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Seasonal and year-to-year variation of mercury concentration in perch (*Perca fluviatilis*) in Boreal lakes

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Abstract

We examined the seasonal and year-to-year variations of mercury (Hg) concentrations in populations of perch (*Perca fluviatilis*) from two Boreal freshwater lakes in southeast Norway. Fish Hg concentrations were determined seasonally (spring, summer, autumn) over three years (2010, 2011, 2012), to test the hypothesis that there are substantial changes in fish Hg concentrations over the year (seasonal variation) as well as annually. Concentrations were significantly ($p < 0.0001$) different in the two study lakes, with mean seasonal concentrations varying from 0.24 to 0.36 mg/kg and from 0.29 to 0.37 mg/kg, respectively. The Hg concentrations of both perch populations showed significant year-to-year ($p < 0.0001$) and seasonal variation ($p < 0.01$). The changing fish Hg concentration were + 25 and + 28 % (2010 - 2011) and + 17 and 0 % (2011 - 2012) in the lakes over the three years, respectively. We demonstrate how the significant year-to-year increase is among other variables related to changes in trophic position, shown through stable nitrogen ($\delta^{15}N$) isotope data. The seasonal variation is related to summer growth dilution. Our results highlights the clear need for yearly studies of fish Hg concentrations, rather than the three-year cycle suggested in the current European policy proposed through the Water Framework Directive. Avoiding yearly sampling of fish may result in erroneous conclusions regarding fish Hg concentration time trends.

Key words

Mercury, methylmercury, freshwater fish, temporal change, general linear models

1 Introduction

Inorganic mercury (Hg) can undergo methylation into the toxic and bioaccumulative species methylmercury (MeHg; [1]). MeHg is accumulated in organisms and biomagnified in the aquatic food chain with potential harmful effects on organisms [2], [3]. For humans [4], the Hg toxicity is primarily linked to intake of Hg through fish consumption due to high levels of Hg in predatory fish at the top of the food chain [5]. Norway is one of the many countries that have nation-wide consumption advice for consumption of fresh water fish due to high Hg concentrations [5], [6].

Several recent studies of remote Boreal lakes, both from North America [7], [8] and Scandinavia [9], [10], [11], reports increasing concentrations of Hg in fresh water fish over the last few decades. In Scandinavia, the increase has occurred in a period where reduced or unchanged atmospheric deposition of Hg is reported, due to emission reductions in Europe [12], [13]. Hence, Hg contamination of freshwater fish continues to be an environmental and human health concern in Boreal regions of the world.

A large range of physical, chemical and ecological mechanisms are shown to influence variations of Hg concentrations in fish both seasonally, annually and on a longer time scale (i.e. decades). Examples are catchment distresses; e.g. land use [14] and harvesting [15], water chemistry; e.g. nutrient status [14], levels of organic matter and pH [16], [17], climate impacts; e.g. temperature [18], atmospheric Hg deposition patterns [16], and direct biological influences; e.g. fish size and diet [19], and density of phytoplankton and zooplankton [20]. The large range of mechanisms involved in controlling Hg concentrations in fish indicates the need for studying these variations in detail.

MeHg concentrations increase with trophic position [21], calculated from the ratio of heavier to lighter stable isotopes of nitrogen ($^{15}N/^{14}N = \delta^{15}N$, [22], [23]. $\delta^{13}C$ ($^{13}C/^{12}C$) values provide information on the major source of energy for an organism, and is used to determine what food chain the organisms belong to [24]. The three main lake habitats littoral, pelagial and profundal show contrasting quality of carbon and nutrients [25], leading to differences in MeHg concentrations of primary consumers [25], [26].

Perch (*Perca fluviatilis*) undergo an ontogenetic shift in diet from young to adult [27] shifting from being pelagic zooplankton feeders as juveniles, to benthic invertebrate feeders at intermediate sizes (70 – 125 mm), and becoming piscivorous when large enough (90 – 240 mm, [28]). Hence, a perch may feed at different trophic levels through its lifetime and thus links the food chain transfer of Hg in fresh water lakes. Additionally, perch are generally non-migratory [27], which makes this fish ideal for examining patterns of local Hg concentrations in fresh water species.

In the present study we focus on two Boreal humic lakes, to test the hypothesis that there are significant changes in fish Hg concentrations both seasonally (spring, summer, autumn) and annually. Our main goal was to study the temporal trends in detail over three years (2010, 2011 and 2012) and whether they were consistent across specific sites and sampling events (seasonally and annually). The specific processes and mechanisms involved in explaining the changing Hg concentrations are discussed in detail.

2 Methods

2.1 Study area

We chose the two lakes Breidtjern (59° 6’51’’N 11° 40’42’’E) and Tollreien (60° 17’25’’N 12° 19’8’’E) as study sites, because they both are located in southeast Norway, representing pristine Boreal areas. Additionally, both lakes have previously been included in studies indicating substantial levels of Hg in freshwater fish [11], [29], but relatively low surface water concentrations of Hg (TotHg < 10 ng/L, MeHg < 0.5 ng/L, [30]). This makes both sites ideal for studying Hg bioaccumulation in Boreal freshwater fish.

Both lake catchments are dominated by forest with presence of wetlands, but no agriculture (Table 1). The two lakes are clearly different when comparing sizes of surface water area and catchment area, with Tollreien being the larger lake catchment system (Table 1).

