth (m)	Max depth (m)	MeHg (ng L^{-1})	SD	iHg (ng L^{-1})	SD	THg (ng L^{-1})	% MeHg	Temperature (°C)	Salinity CTD[a]	Salinity (Water sample)	Oxygen (%)	Oxygen (mg L^{-1})	Latitude	Longitude
1	15	380	0.04	0.0011	0.40	0.01	0.45	9.33	7.25	31.762		97.62	10.10	60°	6° 35,747
	50		0.25	0.0146	0.53	0.01	0.78	32.17	8.22	34.65		50.47	5.01	14,656	
	300		0.11	0.0102	1.45	0.05	1.55	6.98	7.65	34.963	34.95	49.08	4.93		
2	15	780	0.02	0.0011	0.24	0.01	0.25	7.17	7.00	31.656		96.48	10.05	60° 26,58	6° 34,33
	50		0.15	0.0059	0.25	0.01	0.39	37.18	8.28	34.713		56.54	5.61		
	300		0.07	0.0014	0.17	0.01	0.24	29.45	7.68	34.98	34.98	61.30	6.15		
3	15	800	0.01	0.0002	0.13	0.01	0.14	7.68	7.25	31.762		97.62	10.10	60° 23.40	6° 20,43
	50		0.13	0.0030	0.31	0.02	0.45	29.39	8.22	34.65		50.47	5.01		
	300		0.06	0.0003	0.11	0.01	0.17	33.01	7.71	35.012	34.99	61.15	6.13		
	770		0.10	0.0015	0.20	0.01	0.30	32.40	7424	35.058	35.05	53.27	5.37		
4	15	500	0.01	0.0001	0.13	0.00	0.15	9.46	7.04	31.630		99.43	10.35	60° 15.55	6° 11,43
	50		0.07	0.0010	0.21	0.02	0.28	25.31	8.39	34.721		61.23	6.06		
	300		0.03	0.0011	1.63	0.04	1.65	1.53	7.75	34.999	34.99	68.17	6.83		
5	15	650	0.01	0.0004	0.20	0.01	0.21	6.25	7.09	31.580		100.50	10.45	60° 09.12	6° 04,73
	50		0.06	0.0046	0.13	0.01	0.19	32.46	8.47	34.684		64.91	6.41		
	300		0.04	0.0013	0.22	0.01	0.26	16.54	7.72	34.996	34.99	67.76	6.79		
6	15	500	0.04	0.0003	0.20	0.01	0.24	16.63	7.01	31.568		98.46	10.26	60° 00.47	5° 56,15
	50		0.05	0.0016	0.16	0.01	0.21	23.82	8.53	34.693		67.93	6.70		
	300		0.02	0.0006	0.15	0.01	0.17	9.31	7.65	34.974	34.97	75.50	7.58		
7	15	500	0.04	0.0005	0.24	0.01	0.27	13.30	7.06	31.644		99.14	10.31	59° 55.07	5° 45,15
	50		0.04	0.0013	0.27	0.02	0.31	12.93	8.51	34.713		68.57	6.77		
	300		< LOD	< LOD	0.21	0.01	0.21	<LOD	7.50	34.951	34.95	79.60	8.02		
8	15	330	0.02	0.0005	0.21	0.01	0.23	7.53	7.97	32.802		95.16	9.62	59° 44.45	5° 30,38
	50		0.02	0.0003	0.09	0.01	0.10	14.69	7.86	34.726		77.32	7.74		
	300		0.01	0.0001	0.15	0.01	0.16	5.23	7.22	35.079	35.08	81.81	8.29		
9	15	330	0.01	0.0002	0.25	0.01	0.26	4.21	8.74	31.792		99.73	9.98	59° 35.72	5° 15,72
	50		0.04	0.0003	1.16	0.02	1.20	3.26	7.69	34.619		79.00	7.94		
	300		0.01	0.0002	0.28	0.01	0.29	2.44	7.24	34.9	34.91	79.81	8.09		

LOD: limit of detection.
[a] An offset of 0.12 was added.

