Mercury concentration trend as a possible result of changes in cod population demography

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Abstract

Mercury (Hg) in Atlantic cod (*Gadus morhua*) is one of many parameters that are monitored through OSPAR's Joint Assessment and Monitoring Programme. Time series for cod in the Inner Oslofjord (Norway) go back to 1984. Until 2014, annual median Hg-concentrations in cod from the Inner Oslofjord showed both significant upward long-term (whole time series) and short-term (recent 10 years) trends (when 2015 was included, the short-term trend was not significant). However, the median length of the cod sampled also showed upward trends. This may have been caused by low cod recruitment in the area since the start of the 2000s, as indicated by beach seine surveys. To investigate how length would impact the trend analysis, the Hg-concentrations in the cod were normalised to 50 cm. No significant short-term trend in Hg-concentrations could be detected for length-normalised concentrations. The results indicated that most of the upward trend in Hg-concentrations could be attributed to the sampling of larger fish. The reasons for the apparent change in the cod population demography are not conclusive, however, sampling bias must also be considered.

Key Words: *Gadus morhua*, Mercury, the Oslofjord, Body size, Fish, Pollution monitoring, Population characteristics, Sampling

1. Introduction

Mercury (Hg) is an element entering the biosphere from natural and anthropogenic sources. In aquatic systems, anoxic conditions favour the bacterial transformation of inorganic Hg to methylmercury (MeHg), which is the most toxic form of Hg. It acts as a neurotoxin and may cause harmful effects on organisms (Dietz et al., 2013). Methylmercury also has a greater potential for bioaccumulation than elemental Hg and is subject to biomagnification (i.e. the concentration in an organism exceeds that in the organism´s diet due to dietary absorption, thus the concentration increases with higher trophic level; Kidd et al., 2012; Ruus et al., 2015). Therefore, high concentrations of Hg (mostly in the form of MeHg) may accumulate in fish tissues (Julshamn et al., 2011; Teffer et al., 2014). Since fish is a main route of Hg exposure to humans (WHO, 1990), high concentrations of Hg in fish food is of concern in a human health context.

Mercury in Atlantic cod (*Gadus morhua*) is one of many parameters that are monitored through the Norwegian contribution to the Hazardous Substances Theme of OSPAR's (Oslo and Paris Commission) Joint Assessment and Monitoring Programme (JAMP). This contribution is conducted by the Norwegian Institute for Water Research (NIVA) by contract from the Norwegian Environment Agency. JAMP has protocols for sampling and data treatment to facilitate common practice among the contracting countries that border the Northeast Atlantic Ocean (OSPAR, 2008, 2012). The current focus of the Norwegian contribution is on the levels, trends and effects of hazardous substances. The results from Norway and other OSPAR countries provide a basis for a holistic assessment of the state of the marine environment in this region. OSPAR receives guidance from the International Council for the Exploration of the Sea (ICES).

Due to improved regulations, the loads of Hg entering the European marine environment has declined substantially since 1990 (OSPAR, 2009). For instance, riverine inputs of total Hg to the North Sea and Celtic Sea decreased by 75% and 85%, respectively, during the period 1990-2006. In some areas these numbers are partly impacted by changes in the analytical limit of detection (LoD), however, in other areas where LoD was not an issue, there were similar trends, e.g. Rhine/Meuse (71% decrease) and Elbe (69% decrease; OSPAR, 2009). These trends are not always reflected in corresponding trends in biota concentrations. One example is the Inner Oslo fjord, where observations have indicated an upward time trend for Hg in cod muscle in recent years (e.g. Green et al., 2014). A reason for this apparent upward trend may be year-to-year variations in the size of the sampled cod. Mercury accumulates in fish tissue and older and larger fish thereby tend to have higher Hg-concentrations (e.g. Green and Knutzen, 2003; Sackett et al., 2013).

The objective of this study was to describe temporal changes in the Hg-concentrations in cod muscle from the Inner Oslofjord, based on annual sampling since 1984, and analyse how the annual size distribution of the cod may influence time trends.

2. Material and Methods

2.1. Study site and sampling

Oslo, Norway’s largest city (urban area population: 942 000; Figure 1), is located in the Inner Oslofjord. In addition to municipal discharges, the Inner Oslofjord is also affected by industry, leisure boats, ferries, freighters, and cruise ships. Various compounds enter the Inner Oslofjord *inter alia* through surface water/storm water (Ruus et al., 2016). The sediments of the fjord are also contaminated with various persistent “legacy” contaminants, and rivers discharge contaminants from industrial areas and contaminated sites from past industry, as well as from long-range transport to the catchment area (Skarbøvik et al., 2015). There are also two large sewage treatment plants in the vicinity of Oslo, that use the Inner Oslofjord as a recipient. Environmental monitoring and screening for emerging contaminants have shown that different compartments of the fjord (water, sediment and/or organisms) are contaminated with *inter alia* metals, organochlorine compounds, brominated compounds, organotin, polycyclic aromatic hydrocarbons, pharmaceuticals, siloxanes, bisphenols and UV filter chemicals (Ruus et al., 2016; Thomas et al., 2014). The Inner Oslofjord is connected to the Skagerrak region of the North Sea through a narrow sound (the Drøbak sound) with a sill depth of 20 m, which limits water exchange (Staalstrom and Roed, 2016).