The mean yearly temperature and precipitation is typical for southeast Norway (Table 1); below 6 °C and 900 mm, respectively. Atmospheric deposition patterns reveal the typical south-north gradient of deposition seen in Norway [31], with higher deposition rates of nitrogen (N) and sulphur (S) in Breidtjern (the lake located furthest south). This distribution is also found when comparing temperature and precipitation, with Tollreien located in a colder and dryer area than Breidtjern. There is little difference in the top sediment Hg concentrations (0.30 and 0.33 µg/g, respectively for

Breidtjern and Tollreien) and loading of Hg to the two lakes are assumed similar. This is confirmed with patterns of concentrations of Hg in moss (*Hylocomium splendens*, [32]).

2.2 Sampling and sample preparation

Sampling of fish was conducted seasonally (spring, summer and autumn) from 2010 to 2012, providing a total of 9 sampling events for each lake (Supporting Information (SI), Table S1). Sampling of fish focused on populations of perch (*Perca fluviatilis*) as this species is of major relevance regarding exceeding the Norwegian recommended human consumption limits [6]. Perch is also common in south east Norway and is easily caught in an appropriate sample number. Approximately 25-30 fish were collected from each lake at the specified sampling dates. The total number of fish samples included in the study were n = 283 and n = 279 for Breidtjern and Tollreien, respectively (SI, Table S1). Perch were the only species present in Breidtjern, while both northern pike (*Esox lucius*, a small population) and common roach (*Rutilus rutilus*) were present in Tollreien. However the latter two species were not investigated in the present study.

Gill nets composed of different mesh sizes were used for fishing, so a broad distribution of perch sizes could be targeted. All fish were frozen immediately after sampling and kept at - 18 °C until analysis. Recording of fish data (length, weight and sex) and sampling of muscle tissue, otoliths and operculum were conducted according to the EMERGE (European mountain lake ecosystems: regionalisation, diagnostic and socio-economic evaluation) manual [33]. For fish age determination we used opercula. Fish maturity stage was determined, modified from Dahl, 1917 [34], described in Jonsson and Matzow, 1979 [35].

Sampling of surface water for general water chemistry and Hg speciation was conducted in parallel with the fish sampling. Additional water samples were collected throughout each year, giving approximately 4-5 water samples for each lake each year (Table 2). Samples for Hg speciation were collected using 250 mL fluoropolymere (FLPE) bottles, following ultraclean sampling procedures to avoid contamination [36]. All bottles were previously unused and pre-tested for traces of TotHg (quality tested by Brooks Rand Labs; mean TotHg concentrations = 0.02 ng/L). TotHg and MeHg were sampled in individual bottles to avoid errors caused by loss of Hg during preservation [37], [38]. Samples were stored cold and kept in double plastic bags. Preservation techniques are based on United

States Environmental Protection Agency (USEPA) method 1630 for MeHg [39] and method 1631 for TotHg [40]. Hydrochloric acid (concentrated trace level grade, 1 mL) was added to yield a 0.4 % solution for the MeHg samples. All samples used for TotHg analysis were oxidized with bromine monochloride (BrCl) within 48 hours after sampling.

Samples collected for general water chemistry were collected at the same time and depths (0.5 m below surface) as the Hg samples, but in individual bottles (500 – 1000 mL). All samples were collected at the centre of the lake.

2.3 Fish mercury analysis

More than 90 % of Hg in fish is shown to be present as MeHg [1], and Hg concentrations in fish were determined as TotHg. Wet samples of muscle tissue were analysed by thermal decomposition and direct atomic absorption spectrophotometry (AAS, Lumex Mercury Analyser RA915). For every 10 samples of Hg analysis quality assurance and quality control (QA/QC) measures included method blanks sample duplicates (n = 2) and certified reference material (CRM; DORM-3 fish protein; n = 2). The certified Hg concentration of the CRM used was 0.355 ± 0.056 mg/kg (± uncertainty). The relative standard deviation (RSD) of sample duplicates was always < 10 % and recovery of the CRM within 90 – 110 %. If QA/QC measures were not met, samples were re-analysed.

All fish analyses were performed at NIVA’s laboratory in Oslo, Norway.

2.4 Chemical water analysis

The analytical method for MeHg in water was based on USEPA Method 1630 [39] for determining MeHg in water by distillation, aqueous ethylation, purge and trap, and cold vapor atomic fluorescence spectrometry (CVAFS). The method for TotHg followed USEPA Method 1631 for determining Hg in water by oxidation, purge and trap and CVAFS [40]. The MDL was 0.02 ng/L and 0.1 ng/L (3 standard deviations of method blanks) for MeHg and TotHg, respectively. For both species automated systems were used for analysis (Brooks Rand Labs MERX automated systems with Model III AFS Detector). Due to low concentrations of particulate matter all samples were analysed unfiltered (as discussed in Braaten et al., 2014 [30]).

For every batch of Hg analysis in water (n = 24 individual samples) quality assurance and quality control measures included method blanks (n = 5), blank spikes (n = 5), sample duplicates (n = 3) and matrix spikes (n =3). The relative difference of sample duplicates was < 10 % and < 20 % for TotHg and MeHg, respectively. Recovery of blank spikes and matrix spikes were 80 – 120 % for MeHg and 90 – 110 % for TotHg.

Samples for determination of general water chemistry were analysed according to Norwegian Standard (NS) and European Standard (EN-ISO). pH was measured by potensiometry (NS4720); alkalinity was measured by titration (NS-EN-ISO9963); total N (Tot-N; NS4743), total phosphorous (Tot-P; NS-EN1189) and nitrate (NS4745) was measured by spectrophotometry; and sulphate was measured by liquid chromatography (NS-EN-ISO10304-1). TOC was measured by infrared spectrophotometry (NS-EN1484).

All water analyses were performed at NIVA's laboratory in Oslo, Norway.