3.7. Does point source of pollution drive the spatial distribution of Hg in Hardangerfjord?

To investigate the effect of the point source, length adjusted Hg concentrations in tusk from different sites were analyzed as a function of distance from the PSP at Odda and the distance from the open ocean. The distance from PSP explained 59% of the variation of mean Hg concentrations in tusk fillet, but distance from the open ocean improved the model to 76% variance explained (Fig. 5C and D). The same trend was observed when Hg concentrations in individual tusk were used (Fig. S6). An explanation for this may be that an increased distance from the open ocean not only increases the effect of the PSP, but also water residence time, freshwater run-off and terrestrial organic matter.

Hg concentrations in sediment were very high near the PSP at Odda and decreased sharply towards the outer parts of Sørfjord (Fig. 5), indicating that Hg pollution from PSP is likely quite local. Probably a limited amount of Hg is transported from PSP, and Hg found in the outer Hardangerfjord and Eidfjord may originate largely from terrestrial run-off (Rua-Ibarz et al., 2019). A study of local soils showed that Hg concentrations around the zinc plant are very high compared with background concentrations, but within a 10–20 km distance from the source, Hg concentrations are comparable to background conditions (Svendsen et al., 2007). Thus, air emissions and atmospheric deposition of Hg from this point source will likely remain mostly inside the catchment area of the Sørfjord, and the major effect of Hg emissions is concentrated at the head of the fjord near Odda as well as the southern half of Sørfjord. However, Hg can also be distributed long distances to outside catchment areas via atmospheric transport (Fitzgerald et al., 1998).

The average MeHg concentration in the sediment in Sørfjord closest to the PSP was approximately 10 times higher than sediments in the inner Eidfjord sector. Even so, the mean concentration of Hg in tusk fillet (length adjusted) in Sørfjord was approximately 30% lower than in tusk from Eidfjord. This suggests that MeHg from different sites may not be equally bioavailable in these branches or that other factors such as trophic position varies across sites. In a mesocosm experiment, using Hg isotope tracers in both inorganic and organic forms, Jonsson et al. (2014) showed that MeHg from terrestrial and atmospheric sources have higher bioavailability compared to MeHg formed in the sediment and that MeHg from terrestrial run-off has a significant effect on MeHg burdens in estuarine biota. These findings could explain the trend in our results, where MeHg produced in sediments from inorganic Hg originating from the PSP appear to be less bioavailable than MeHg in Eidfjord that likely mainly originates from terrestrial run-off and atmospheric deposition, although the effects from local hydropower stations may also be substantial. High Hg concentrations in tusk have been reported from inner Nordfjord (another fjord in western Norway) compared to open ocean habitats (Berg et al., 2000), but further investigations in a fjord ecosystem without a point source are required to fully evaluate this hypothesis. However, it seems likely that the fjord ecosystems favor high Hg accumulation in deep-water, demersal fish compared to pelagic species, with some exceptions. Moreover, life history characteristics, and spatial and temporal variation in trophic complexity in these ecosystems, must also be considered important drivers of Hg in seafood species inhabiting fjords and other coastal environments, especially considering that subtle differences in diet and food web position may lead to substantial differences in Hg bioaccumulation (Bank et al., 2007). The high freshwater input from large catchment areas are believed to deliver highly bioavailable terrestrial MeHg to the fjord. When run-off reaches the fjord, the increase in salinity increases the partitioning of contaminants bound to organic matter in the particulate phase and thus the contaminants sedimentation can be enhanced from suspended particulate matter entering the sediments (Turner and Millward, 2002). MeHg will be retained in the fjord due to limited exchange of bottom water and the presence of a shallow sill. Additionally, Wang et al. (2018) reported the importance of Hg methylation in subsurface water in predicting Hg in marine biota from the Arctic. Future research should evaluate the role of subsurface methylation in relation to Hg dynamics in fjord food webs.