Cod were collected annually in the Inner Oslofjord (Figure 1), in accordance with OSPAR guidelines (OSPAR, 2012)[1]. Prior to 2012, the protocols required 25 individuals to be collected annually, but since 2012 only 15 cod were required annually. According to the sample protocol the age of the cod should preferably be within 1-3 years. Smaller fish should if possible be selected to reflect recent influence and reduce the effect of sex, as age determination without dissection is not possible. When the amount of tissue needed for all analyses within an integrated chemical and biological effect monitoring programme is not sufficient, selection of larger fish, or pooling of samples, may be appropriate. If possible, we aspired to collect cod within five size classes, with the same number of individuals within each class (i.e. 3 individuals in each class since 2012): 370 - 420 mm, 420 - 475 mm, 475 - 540 mm, 540 - 615 mm and 615 - 700 mm.

[1] See also http://www.ospar.org/work-areas/hasec

The fish were caught by benthic trawl (15×6.5 m opening; 1600 meshes; mesh size 20×20 mm; equipped with a separation grid), from R/V Trygve Braarud during autumn (mostly November) each year since 2005. Prior to that, the autumn catch was commissioned through local fishermen. The length and weight of each sampled cod were registered. Tissue samples (muscle for Hg analysis) from each fish were prepared and stored frozen (-20 ºC) until homogenisation and analysis. The age of fish was determined by counting the number of opaque and hyaline zones in otoliths.

2.2. Mercury analysis

Mercury (total) was analysed in the muscle samples of cod at NIVA (1985-2011) and Eurofins (2012-1015), and only in 1984 was the analysis conducted at the Norwegian Veterinary institute. An accredited method (NS-EN ISO 12846) was applied, using Cold-Vapour Atomic absorption spectrometry (CV-AAS; Green et al., 2008). Samples of cod muscle were homogenised, using an ultra Turrax™. Homogenised samples (0.1–1 g) were subsequently digested in nitric acid (HNO_3; 5–10 ml) in a microwave oven and diluted to 50–100 ml with distilled, de-ionised water (according to Standard NS-4770). Mercury was then analysed by CV-AAS, at NIVA using a Perkin-Elmer FIMS-400 (Flow Injection Mercury system), at Eurofins using a Teledyne CETAC Technologies QuickTrace M-8000 Mercury Analyser. The analytical Limit of Detection (LoD) was determined as 3 times the standard deviation of the signal:noise ratio in 10 validation samples, and has been 0.005 mg/kg the recent years. NIVA's laboratory and Eurofins are accredited by the Norwegian Accreditation as a testing laboratory according to the requirements of NS-EN ISO/IEC 17025 (2005). Analytical quality was ensured by running blanks (de-ionised water and/or acid) and Certified Reference Materials (CRM; DORM-3 with CRM value 0.382 ± 0.060 mg/kg dry wt., and DORM-4 with CRM value 0.410 ± 0.055 mg/kg dry wt.; National Research Council Canada, Division of Chemistry, Marine Analytical Chemistry Standards) with the samples. If the concentration in the blank samples exceeded twice the LoD, or the analytical result of the CRM fell outside the acceptable range (± 3 standard deviations of ≥20 previous subsequent measurements), a subset of samples was digested/prepared and analysed. If the results of these samples differed more than 20% from the first results, a new subset of all samples were digested/prepared and analysed. Concentrations measured in the CRMs were as follows: In 2011 (the last year of analysis at NIVA; DORM-3) the mean concentration (± standard deviation) was 0.42 ± 0.028 mg/kg dry wt. (n=24 samples analysed over a 29-week period). In 2015 (the last year of analysis at Eurofins; DORM-4) the mean concentration (± standard deviation) was 0.38 ± 0.04 mg/kg dry wt. (n=52 samples analysed over a 35-week period). In 2012 a set of samples was analysed at both NIVA and Eurofins to ensure accordant results between laboratories. The results for Hg showed good agreement between the two laboratories, the difference being less than 20% (Green et al. 2013). Analytical standards were also certified by the participation in international calibration tests, including QUASIMEME twice per year.

2.3. Data treatment and statistical methods

In the JAMP monitoring programme, temporal trends in contaminant concentrations are reported using the OSPAR method (OSPAR, 2008). This model approach has been developed to study time trends for contaminants in biota based on median concentrations (ASMO, 1994). When there are at least 7 years of data, time trends are assessed by fitting either a linear or a non-linear (depending on Akaike Information Criterion values; AIC) regression line to the annual median log-concentrations as a function of time. The non-linear fit is a Loess smoother based on a running six-year interval (Nicholson et al., 1994; Nicholson et al., 1997; Nicholson et al., 1991) with revisions by Fryer and Nicholson (1999). For statistical tests based on the fitted smoother to be valid, the variance should be constant and the residuals for the fitted model should be log-normally distributed (Nicholson et al., 1998). For non-linear trends, the trend is said to be upward or downward if the first and last years are significantly different, using a t-test. The significance level used is $\alpha = 0.05$. For the linear fit, we used ordinary linear regression (function *lm* in R), while for the non-linear fit we used code written specifically for the purpose based on the articles referenced above (code available from the authors on request). The concentrations and trends (using the above mentioned OSPAR method) from the Norwegian contribution to the JAMP monitoring programme are reported each year (e.g. Green et al., 2014).