2.5 Stable C and N isotopes analysis

All stable isotope ($^{13}C/^{12}C$; $\delta^{13}C$ and $^{15}N/^{14}N$; $\delta^{15}N$) analysis of fish muscle was conducted at the Institute of Energy Technology at Kjeller, Norway. Dried and grinded samples (approximately 1 mg) were combusted in the presence of O_2 and Cr_2O_3 at 1700 °C (Eurovector EA3028 element analyser). Reduction of NO_x to N_2 was done in a Cu oven at 650 °C. Water was removed in a $Mg(ClO_4)_2$ trap before separation of N_2 and CO_2 on a gas chromatography (GC) column (2 m Poraplot Q). The C/N ratio was quantified on the basis of the TCD (thermal combustion detector) results from the GC. N_2 and CO_2 were directly injected on-line to a Horizon Isotope Ratio Mass Spectrometer (IRMS; Nu-Instruments) for determination of $\delta^{13}C$ and $\delta^{15}N$.

To compare inter-lake differences in trophic position, baseline corrections are needed for all $\delta^{15}N$ biota values. Such a baseline correction is normally done with long lived primary consumers such as snails or mussels [41]. However, due to lack of such organism groups in our study lakes we chose to do a baseline adjustment to trophic level 3 (small planktivorous perch) by using the minimum $\delta^{15}N$ values in our data material to correct for inter-lake differences. The specific values of the carnivore fish used for this purpose were 3.9 ‰ (Breidtjern) and 5.4 ‰ (Tollreien). All individual fish data were corrected by subtracting the lake-specific baseline value.

We use trophic magnification slope (TMS) to calculate bioaccumulation in our fish populations, which can be found as the slope b in the following equation [42]:

$$\log_{10}[\mathrm{MeHg}] = \delta^{15}\mathrm{N}(b) + a \qquad (1)$$

2.6 Data treatment and statistical analysis

When Hg concentration in fish is to be compared between lakes, years and seasons, a length and/or age adjustment is needed due to the strong co-variation between Hg concentration and fish size (i.e. length and weight; [19], [43]) and hence, also age (present work). To investigate the Hg concentration variations, we utilised a covariance analysis creating a general linear model. Potential explanatory variables to the model included season and year of sampling, as well as the fish characteristics; length, weight, age, sex, maturity stage and δ^{13}C and δ^{15}N . To evaluate potential changes in the relationship between fish length and Hg concentrations (length*season and length*year) and between fish age and Hg concentrations over time (age*year), interaction terms were also included in the model (also season*year). Additionally, we included the interaction term evaluating change in relationship between δ^{15}N data and Hg concentrations over time (δ^{15}N*year). Other terms that were tested, but not included in the final model due to lack of significance, were lake-specific effects of age (lake*age) and length (lake*length), and remaining stable isotope interactions with year, season and lake (δ^{15}N*season, δ^{15}N*lake, δ^{13}C*year, δ^{13}C*season, δ^{13}C*lake). Specific model variable estimates (t ratios), effects tests (*p*) and residual and actual by predicted plots are shown in SI (including the final and chosen model expression, Table S5, Table S6, Figure S3).

Explanatory variables were chosen, evaluated and included in the model based on significance and the Akaike Information Criterion (AIC). To avoid influence from non-normality and reduce heteroscedasticity in the statistical analysis, the numerical data variables fish Hg concentrations, length, weight and age were transformed to a logarithmic scale. The final adjusted Hg concentrations model specifications include $r^2 = 0.81$, root mean square error (RMSE) = 0.23 and AIC = -5.8. The length and age adjusted population means (least square means) of perch Hg concentrations were adjusted to 13.9 cm and 3.7 years (sample geometric means), respectively.

We also performed a power analysis on our model variables to ensure representativeness of the samples (discussed and presented in SI). All statistical analyses and calculations were performed in JMP 9.0 with a significance level $\alpha = 0.05$, unless otherwise mentioned. Specific statistical tests are described in the text.

2.7 Data sources

Catchment area and wetland area were determined using Geographical Information System (GIS) software (ESRI ArcMap 10.0). The GIS software was used in combination with Web Map Services (WMS) available from The Norwegian Geo Network. Background lake data (i.e. lake size, lake identification number and elevation) were gathered from the National Lake Database of The Norwegian Water Resources and Energy Directorate (NVE).

Atmospheric deposition data for S and N were supplied by The Norwegian Institute for Air Research (NILU). The data set is based on interpolated data from the period 2007 to 2011 ([44]; samples collected on a daily or weekly basis). Top sediment (0 – 0.5 cm) TotHg concentrations in the two lakes are gathered from [30], where concentrations were interpolated by kriging, based on measurement of sediment TotHg in Norway during 2006 – 2008 [45]. Investigations of lake sediments indicated considerable enrichment of Hg in top sediments compared with preindustrial sediments, and correlations between contents of moss Hg and Hg in top sediments, indicate that the top sediment TotHg concentrations can be used as an proxy for TotHg deposition [46]. No quantification of TOC content of the sediments was done.

Temperature and precipitation is presented as the yearly average value for each lake between 1961 and 1990, based on procedures described by World Meteorological Organisation [47]. We chose data from the last available standard reference period in climatology as it represents the “normal” climate conditions in a specific area. The data is available from Norwegian Meteorological Institute [48]. Run-off was estimated for each lake based on models from NVE [49] and shows the annual average between 1961 and 1990 [50].

Daily mean temperature is also available from Norwegian Meteorological Institute [48]. For each of the two study lakes, the geographically closest available meteorological station was chosen to represent the specific lakes.