Using a linear model, distance from the open ocean explained 76% of the variation in Hg concentrations in tusk fillets. The linear model can be used to estimate the range of Hg contamination in deep-water species such as tusk. Therefore, tusk can be considered an important bioindicator for Hg contamination in high trophic species inhabiting fjord ecosystems especially since they have a very wide distribution and low vagility.

3.8. Comparison of Hardangerfjord seafood with the EU maximum level

Hg concentrations in fish and fishery products, including claw and tail meat of crustaceans, are regulated by the EU in different categories and should be below the maximum level (EUML) of 0.5 mg kg^{-1} ww for all species investigated in this study (EC, 2006). Tusk and blue ling collected from all sites in both inner and outer Hardangerfjord had 2–3 times higher average Hg concentrations than the EUML and all individual measurements in samples collected from inner Hardangerfjord exceeded the EUML (Table 3). Mean Hg concentrations in common ling from the inner part of the fjord exceeded the EUML by ~2-fold, and mean Hg concentrations in samples from the outer part were close to EUML (0.49 mg kg^{-1} ww). Hg levels in wolffish and sprat were well below the EUML. The sampled crustacean species had average Hg concentrations below the EUML, except for European lobster caught in Sørfjord that had 0.62 mg Hg kg^{-1} ww and four out of five specimens had concentrations above EUML. The EUML regulates commercial fishery in this area, as it is illegal to sell food exceeding EUMLs. To protect local recreational fishers and their families from MeHg exposure, the Norwegian Food Safety Authority (NFSA) has issued a consumption advisory to avoid blue ling and tusk from the whole Hardangerfjord and common ling from Sørfjord. Further, pregnant and nursing women are advised by NFSA to avoid consumption of crab, European lobster and sentinel fish species from Sørfjord (www.miljostatus.no).

Recently, Selenium Health Benefit Value (HBV_{Se}), was suggested as a comprehensive human health index considering the Se co-exposure that potentially reduces bioavailability, exposure and toxicity of MeHg (Ralston et al., 2016). Negative HBV_{Se} values imply higher molar concentration of Hg than Se, and consumption of seafood with negative values may be more detrimental for human health than consumption of seafood with positive values. In this investigation only blue ling and tusk from inner Hardangerfjord had HBV_{Se} with negative values of −1.7 and −0.6, respectively.

In general, crustaceans had higher HBV_{Se} values than fish species (~3 times higher in the outer part of the fjord and ~9 times higher in the inner sectors) since they contain less Hg and more Se (Table 2). Although Hg and Se concentrations were correlated in both tusk and blue ling from the inner part of Hardangerfjord, tusk with higher Hg concentrations had higher HBV_{Se} values than blue ling. This may be due to differences in bioaccumulation mechanisms and toxicokinetics of Se and Hg across taxa which have important implications for seafood safety and overall food security.

4. Conclusions

Hardangerfjord is a Hg impacted fjord with a pollution source at the end of its inner sector and provides a unique opportunity to investigate Hg bioavailability in seafood species commonly consumed by humans. Although the direct release of jarosite containing contaminants from the zinc plant into Hardangerfjord was stopped in 1986, legacy Hg is still present in the environment and concentrations in seawater and sediment were highest close to this point source at the inner most part of Sørfjord (Fig. 1). Tusk, blue ling and common ling from the entire Hardangerfjord area and European lobster from the inner part of the Hardangerfjord are highly polluted by Hg and well above the EUML. Concentrations of Hg in both seafood, sediment, and seawater increased from the open ocean to the inner part of the fjord. Although sediment concentrations were ten times higher in the inner fjord branch with a PSP (Sørfjord) compared to an adjacent fjord branch that may have been influenced by freshwater inputs, Hg concentrations in the demersal fish species tusk sampled from each branch were similar. Although Hg originating from the point source was methylated in sediments and Hg contamination in both fish and crustacean species increased towards the PSP, atmospheric Hg transferred by run-off and hydroelectric power stations cannot be ruled out as important sources of Hg to biota.