In this study, we extended the trend analyses of Hg-concentrations in cod muscle from the Inner Oslofjord by including the effect of fish length on concentrations. The relationship between length and Hg-concentrations on the individual level was used to find the expected Hg-concentration for fish of a standard length, which then was used to recalculate annual medians, before re-analysing time trends. For this, we applied seven alternative linear regression models for explaining log(Hg), where log(Hg) could be a linear function of year (as a categorical variable), body length, and/or a second-order polynomial function of body length, and possibly an interaction between year and length (Table 1). The best of these seven models (based on AIC corrected for small sample size, AIC_C) was used to calculate the expected log(concentration) for a standard length (in the case of a year×length interaction, we would calculate expected concentrations for two standard lengths). In other words, the log(concentration) for each fish was projected parallel to the regression line for the respective year. Then we recalculated the annual median, and performed analysis of time trends following OSPAR procedures. Data treatment and statistics were performed using R software (Ver. 3.3.2.).

3. Results and Discussion

Until year 2014, annual median (unadjusted) Hg-concentrations in cod muscle from the Inner Oslofjord showed both significant upward long-term (whole time series) and short-term (recent 10 years) trends ($p < 0.05$). When the year 2015 was included, the short term trend became non-significant ($p = 0.08$, Figure 2).

There are in general several mechanisms that can be hypothesized to explain increases in fish Hg-concentrations. Upward trends in Hg-concentrations have also been registered in freshwater fish species in Norway (see Fjeld et al., 2010). Fjeld et al. (2010) noted that the atmospheric deposition of Hg in the south eastern part of Norway had decreased in recent years (Wängberg et al., 2010), and therefore they anticipated to observe a decrease (or unchanged concentrations) of Hg in fish from inland lakes. They further suggested that increased wash-out of humus substances in inland waters could have led to increased Hg-methylation because of increased microbial activity in the sediments, and thus increased Hg bioavailability. The factors governing methylation processes in sediments are not well understood, and in general, it could be hypothesized that changes in organic carbon input and deep water renewals could alter redox conditions, and thus methylation processes, at the sediment-water boundary (Hammerschmidt and Fitzgerald, 2006). However, there is evidence that the amount of particles in the surface water in the Inner Oslofjord has been reduced during the last decades (Berge et al., 2013), with the likely consequence of reduced organic carbon input to the Oslofjord sediments, which would lead to less favourable conditions for Hg methylation in the Oslofjord sediments (Hammerschmidt and Fitzgerald, 2006). Yet other theoretical mechanisms for increased Hg-concentrations could be reduced photodemethylation in surface waters (Poste et al., 2015) or altered trophic relationships in the Inner Oslofjord (Barcelo et al., 2016), leading to a shift in cod diet to prey items with higher Hg-concentrations. Cod is a generalist, and a comprehensive study of the diet of cod conducted in the Inner Oslofjord (Heggelund, 2001) showed that it forages on a wide variety of prey organisms, such as shrimps (*Caridea*), crabs (*Brachyura*), polychaetes (*Polychaeata*), gadoid fish (*Gadiformes*) and krill (*Euphausiacea*), much like what is shown elsewhere (Heggelund, 2001). It has been shown that trophic relationships can be defined by use of relative abundances of naturally occurring stable isotopes of nitrogen ($^{15}N/^{14}N$, expressed as $\delta^{15}N$ values). The increase in $\delta^{15}N$ is generally 3 to 5‰ between trophic levels (Minigawa and Wada, 1984). The parameter $\delta^{15}N$ has been measured routinely in cod from the Inner Oslofjord only for the past few years (mean ± standard deviation was 17.14 ± 0.65 and 17.56 ± 0.48 in 2013 and 2015, respectively; Green et al. 2014; Ruus et al. 2016), so there is no basis for retrospective comparison. However, in the Outer Oslofjord, close to the Swedish border, $\delta^{15}N$ was measured in cod caught in 1998, showing slightly lower values ($\delta^{15}N$ = 15.33 ± 0.97; Ruus et al. 2002). Though, this can hardly be interpreted as an indication of a slightly higher trophic position of the Inner Oslofjord cod in recent years. It is known that background levels of ^{15}N and ^{14}N may be affected by human activities such as leaching of agricultural fertilizers or sewage plant discharges, and it is shown that the geographical variation in the $\delta^{15}N$ baseline is >5‰ along the Norwegian coast (Green et al. 2014). The Outer Oslofjord is recipient of water from Norway's largest river, the Glomma, which runs through rich agricultural areas.

However, apart from the Hg-concentrations in cod muscle, the median length of the cod sampled has also shown upward trends (Figure 3; Whole time series: $p < 0.04$; 2005-2015: $p = 0.086$; 2004-2014: $p < 0.04$). The correlation between fish length and Hg-concentrations is well known (e.g. Eikenberry et al., 2015; Green and Knutzen, 2003; Jones et al., 2013;

Julshamn et al., 2013; Sackett et al., 2013), and Jones et al. (2013) noted that detecting the influence of changes in Hg exposure will depend on how fish biometrics (length, age and growth rates) are taken into account. Therefore, length was examined more extensively for cod from the Inner Oslofjord.