3 Results and discussion

3.1 Modelled fish Hg concentrations

The chosen general linear model was used to calculate length (13.9 cm) and age (3.7 years) adjusted mean concentrations of Hg in fish for each population (seasonally for both lakes, Figure 1). The yearly (2010, 2011 and 2012) and seasonal (spring, summer, autumn) adjusted concentrations of Hg varied from 0.24 ± 0.02 mg/kg (mean ± 95 % confidence interval) to 0.36 ± 0.03 mg/kg and from 0.29 ± 0.02 mg/kg to 0.37 ± 0.03 mg/kg for Breidtjern and Tollreien, respectively Modelled fish Hg concentrations, across years and seasons, were significantly ($p < 0.0001$) higher in the Tollreien fish population (least square mean: 0.31 ± 0.03 mg/kg) compared to the Breidtjern population (0.26 ± 0.03 mg/kg).

The adjusted Hg concentrations calculated in the present study are similar to results in other studies of Hg in perch from the last decade in similar Scandinavian areas ([9]: 0.45 mg/kg, [17]: 0.28 ± 0.14 mg/kg, [18]: 0.10 – 0.15 mg/kg, [29]: 0.08 – 0.61 mg/kg). Concentrations are also similar to what is documented in similar fish species (yellow perch, *Perca flavescens*) in North America ([51]: 0.35 ± 0.20 mg/kg, [52]: 0.02 – 0.22 mg/kg). All studies represent fish Hg concentrations in perch from areas without local point sources of Hg.

Mean yearly concentrations in Breidtjern and Tollreien between 2010 and 2012 are always lower than the EU’s health advisory limit of 0.5 mg/kg [5]. Calculations show that the advisory limit of 0.5 mg/kg is reached for perch at an age of approximately 8 years (length and weight 21 cm and 18 cm, and 93 g and 64 g for Breidtjern and Tollreien, respectively) in both lakes (Figure 2). Of the total fish collected, 11 and 26 % of the individuals were at this threshold age for Breidtjern and Tollreien, respectively. This is related to the fact that all collected fish from Breidtjern is longer than fish from Tollreien in all age groups, while weight versus length regression lines intersects at relatively low weight (Figure 3). Additionally, the fish seem to live longer in Tollreien compared to Breidtjern (SI, Table S4).

3.2 Fish Hg concentrations related to morphological characteristics

Specific morphological fish characteristics are shown in *Supporting Information* (SI), including length (Table S2), weight (Table S3), age (Table S4), sex, maturity stage (Figure S1) and growth rates. Following here is a short and population-generalised description and discussion of the material, with attention drawn to implications for fish Hg concentrations in the two study populations.

3.2.1 Fish Hg concentrations and fish size

The size (i.e. length and weight), age and sex distributions (Figure 3) are representative indicators of the perch present in typical Boreal dystrophic lake systems in Scandinavia [9], [11], [29], [53]. As expected from accumulation of Hg, model predicted Hg concentrations are significantly correlated to length, weight and age (length and age in Figure 2) in both lakes. Our collected fish from Tollreien are significantly larger, i.e. heavier (50.2 ± 114.6 g) and longer (14.4 ± 4.9 cm) than the fish in Breidtjern (36.2 ± 36.8 g, 14.0 ± 4.0 cm, data in SI, Table S2 and Table S3). Even though the slope of the linear regression of weight and length (Figure 3) for Breidtjern and Tollreien is only weakly different (3.01 and 3.19, respectively), concentrations of Hg is significantly higher in the Tollreien fish populations (Figure 1). A possible explanation could be related to a larger amount of small fish in Breidtjern (e.g. due to absence of piscivorous adult fish), which leads to less stress and lower bioaccumulation at lower trophic levels.

3.2.2 Fish Hg concentrations and fish sex

Recently, sex has also been pointed out as an important characteristic when comparing fish Hg concentrations in different populations [54]. In the present study we see that the mean female fish caught is significantly ($p < 0.0001$) larger (54.6 ± 104.3 g; 15.3 ± 4.9 cm) than the mean male fish (26.6 ± 22.3 g; 13.0 ± 3.2 cm, Figure 3). Following the arguments provided above, this should mean that also Hg concentrations are significantly higher in female fish (all data: 0.38 ± 0.13 mg/kg) compared to male fish (0.32 ± 0.13 mg/kg). However, this only applies to fish from Tollreien ($p < 0.0001$), and not the fish from Breidtjern ($p = 0.38$).

Jarv et al., 2013 [55] show that small perch (< 15 cm) in a brackish environment show no significant difference in either length or weight between male and female. However, for the group of fish longer than 15 cm, female fish are significantly longer and heavier than male fish. This is similar to what we found in the present study where both the mean distributions of length and weight (male: 13.0 ± 3.2 cm/27 ± 22 g; female: 15.3 ± 4.9 cm/55 ± 104 g) and maximum values (male: 24.4 cm/175 g; female: 42.1 cm/1004 g) indicate the same pattern. However, the significant difference between female and male fish Hg concentrations is only significant for the Tollreien populations, and not the Breidtjern population. This is likely related to the fact that the perch live to be older in Tollreien, providing the pattern of size versus Hg concentrations discussed above.