The effects of the PSP, run-off and organic matter input from the catchment, anaerobic conditions, and residence time gradually increased in the same direction (towards inner parts) and therefore it is difficult to separate the effect of these different Hg pools on biota. Adding a study in another fjord with similar conditions, but without a pollution point source or conducting Hg stable isotope analysis on terrestrial and marine ecosystem compartments from Hardangerfjord will likely help to better understand the relationship between different sources of Hg, local biogeochemistry patterns and overall bioavailability, fate, and transport of MeHg.

Acknowledgments

The authors wish to thank the technical staff in the laboratories at the Institute of Marine Research (IMR) for sample preparation and analyses. We also thank Otte Bjelland at IMR for organizing the fishing cruises and master student Michael Lindgren for analyzing part of the fish and crustacean samples. Photos used in the graphical abstract were reprinted with permission; European lobster (photo credit: E. Senneset, IMR) and brown crab (photo credit: O. Paulsen, IMR). We thank Arne Duinker for assistance with preparing the study area map. This study was supported by funding from three organizations including: Norwegian Ministry of Trade, Industry and Fisheries, Norwegian Food Safety Authority and Institute of Marine Research.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.02.352.

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Supplementary materials

Spatial distribution of mercury in seawater, sediment, and seafood from the Hardangerfjord ecosystem, Norway

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Table S1. Linear regression between log Hg levels and size (length and weight) of the fish and crustacean species collected from the Hardangerfjord ecosystem in 2011. NS = not significant.

		logHg vs length			logHg vs weight		
Species	N	Slope	r^2	*P*-value	Slope	r^2	*P*-value
Blue ling	41	0.008	0.18	<0.01	0.0001	0.20	<0.01
Common ling	30	0.016	0.60	<0.0001	0.0002	0.40	<0.001
Tusk	138	0.015	0.35	<0.0001	0.0001	0.20	<0.0001
All fishes	218	0.012	0.31	<0.0001	0.0001	0.24	<0.0001
Brown crab	20	-0.029	0.04	NS	-0.0002	0.03	NS
European lobster	26	0.052	0.14	NS	0.001	0.19	<0.05
Norway lobster	10	-0.028	0.42	<0.05	-0.001	0.68	<0.01
All crustaceans	56	0.017	0.13	<0.01	-0.0001	0.002	NS

Table S2. Student's *t*-test comparison of Hg levels in fish and crustacean species between samples collected from inner vs outer Hardangerfjord. All species showed no difference ($P > 0.05$) in length (cm) between inner and outer Hardangerfjord.

	Hg (mg kg-1 ww)			Length (cm)		
Species	*t*-value	df	*P*-value	*t*-value	df	*P*-value
Blue ling	-1.83	39	NS	-0.28	37	NS
Common ling	-1.37	28	NS	-0.30	28	NS
Tusk	-8.09	136	<0.0001	0.55	136	NS
European lobster	7.48	24	<0.0001	-1.43	24	NS
Brown crab	2.34	18	<0.05	-0.57	18	NS
All species	-5.30	272	<0.0001	1.70	270	NS

Table S3. Mercury species and selenium concentrations in sediment samples collected from the Hardangerfjord ecosystem in 2015.