Of the seven regression models we fitted to log(Hg), based on measurements from 806 cod, the optimal model in terms of AIC_C was a model with a non-linear (second-order polynomial) effect of length and an additional effect of year as a categorical variable (Model 1 in Table 1). The other models were a lot poorer ($\Delta AIC_c >> 2$; negligible Akaike weights). According to this model, the expected Hg-concentration follows the equation:

$$\mathrm{Log(Hg)} = 7.61 \cdot 10^{-3} \cdot \mathrm{Length} - 4.34 \cdot 10^{-6} \cdot \mathrm{Length}^2 + \mathrm{Constant}_{\mathrm{Year}} \qquad \text{(Eq. 1)}$$

where $\mathrm{Constant}_{\mathrm{Year}}$ is a year-specific constant. Thus, this model has the same relationship between length and concentrations (on a logarithmic scale) for all years, but the curve is shifted vertically depending on year (Figure 4; See also Figure S1; Supplemental data). It should be noted that if we assume a linear relationship between log(concentration) and length, the model with interaction (Model 3) is slightly better than a model without (Model 4), due to the different length ranges between years (Figure 4). As Model 1 has no interaction, time trends in estimated log(concentration) for a given length does not depend on which length we choose for normalisation; we chose 50 cm, since this length was represented most years. As mentioned, the normalisation was done by projecting the Hg-concentration in each fish parallel to the regression line for the respective year. The resulting median values (Figure 5b) for 50 cm cod showed some differences from the “raw” medians (Figure 5a; see also Figure S2; Supplementary data). In particular, the length-normalised Hg-concentrations show an increase during the 1990s to approximately 2000, and a fairly stable level thereafter. In contrast, the non-normalised concentrations show an increase until approximately year 2012, and possibly a decrease thereafter. As opposed to non-normalised Hg-concentrations, the length-normalised Hg-concentrations showed no significant changes on both short (2005-2015; p = 0.10) and long (1984-2015; p = 0.10) term (in both cases using a non-linear model following the OSPAR method). This is also valid if year 2015 is not included (p = 0.08 and 0.12, respectively). Excluding the outlying value (1987) from the analysis yielded similar conclusions (p = 0.10 and 0.08, respectively).

From this it was concluded that most of the upward trend in Hg-concentrations in cod muscle from the Inner Oslofjord over the last 10-20 years could be attributed to the sampling of larger fish. When analysing the variation in all cod over the years, 31% of the total variation in Hg-concentration could be explained solely by variation in length, while 41% of the variation could not be explained by year and length. Therefore, more research is needed to scrutinise the influence of other explanatory variables than length.

The sampling of larger fish (on average) in recent years is consistent with results of beach seine surveys carried out in the Inner Oslofjord (Espeland and Knutsen, 2014), showing that cod recruitment in the fjord has been low since the start of the 2000s (especially recruitment since 2008). No cod recruits were observed in the Inner Oslofjord in 2014. Barcelo et al. (2016) showed that the juvenile fish community in nursery habitats along the Norwegian Skagerrak coast has undergone significant shifts in community composition, including a shift to a “warm community” (more pelagic/planktivorous and less demersal species) in the past two decades, involving negative effects on gadoids. It should also be noted that cod in the

Inner Oslofjord may represent different populations with different life histories and size distributions, as shown by genetic analyses (Freitas et al. unpublished results).

There is also the likelihood that changes in sampling bias, albeit small, may impact the trend analyses. During the monitoring programme, there has been an increasing need of tissue, driven by an increasing number of chemical parameters to be analysed (e.g. "emerging contaminants"), potentially creating false patterns in the data. The OSPAR guidelines emphasise that the sampling strategy should take into account the specific objectives of the monitoring programme, including quantitative objectives. Natural variability between the samples should be reduced by an appropriate sampling design. Furthermore, the sampling strategy should cover the demands of as many purposes as possible for both OSPAR and the EU Marine Framework Strategy Directive (MFSD; EU, 2008), as well as meet the requirements of the EU Water Framework Directive (WFD; EU, 2000). As such, it is pointed to the importance of the fish sampled being of reasonable size, giving adequate amounts of tissue for chemical, biochemical and physiological analyses. For cod it is specified that the number of individuals should be at least 12, the size should be within a narrow length range, and the age should, as mentioned, preferably be within 1-3 years. When the amount of tissue needed for all investigations within an integrated chemical and biological effect monitoring programme is not sufficient, selection of larger fish may be appropriate (OSPAR, 2012). We have aspired to collect cod within 5 size classes mentioned above. Any bias towards sampling more of the largest individuals within each size class may in theory cause an increasing annual median of the cod length, and as a result increased Hg-concentrations in this species.