3.2.3 Fish Hg concentrations and fish maturity

For both Breidtjern and Tollreien populations (all data included), the age at 50 % maturity is 1.8 years (SI, Figure S2). The maturity age is similar to what is found for perch populations in previous studies of Swedish [53] and Norwegian lakes [56]. The patterns of maturity in our two lakes indicate similar development of life patterns for perch in the two systems. However, for all age groups in the collected material fish were longer in Breidtjern than in Tollreien (Figure 3). Despite this, both lake's fish Hg concentrations showed similar relationships with age (Breidtjern $r^2 = 0.82$ and Tollreien $r^2 = 0.83$, $p < 0.0001$, Figure 2), identifying the significant relationships between fish weight, length and age (Figure 3). The different sizes at identical age groups between the lakes could reflect different top predator patterns. The fish in Breidtjern is less exposed to piscivorous adult fish.

3.3 Year-to-year variations of fish Hg concentrations

The fish populations from both Breidtjern and Tollreien show a significant increase in Hg concentrations from 2010 to 2012 ($p < 0.0001$), with spring 2012 concentrations being the highest in the data material (Figure 1). Model predicted Hg concentrations in perch from 2012 (least square mean Breidtjern: 0.35 ± 0.03 mg/kg, Tollreien: 0.36 ± 0.03 mg/kg) are higher than any of the previous two years. Based on autumn data, the concentration increase between 2010 and 2012 is 45.8 % and 27.6 % in Breidtjern and Tollreien, respectively.

Interestingly, the concentration change in the two lakes is differently distributed between the three years. In Breidtjern the increase was 25.0 % from 2010 to 2011, and 16.7 % from 2011 to 2012. Similar numbers for Tollreien are 27.6 % from 2010 to 2011, while there was no change between 2011 and 2012 (0.0 %). Possible explanations for *i*) the general increase in Hg concentrations from 2010 to 2012, and *ii*) the year-specific increase from 2010 to 2011 and from 2011 to 2012, will be provided and discussed further on.

While studies linking historical fish Hg concentrations with new data often compare selected years in the study period, our data highlights the need for yearly monitoring of fish populations to fully understand the Hg dynamics. The EU Water Framework Directive [57] advises monitoring every three to six years, which means that the variation seen yearly from 2010, 2011 and 2012 in the present study might go unnoticed. This in turn may result in erroneous conclusions regarding the dynamics of Hg concentrations in fish, and also our understanding of the biogeochemical cycling of Hg in the Boreal environment in general. Based on fish Hg concentrations found in Breidtjern and Tollreien between 2010 and 2012, it is our recommendation that yearly monitoring of both fish and other possible explanatory variables for fish Hg concentrations is needed.

3.4 Seasonal variations of fish Hg concentrations

Seasonal variation of adjusted Hg concentrations in the fish populations showed significantly ($p < 0.01$) changing concentrations between spring, summer and autumn populations. Mean fish Hg concentrations are higher in the spring compared to the summer and autumn (Student's t-test: $p < 0.05$). Least square means Hg concentrations were lower in the autumn compared to the summer, but not significantly different (Student's t-test: $p = 0.10$, Figure 1).

For both lakes, the seasonal fish Hg concentrations are always higher in the spring compared to the summer (Figure 1). Spring Hg fish concentrations are 0.29 ± 0.02, 0.28 ± 0.02 and 0.36 ± 0.03 mg/kg for 2010, 2011 and 2012 in Breidtjern, and 0.34 ± 0.03, 0.35 ± 0.03 and 0.37 ± 0.03 mg/kg in Tollreien. Possible explanations for the spring maximums include energy-demanding spring spawning [58] and summer growth dilution [52]. Because concentrations increase again from summer to autumn (not in 2010), a summer growth dilution is the most likely cause. The growth dilution is a result of faster growth during warmer months [52], which causes fish Hg concentrations to decrease. For

Breidtjern and Tollreien the mean body weight of the collected fish increases from 35.0 to 40.2 g and from 33.6 to 59.6 g, respectively (variations not significant), between spring and summer (all data included), supporting the growth dilution hypothesis.

The significantly changing seasonal fish Hg concentrations have major impacts on timing of sampling for fish Hg monitoring. Based on the present data it is important to sample fish consequently at the same time of year to get comparable yearly data. Future studies and monitoring programmes for fish Hg concentrations should be careful as to when samples are collected. Since fish Hg concentrations are highest in the spring and autumn, it is our recommendation that one of these two seasons is chosen. However, the two seasons show significantly different concentrations (spring generally higher than autumn), and long-term monitoring studies should stick to one chosen season. Based on the fact that perch are a spring spawning species, sampling in the autumn is preferable to make sure the populations are not undergoing spawning at the time of sampling.

3.5 Explanatory variables for changing fish Hg concentrations

Since less Hg is shown to be atmospherically transported and deposited over the last decades in the regions where Breidtjern and Tollreien (and other Scandinavian lakes showing increased fish Hg concentrations) are located [12], the increase of Hg concentrations in fish populations are related to processes in the catchment or within the lake itself. As both lakes showed an increase from 2010 to 2012, and similar seasonal patterns (Figure 1), processes on a regional scale could be suggested. But, since our two lakes show different increases the three study years, processes on a smaller scale are also likely to occur. The mechanisms controlling these seasonal and yearly variations of fish Hg concentrations are however not clearly defined in the literature. Parameters suggested to influence temporal fish Hg dynamics are water chemistry (organic matter and pH: [10], [17], [59]), climate factors (temperature: [18]), dietary patterns and trophic position [18].