Site	THg (mg kg^{-1} dw)	MeHg (µg kg^{-1} dw)	% MeHg	Se (mg kg^{-1} dw)	Latitude	Longitude
1	2.26	8.4	0.37	0.57	60° 04.790	6° 31.818
2	0.715	2.1	0.29	0.24	60° 09.682	6° 33.599
3	0.025	0.22	0.88	0.09	60° 17.878	6° 36.231
4	0.05	0.12	0.24	0.16	60° 24.641	6° 30.693
5	0.072	0.2	0.28	0.22	60° 28.932	6° 58.696
6	0.173	0.82	0.47	0.37	60° 28.860	7° 05.163
7	0.015	0.15	1.00	0.11	59° 58.795	6° 00.459

Table S4. Nonparametric correlation matrix between sediment Hg species, Se concentrations and distance from the point source of pollution (PSP) and the open ocean. Samples collected in 2015 from Hardangerfjord, Norway. Kendall tau coefficient for significantly correlated cases are shown in bold.

Variable	Distance (PSP)	Distance (ocean)	Se	% MeHg	MeHg	THg
Distance (PSP)	1.000000					
Distance (ocean)	-0.523810	1.000000				
Se	-0.238095	**0.714286**	1.000000			
% MeHg	0.238095	-0.142857	-0.047619	1.000000		
MeHg	-0.523810	**0.809524**	0.523810	0.047619	1.000000	
THg	-0.428571	**0.904762**	**0.809524**	-0.238095	**0.714286**	1.000000

Table S5. Nonparametric correlation matrix of seawater Hg species and ancillary variables measured at 15m, 50m and 300m depths in Hardangerfjord, 2018. Kendall tau coefficient for significantly correlated variables are shown in bold.

A. 15m depth

Variable	MeHg (ng L^{-1})	iHg (ng L^{-1})	THg (ng L^{-1})	MeHg %	Temperature (°C)	Salinity CTD	Oxygen (%)	Oxygen (mg L^{-1})	Distance (PSP)
MeHg (ng L^{-1})	1.00000								
iHg	0.44444	1.00000							
THg	**0.55556**	**0.88889**	1.00000						
% MeHg	0.44444	-0.11111	0.00000	1.00000					
Temperature (°C)	-0.30989	0.14086	0.02817	-0.42258	1.00000				
Salinity CTD	-0.14086	0.19720	0.08452	-0.36623	**0.60000**	1.00000			
Oxygen (%)	-0.25355	-0.02817	-0.02817	-0.02817	0.02857	-0.37143	1.00000		
Oxygen (mg L-1)	-0.02817	-0.25355	-0.14086	0.30989	-0.31429	**-0.71429**	**0.65714**	1.00000	
Distance (PSP)	-0.22222	0.22222	0.11111	-0.11111	0.30989	0.02817	0.25355	-0.08452	1.00000

B. 50m depth

Variable	MeHg (ng L^{-1})	iHg (ng L^{-1})	THg (ng L^{-1})	MeHg %	Temperature (°C)	Salinity CTD	Oxygen (%)	Oxygen (mg L^{-1})	Distance (PSP)
MeHg (ng L^{-1})	1.00000								
iHg	0.27778	1.00000							
THg	0.33333	**0.94444**	1.00000						
% MeHg	**0.66667**	-0.05556	0.00000	1.00000					
Temperature (°C)	-0.08452	-0.36623	-0.42258	0.14086	1.00000				
Salinity CTD	-0.22866	**-0.57166**	**-0.57166**	0.00000	0.23191	1.00000			
Oxygen (%)	**-0.87333**	-0.25355	-0.30989	**-0.64795**	0.08571	0.23191	1.00000		
Oxygen (mg L-1)	**-0.87333**	-0.25355	-0.30989	**-0.64795**	0.08571	0.23191	**1.00000**	1.00000	
Distance (PSP)	**-0.94444**	-0.22222	-0.27778	**-0.72222**	0.02817	0.17150	**0.92967**	**0.92967**	1.00000