4. Concluding remarks

It is concluded that most of the apparent increase in Hg-concentrations in cod muscle from the Inner Oslofjord during the last 10-20 years could be attributed to the sampling of larger fish. More research is needed to investigate the influence of explanatory variables other than length. Thus, there is not merely a question of whether Hg is increasing in cod, but also a question of why the size of sampled cod is increasing. Two possible explanations (which are not mutually exclusive) for apparent changes in the size distribution are: (1) changes in the population structure of the fish, or (2) changes in sampling bias. Hence, scrutiny of population structure is most likely an important need. In the case of changes in sampling bias, one must acknowledge that the aim of monitoring an ever-increasing number of compounds and emerging contaminants may conflict with the aim of keeping the sampling strategy as constant as possible, especially in face of decreasing funding for sampling and/or decreasing stocks of cod. This can potentially cause problems for the use of the data (without taking into consideration impacting variables, e.g. length) in production of environmental indicators. This also points to the need for knowledge regarding the size distribution of the sampled sub-population in relation to the size distribution of the whole catch. This knowledge could render quantification of sampling bias possible. As an initial step, in the Norwegian JAMP monitoring, the length of all individuals in the catch has been registered since 2016. In general, proper acoustical on board equipment, should also provide valuable information in this regard.

Acknowledgements

The data for this study were gathered through the Norwegian contribution to the Joint Assessment and Monitoring Programme (JAMP), carried out by the Norwegian Institute for Water Research (NIVA) by contract from the Norwegian Environment Agency. The authors thank Ian Allan, Tore Høgåsen, Jarle Håvardstun, Espen Lund, Lise Tveiten and Kine Bæk for their skillful assistance.

Funding: This work was supported by the Norwegian Research Council, through Grant number 234388 (COCO).

Supplementary data

Figures S1 and S2

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Table 1. Parsimony (Akaike Information Criterion; AIC) for eight models (the null model and seven other models) for mercury concentration (Conc) as a function of cod body length and year, pertaining to cod (*Gadus morhua*) from the Inner Oslofjord. The table shows the number of degrees of freedom of the model, the number of model parameters (K), Akaike's Information Criterion adjusted for small sample size (AIC_c), the difference between AIC_c and the minimum AIC_c (ΔAIC_c), Akaike weights based on ΔAIC_c values, and residual sum of squares (Resid. SS). Sample size (N) = 806. Model 4 was considered to be the global model. The models are arranged with decreasing Akaike weights.

Model	Equation	df	K	AIC_c	ΔAIC_c	Akaike weight	Resid. SS
1	log(Conc) ~ Length + Length^2 + Year	35	34	783.28	0	> 0.99	113.88
2	log(Conc) ~ Length + Year	34	33	805.59	22.31	$1.43 \cdot 10^{-5}$	117.39
3	log(Conc) ~ Length×Year	65	64	813.43	30.15	$2.83 \cdot 10^{-7}$	108.61
4	log(Conc) ~ Length×Year + Length^2×Year	97	96	841.55	58.27	$2.22 \cdot 10^{-13}$	101.93
5	log(Conc) ~ Year	33	32	1044.55	261.27	$1.84 \cdot 10^{-57}$	158.33
6	log(Conc) ~ Length + Length^2	4	3	1239.59	456.31	$8.2 \cdot 10^{-100}$	217.49
7	log(Conc) ~ Length	3	2	1244.42	461.14	$7.33 \cdot 10^{-101}$	219.35
8	log(Conc) ~ mean (null model)	2	1	1490.98	707.7	$2.11 \cdot 10^{-154}$	298.59

Figure Legends:

Figure 1. Map showing the Inner Oslofjord. Oslo, the capital of Norway, is situated in the innermost part of this area. The sampling area of cod (*Gadus morhua*) is indicated.

Figure 2. Mercury concentrations (mg/kg wet wt.) in Atlantic cod (*Gadus morhua*) muscle from the Inner Oslofjord (Norway), 1984-2015. The graphs show "raw" data for each year (points), annual medians (bars), and the time trend (with 95% confidence interval) using the OSPAR method (lines). The increase for the whole period (1984-2015) was not statistically significant ($p = 0.08$), while the increase for the last 10 years (2005-2015) was ($p < 0.05$). Excluding the year 2015 would yield both trends significant ($p < 0.05$).

Figure 3. Length (mm) of Atlantic cod (*Gadus morhua*) sampled from the Inner Oslofjord (Norway), 1984-2015. Median and quartiles (box) are depicted. Whiskers represent the largest/smallest value within 1.5·IQR (Inter-Quartile Range) from the quartiles. Data outside the whisker range are shown as individual data points. The increase for the whole period (1984-2015) was statistically significant ($p < 0.04$), but the increase for the last 10 years (2005-2015) was not ($p = 0.086$). Excluding the year 2015 would yield both trends significant ($p < 0.04$).

Figure 4. Mercury concentrations (mg/kg wet wt.; in muscle) as a function of body length (mm) of Atlantic cod (*Gadus morhua*) from the Inner Oslofjord, according to the optimal model (Model 1 in Table 1) for the decades 1984-1989, 1990-1999, 2000-2009 and 2010-2015. The lines represent the concentration (log scale) as a function of length, in a single year.

The black lines in each subplot show the regression fits for each year in the respective decade. The grey lines show regression lines for all years (shown in all plots/decades, for comparative purposes). The horizontal extent of each regression line corresponds to the range of fish lengths that year.

Figure 5. Mercury concentrations (mg/kg wet wt.; log scale) in Atlantic cod (*Gadus morhua*) muscle from the Inner Oslofjord (Norway), 1984-2015. (a) Median concentrations for all cod ("raw" data). (b) Median length-normalised concentrations for cod of 50 cm length. The non-linear regression lines are fitted using Loess regression, according to OSPARs guidelines, for all years 1984-2015 and for the last 10 years 2005-2015. In the left figure (a), the trend is significant ($p < 0.05$), while in the right figure (b), there is no significant trend ($p \geq 0.10$). Trends were analysed using the OSPAR approach, applying a Loess smoother based on a running six-year interval (see Materials and Methods for details; Short term trends are superimposed in Figure S2 in Supplementary data).