3.5.1 Water chemistry

We observe a significantly higher fish Hg concentration in Tollreien compared to Breidtjern every three study years (Figure 1). Mean fish Hg concentrations for all data in the study is 0.26 ± 0.03 and 0.31 ± 0.03 mg/kg for Breidtjern and Tollreien, respectively. This reflects the trends of different surface water chemistry (Table 2), with mean annual concentrations of TotHg, MeHg and %MeHg being significantly higher in Tollreien than Breidtjern for all three study years (t-tests, $p < 0.001$). Tollreien is also more humic (mean TOC concentrations range 13.1 - 16.0 mg/L) than Breidtjern (8.1 – 9.6 mg/L). Together this highlights how the availability of aqueous Hg species to freshwater food chains (through catchment TOC transport: [60], and Hg speciation in water: [43], [61]) controls the general fish Hg concentration levels. However, as there is no significant yearly variation seen in the water chemistry, this does not explain the short-term (yearly and seasonally) variation of fish Hg concentrations. The same arguments apply to other water chemistry parameters thought to influence Hg speciation in lake water and subsequently fish growth rates, including pH and alkalinity [62], Tot-N and Tot-P [30], and sulphate [63].

3.5.2 Climate factors

Of morphological characteristics that could influence fish Hg concentrations (in the present study: length, weight and age, Figure 2), particularly growth rates are previously shown to be influenced by climate factors (e.g. temperature, [64]). Breidtjern is located further south than Tollreien (Table 1), and the mean annual temperature is higher at the Breidtjern location (5.1 and 3.4 °C, respectively, yearly average between 1961 and 1990). However, the mean annual (Table 1) and monthly (data in SI, Figure S4 and S5) temperature did not significantly vary between the three study years (comparisons for each pair by Student's t, all $p > 0.05$), and cannot explain the significant yearly variation between 2010 and 2012. This is also evident when studying mean annual fish weights; neither in Breidtjern nor Tollreien does the fish weight change significantly from year to year ($p > 0.05$, specific data not shown). However, the significantly warmer summer months compared to the spring and autumn months (Figure S4 and S5), are thought to improve lake conditions leading to increased fish growth rates over summer. This again leads to lower fish Hg concentrations in the summer months (growth dilution, Figure 1), and temperature is at least indirectly responsible for the seasonal fish Hg dynamics discussed above.

3.5.3 Dietary patterns and trophic position

Mean seasonal $\delta^{13}C$ variations show no significant seasonal variations ($p > 0.05$, Figure 4), indicating that the fish populations collected in the present study do not change dietary patterns (within the same year). However, mean yearly $\delta^{13}C$ levels increase (i.e. less negative values) significantly (comparisons for each pair using Student's t, $p < 0.05$) in Breidtjern from 2010 (-28.7 ‰) to 2011 (-28.4 ‰) and from 2011 to 2012 (-28.1 ‰). This small change in mean $\delta^{13}C$ signal could indicate a shift in the carbon sources for the food chain, as previously documented for perch in Finland [18]. This could again lead to changing fish Hg uptake due to habitat specific uptake of MeHg in primary consumers [26].

However, based on present data, it will only influence the fish Hg concentrations seen in Breidtjern, and cannot explain the yearly increase observed in Tollreien. In Tollreien, there is in fact a significant decrease (i.e. more negative values) seen from 2010 (-29.6 ‰) to 2011 (-30.2 ‰) and 2012 (-28.1 ‰) in $\delta^{13}C$ levels. As is also discussed previously, $\delta^{13}C$ did not contribute to significantly increase the explanatory power of our fish Hg concentrations model, and was hence not included in the model. Based on this we conclude that a possible change in $\delta^{13}C$ signal is not responsible for the changing seasonal and year-to-year variation of perch Hg concentrations documented in the present study.

In Breidtjern there is a significant increase in $\delta^{15}N$ levels from 2011 to 2012 ($p < 0.01$, Figure 4), but no difference between 2010 and 2011. Since the fish Hg concentration increase in the lake is relatively large in both years (25 and 17 %, respectively), it is clear that $\delta^{15}N$ patterns cannot explain the increase alone. However, the fish caught in 2012 have a mean trophic position higher than the fish caught in 2010 and 2011, and could at least explain parts of the increasing fish Hg concentrations in Breidtjern.

In Tollreien it is a significant ($p < 0.05$) decrease in $\delta^{15}N$ levels from 2010 to 2011, while the fish Hg concentrations increase with 28 %. From 2011 to 2012, $\delta^{15}N$ levels increase significantly ($p < 0.0001$), while fish Hg concentrations show no increase (0 %). But, since it significantly increased the explanatory power, data of $\delta^{15}N$ were added to our fish Hg concentrations model. This implies, as for Breidtjern, that $\delta^{15}N$ can, if not alone, at least partly, explain the year-to-year increase in mean fish Hg concentration from 2010 to 2012 (Figure 1).

A point that could clarify the observed relationship between year-to-year increase in fish Hg concentrations and $\delta^{15}N$ levels is the bioaccumulation rates in the two fish populations (Figure 5). As is previously discussed, the perch collected from Tollreien is larger than the perch collected from Breidtjern (Figure 2). However, Hg (i.e. MeHg) accumulates at a slower rate in Tollreien (trophic accumulation slope (TMS) = 0.43, all data) compared to Breidtjern (TMS = 0.50). We hypothesise that this is related to the group of large predatory fish in Tollreien that feed on smaller fish. This will lead to shortened life history for the smaller fish due to stress, and they will not accumulate as much MeHg as fish without this top predator pressure (as for Breidtjern perch). Hence, the TMS will be less steep than what is present in Breidtjern where the smaller fish live longer.