C. 300m depth

Variable	MeHg (ng L^{-1})	iHg (ng L^{-1})	THg (ng L^{-1})	MeHg %	Temperature (°C)	Salinity CTD	Oxygen (%)	Oxygen (mg L^{-1})	Distance (PSP)
MeHg (ng L^{-1})	1.00000								
iHg	0.07143	1.00000							
THg	0.14286	**0.88889**	1.00000						
% MeHg	0.42857	-0.50000	-0.42857	1.00000					
Temperature (°C)	0.14286	0.16667	0.27778	0.28571	1.00000				
Salinity CTD	-0.07143	-0.27778	-0.27778	0.21429	0.44444	1.00000			
Oxygen (%)	**-0.85714**	-0.05556	-0.16667	-0.42857	-0.44444	-0.11111	1.00000		
Oxygen (mg L^{-1})	**-0.85714**	-0.05556	-0.16667	-0.42857	-0.44444	-0.11111	**1.00000**	1.00000	
Distance (PSP)	**-0.92857**	-0.11111	-0.22222	-0.35714	-0.38889	-0.16667	**0.83333**	**0.83333**	1.00000

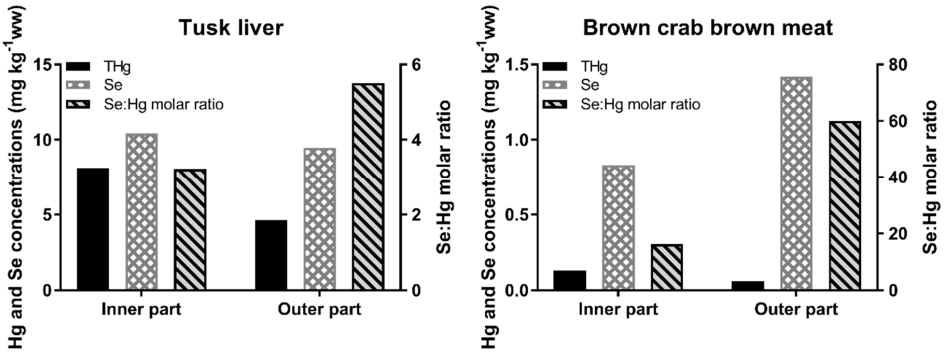

Figure S1. THg and Se concentrations and Se:Hg molar ratios in tusk liver and brown meat of brown crab collected from inner and outer Hardangerfjord in 2011. Tusk liver data are from Lindgren (2012).

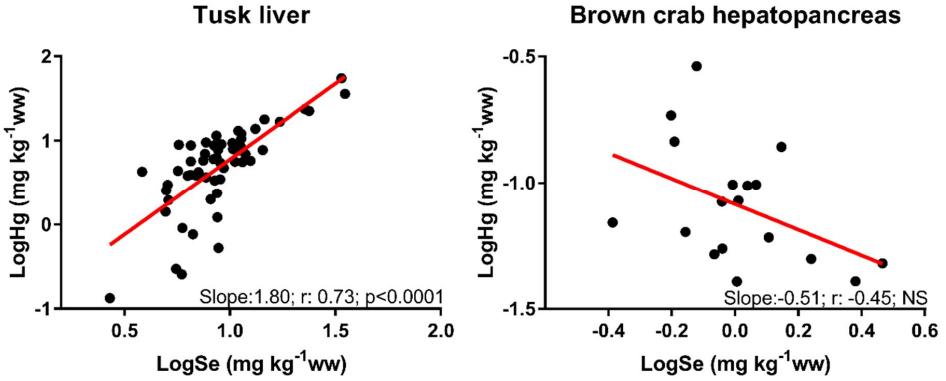

Figure S2. Pearson correlation between log Hg and log Se (mg kg^{-1} ww) in tusk liver and brown crab hepatopancreas collected in 2011 from Hardangerfjord. Slope, *r* and *P* are presented. NS = not significant.