Figure 1.

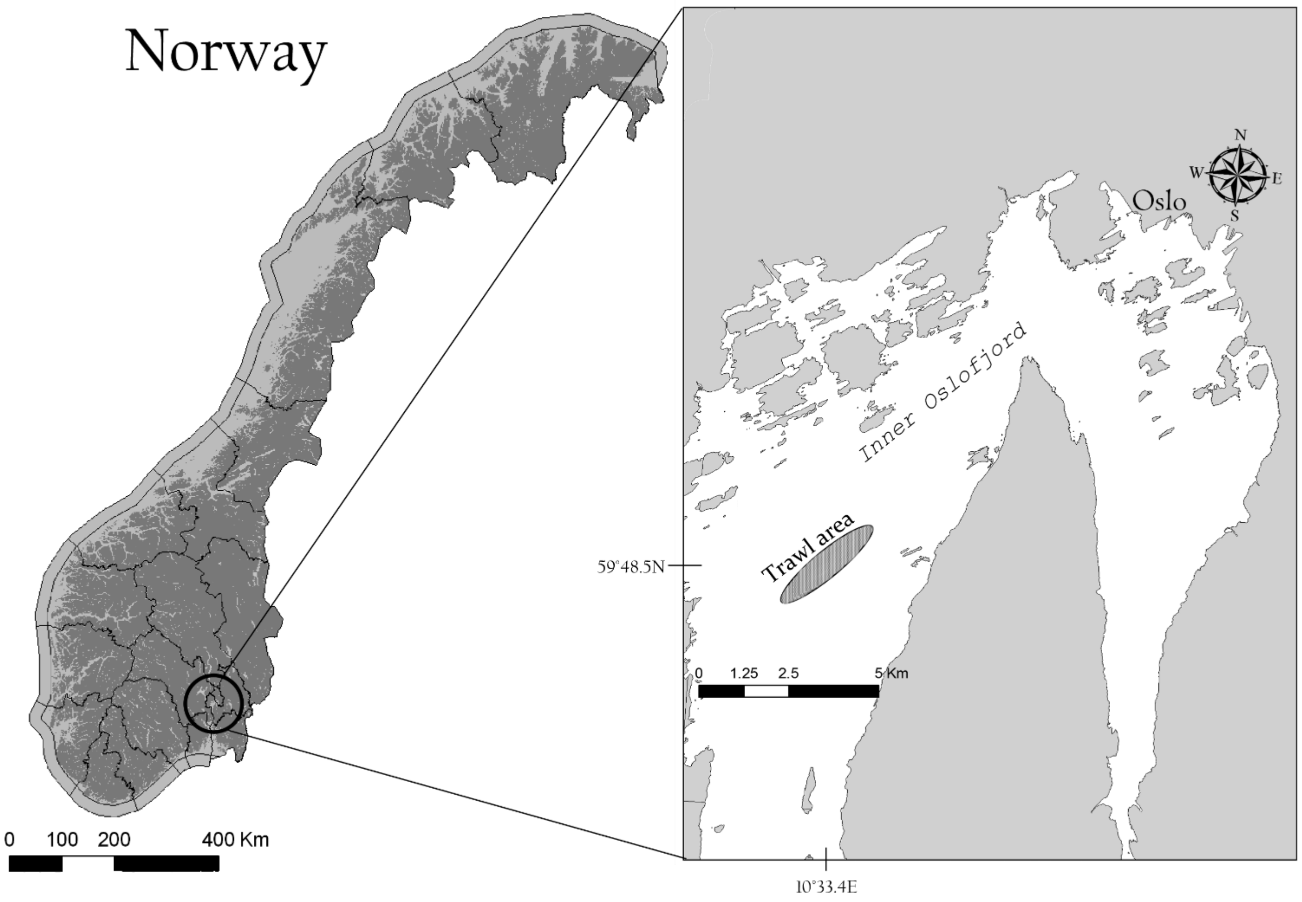

Norway
0 100 200 400 Km
Oslo
N
W
E
S
Inner Oslofjord
Trawl area
59°48.5N
0 1.25 2.5 5 Km
10°33.4E

Figure 2.