The TMS are decreasing in both our lakes from 2010 through 2011 to 2012 (Figure 5). In 2010 TMS values are 0.56 in both Breidtjern and Tollreien, while TMS in 2011 and 2012 are 0.45 and 0.47, and 0.42 and 0.34 in the two lakes, respectively. This indicate that MeHg is accumulating slower in the fish populations every year, suggestion that biological mechanisms are responsible for the changing fish Hg concentrations. An explanation for the reduced TMS could be increased pressure on the fish population, for example from exterior factors we have been unable to access in the present data set, leading to shorter life histories and reduced MeHg accumulation in the fish populations. This reflects again the significant contribution from $\delta^{15}N$ levels on fish Hg concentrations, and hence explains the increasing fish Hg concentrations observed.

4 Conclusions

We document in the present study that populations of perch from two boreal freshwater lakes showed significant year-to-year and seasonal variation of Hg concentrations from 2010 to 2012. We hypothesize that the increase from 2010 to 2012 is, among other variables, related to changes in fish trophic positions, and that seasonal changes are related to summer growth dilution. An increased understanding of these short-term variations of Hg in freshwater lake systems (including water and biota concentrations), is necessary to be able to consider the future long-term development of fish Hg concentrations in Boreal areas.

Our data highlights the clear need for yearly monitoring of fish Hg concentrations, rather than the three-year cycle suggested in the current European policy proposed through the Water Framework

Directive. Avoiding yearly monitoring may result in erroneous conclusions regarding fish Hg concentration trends. Additionally, caution should be made regarding what time of year samples are collected.

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Figure and tabel legends

Figure 1 The seasonal (spring, summer, autumn) and year-to-year (2010, 2011, 2012) variations of fish Hg concentrations for the Breidtjern (filled bars) and Tollreien (open bars) populations. Shown are concentrations for all available data (n = 562). Error bars represent +/- 95 % confidence interval.

Figure 2 Plots of predicted Hg concentrations versus length (left) and age (right). The linear Hg concentrations versus length regression lines are similar for the two lakes; Breidtjern (dotted lines and open circles) $r^2 = 0.79$, $y = -5.1 + 1.4x$; Tollreien (solid lines and open triangles) $r^2 = 0.85$, $y = -4.7 + 1.4x$, both $p < 0.001$. Identical data for the linear Hg concentrations versus age regression lines are; Breidtjern $r^2 = 0.82$, $y = -1.9 + 0.6x$; Tollreien $r^2 = 0.83$, $y = -1.9 + 0.6x$, both $p < 0.001$. Shown are also at what length and age the fish in the two populations exceed European Union limit for maximum Hg concentration in fish (0.5 mg/kg, UNEP, 2002).

Figure 3 The relationship between length and sex (top) for the Breidtjern (left) and Tollreien (right) populations, length and weight (bottom left) and length and age (bottom right) for all data included in the study (n = 562). The linear length versus weight regression (Tollreien; $r^2 = 0.98$, and Breidtjern; $r^2 = 0.99$) is based on log transformed values of both weight and length. Slopes for the Breidtjern data (dotted line; $y = -4.6 + 3.01x$) are similar to the Tollreien data (solid line; $y = -5.1 + 3.19x$). The regression lines intersect at $(x, y) = (12.5, 19.5)$, indicated by the solid lines. The linear length versus age regression is also based on log transformed values; Tollreien (solid line) $r^2 = 0.72$, $y = 2.1 + 0.3x$; Breidtjern (dotted line) $r^2 = 0.85$, $y = 2.2 + 0.4x$. The horizontal line in the length versus sex plots, show the mean length for the lake-specific data sets (14.0 and 14.4 cm for Breidtjern and Tollreien, respectively).

Figure 4 Box plots of seasonal $\delta^{13}C$ (top) and $\delta^{15}N$ (bottom) data in Breidtjern (above bold horizontal line) and Tollreien (below bold horizontal line). The horizontal line inside the box represent seasonal median value, the ends of the box represent 75th and 25th quantiles, and the end of the lines represent +/- 1.5*interquartile range. Values outside this range are shown as circles (Breidtjern) and triangles (Tollreien).

Figure 5 Log Hg concentrations (x-axes, ng/g) versus $\delta^{15}N$ values (y-axes, ‰, adjusted) for all fish included in the present study. Shown are Breidtjern (left panel, circles) and Langtjern (right panel, diamonds). Trophic magnification slopes for the years 2010 (unbroken lines), 2011 (dotted line) and 2012 (dashed line) is 0.56, 0.45 and 0.42 for Breidtjern. Similar numbers for Tollreien are 0.56, 0.47 and 0.34.

Table 1 Location, catchment characteristics, deposition patterns and climate variables presented for the 2 lakes in the study.

Table 2 Mean (± one standard deviation (SD)) annual water Hg speciation and general water chemistry for Breidtjern and Tollreien. Data from 2010, 2011 and 2012 is based on n = 3, n = 5 and n = 3 sampling dates, respectively. Where no SD is indicated, only 1 sample is considered.