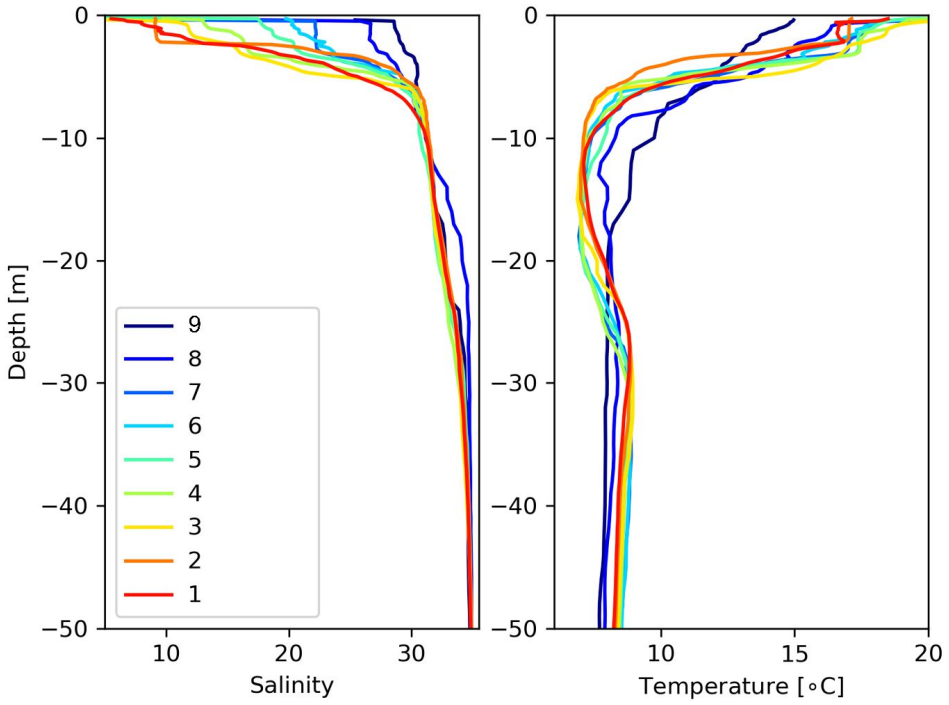

Figure S3. Salinity and temperature in the seawater column from the different sampling sites in Hardangerfjord, 2018. Different sites are presented with different numbers and colors, for details on sampling location refer to Figure 1.