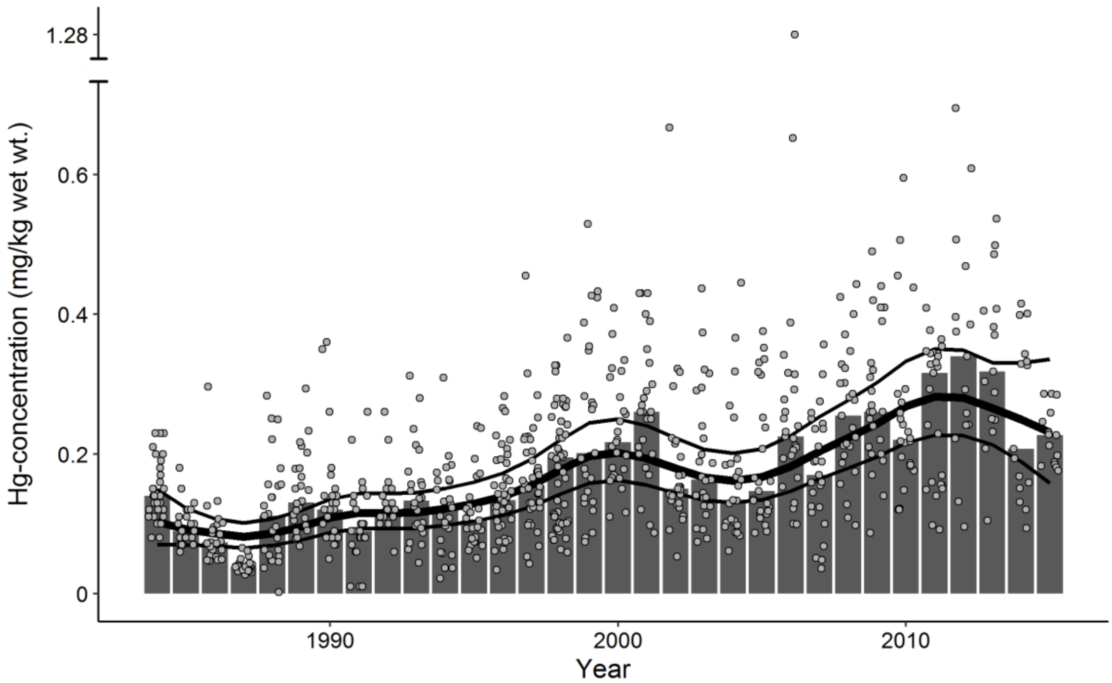

1.28
0.6
0.4
0.2
0
Hg-concentration (mg/kg wet wt.)
1990
2000
2010
Year

Figure 3.

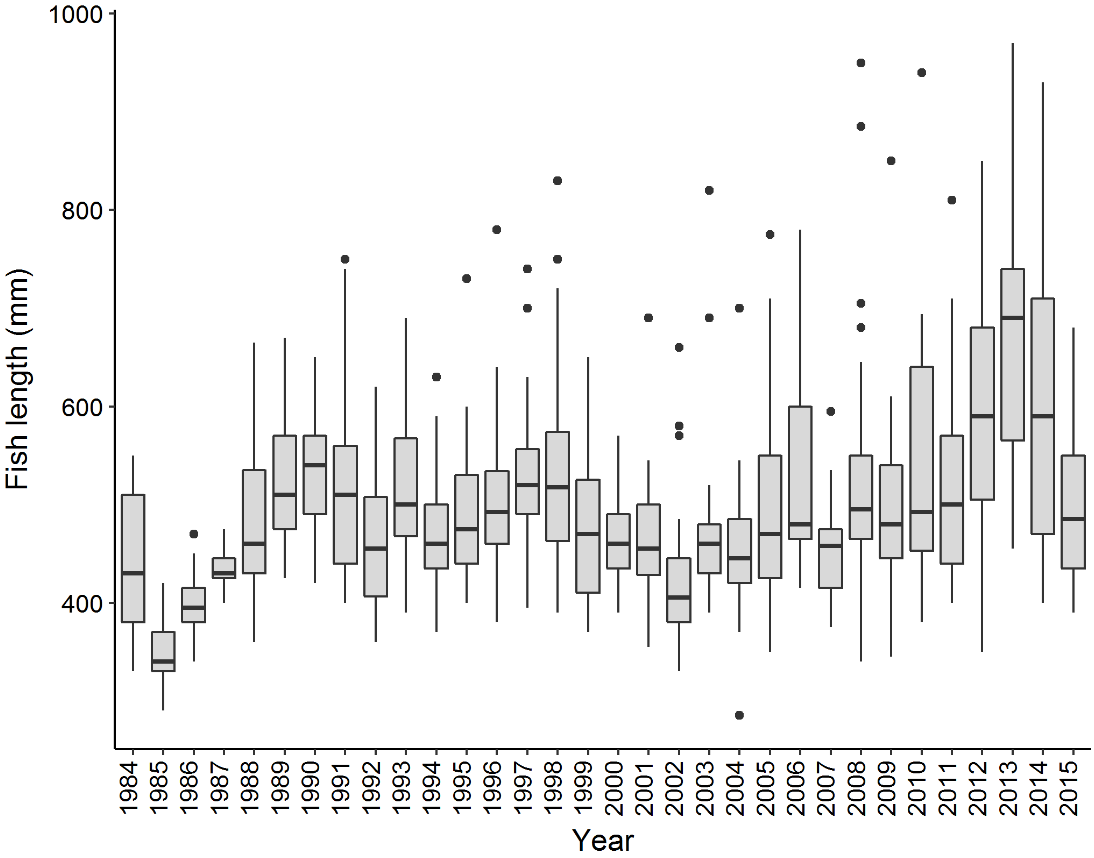

1000
800
600
400
Fish length (mm)
1984
1985
1986
1987
1988
1989
1990
1991
1992
1993
1994
1995
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
Year

Figure 4.