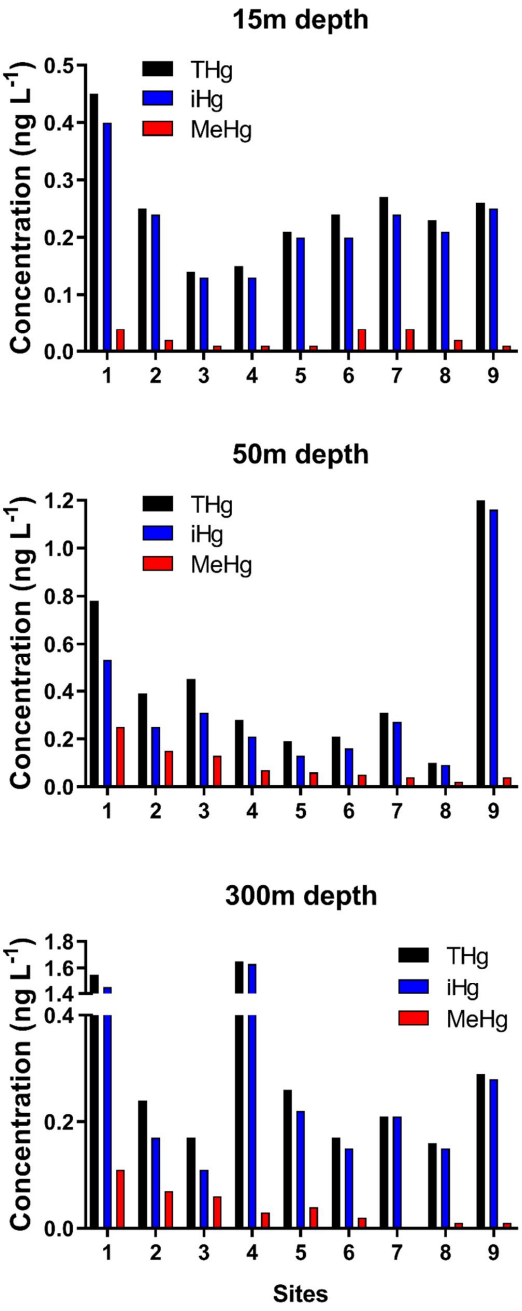

Figure S4. Total mercury (THg), Inorganic mercury (iHg) and methylmercury (MeHg) concentrations (ng L^{-1}) in seawater from different sampling sites in Hardangerfjord at three depths, May, 2018.

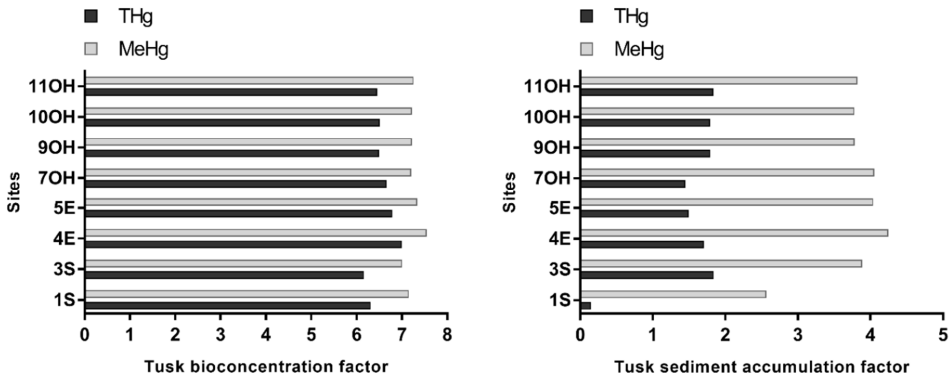

Figure S5. Tusk bioconcentration factors (BCF) and biota-sediment bioaccumulation factors (BSAF) of THg and MeHg sampled from sites in the Hardangerfjord ecosystem, Norway. Seawater was sampled in 2018, tusk in 2011 and sediment in 2015.

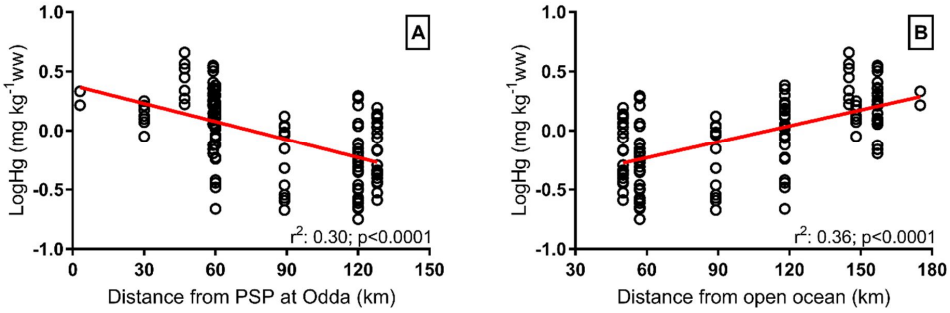

Figure S6. Linear regression between log Hg (mg kg^{-1} ww) in individual tusk fillets collected from different sites in the Hardangerfjord ecosystem in 2011 and (A) distance from point source of pollution (PSP) at Odda, (B) distance from the open ocean.

References

Lindgren M. The distribution of methylmercury in fish and shellfish from the Hardangerfjord. Master thesis, department of biology, University of Bergen, Norway 2012.

Graphic design: Communication Division, UiB / Print: Skipnes Kommunikasjon AS

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ISBN: 9788230863398 (print)
9788230858967 (PDF)