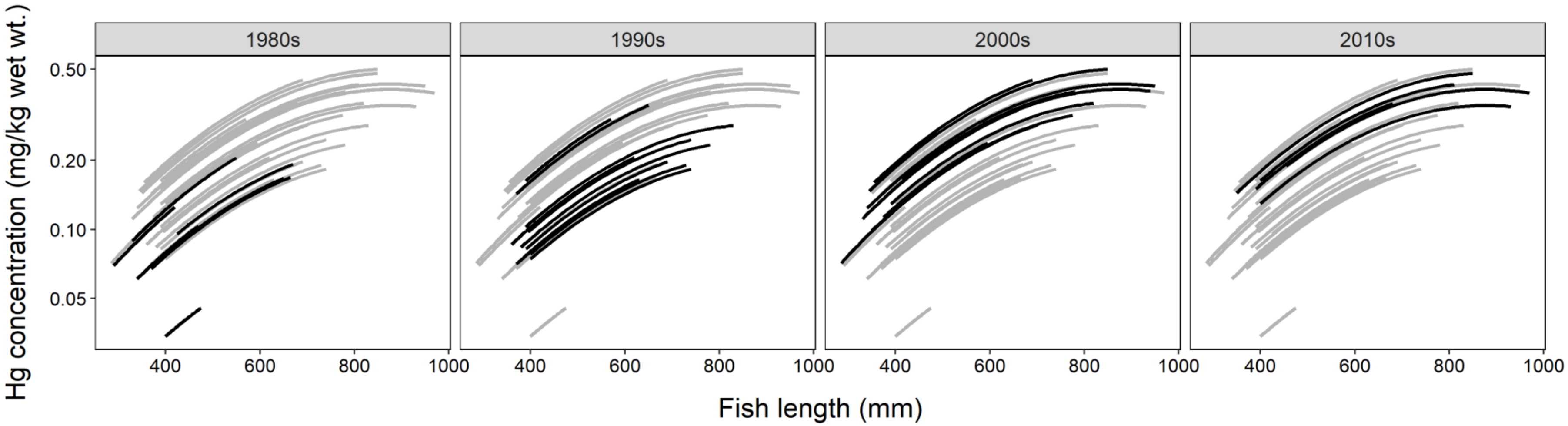
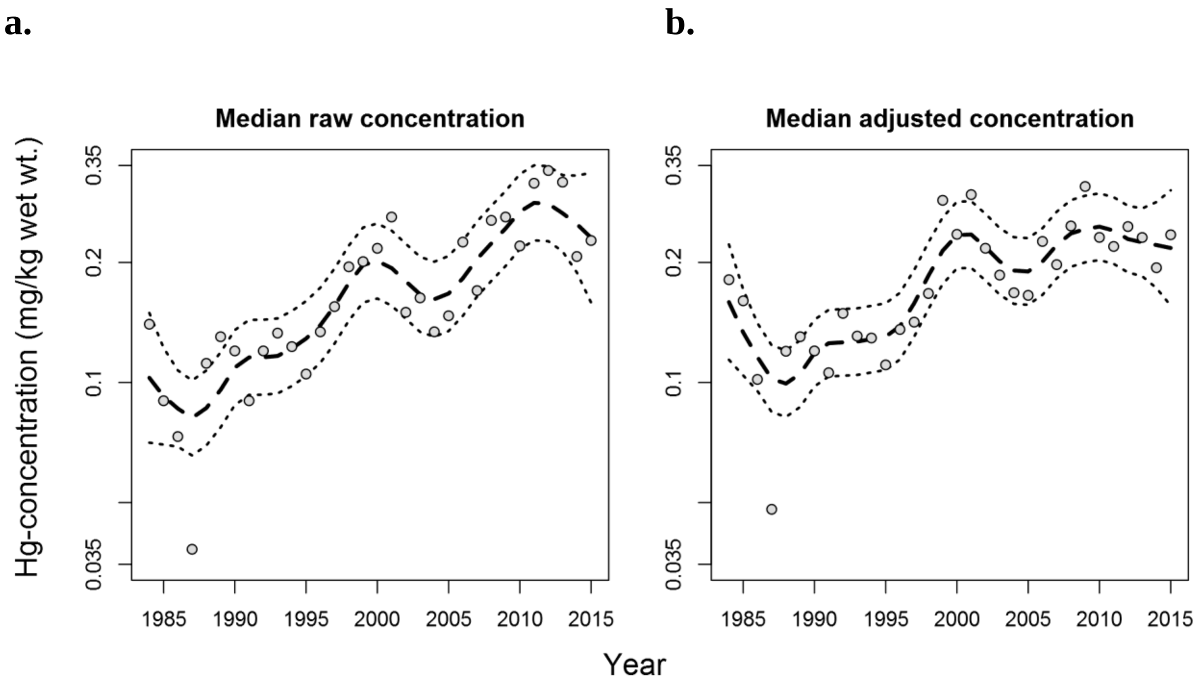

1980s
1990s
2000s
2010s
0.50
0.20
0.10
0.05
400
600
800
1000
Hg concentration (mg/kg wet wt.)
Fish length (mm)

Figure 5.


a.
b.
Median raw concentration
Median adjusted concentration
Hg-concentration (mg/kg wet wt.)
0.35
0.2
0.1
0.035
1985
1990
1995
2000
2005
2010
2015
Year