Figures

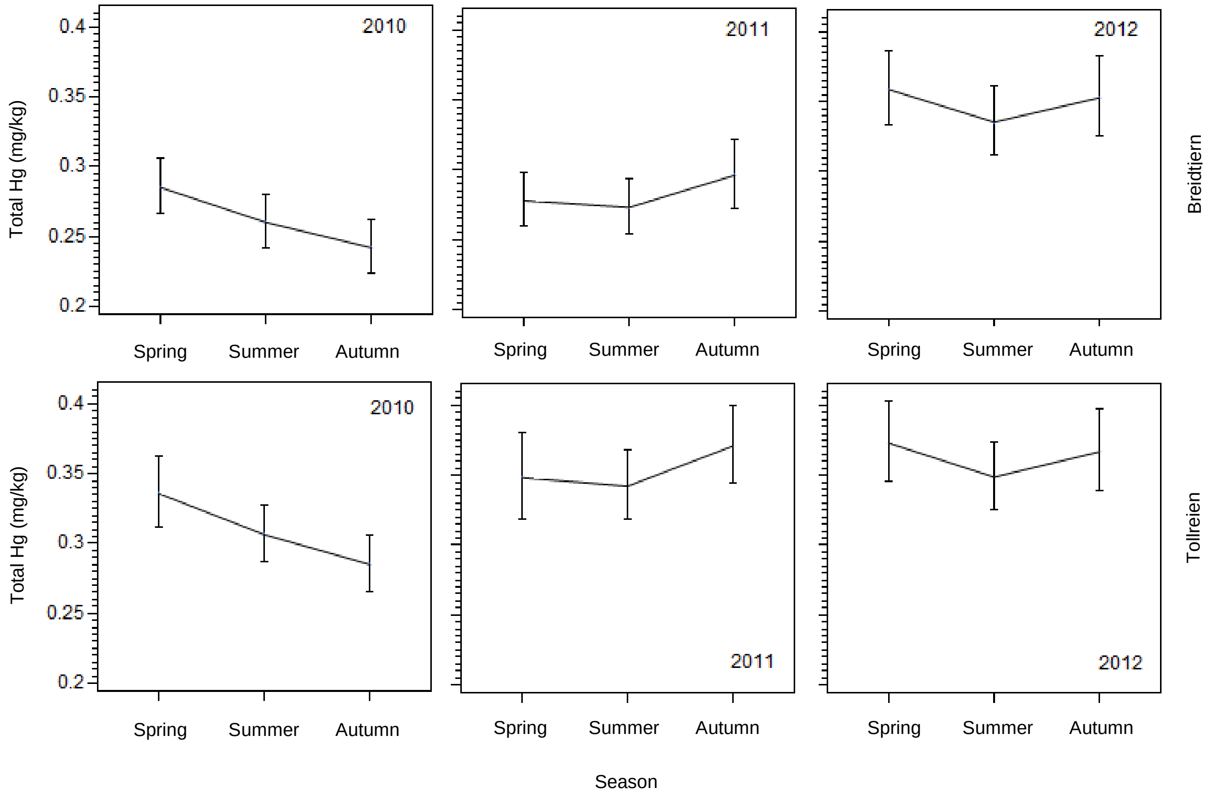

Figure 1 The seasonal (spring, summer, autumn) and year-to-year (2010, 2011, 2012) variations of fish Hg concentrations for the Breidtjern (top panels) and Tollreien (bottom panels) populations. Shown are concentrations for all available data (n = 562). Error bars represent +/- 95 % confidence interval.

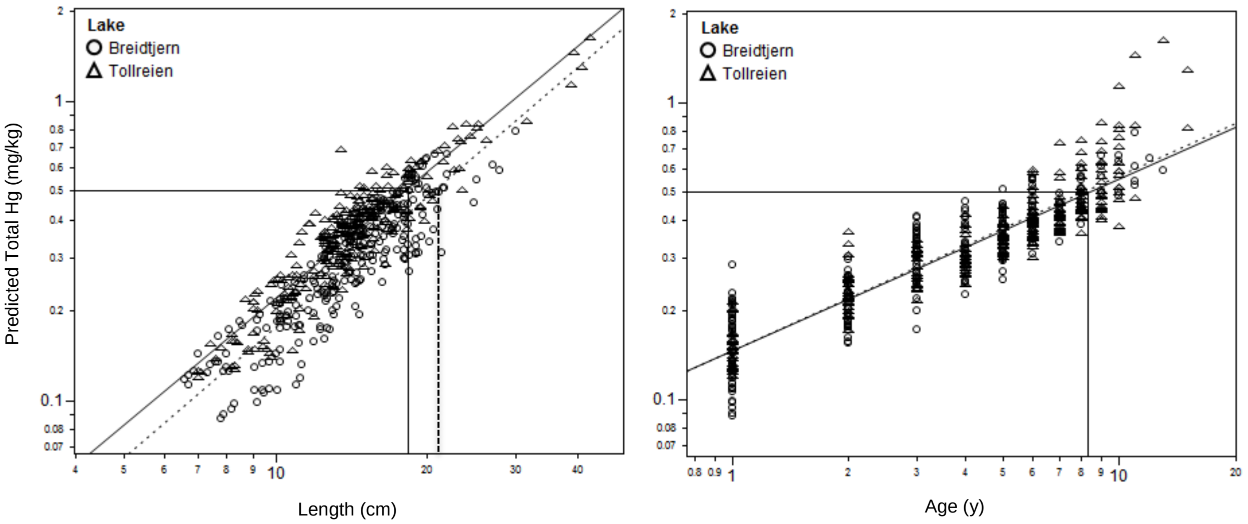

Figure 2 Plots of predicted Hg concentrations versus length (left) and age (right). The linear Hg concentrations versus length regression lines are similar for the two lakes; Breidtjern (dotted lines and open circles) $r^2 = 0.80$, $y = -5.1 + 1.4x$; Tollreien (solid lines and open triangles) $r^2 = 0.85$, $y = -4.7 + 1.4x$, both $p < 0.001$. Identical data for the linear Hg concentrations versus age regression lines are; Breidtjern $r^2 = 0.82$, $y = -1.9 + 0.6x$; Tollreien $r^2 = 0.83$, $y = -1.9 + 0.6x$, both $p < 0.001$. Shown are also at what length and age the fish in the two populations exceed European Union limit for maximum Hg concentration in fish (0.5 mg/kg, UNEP, 2002).

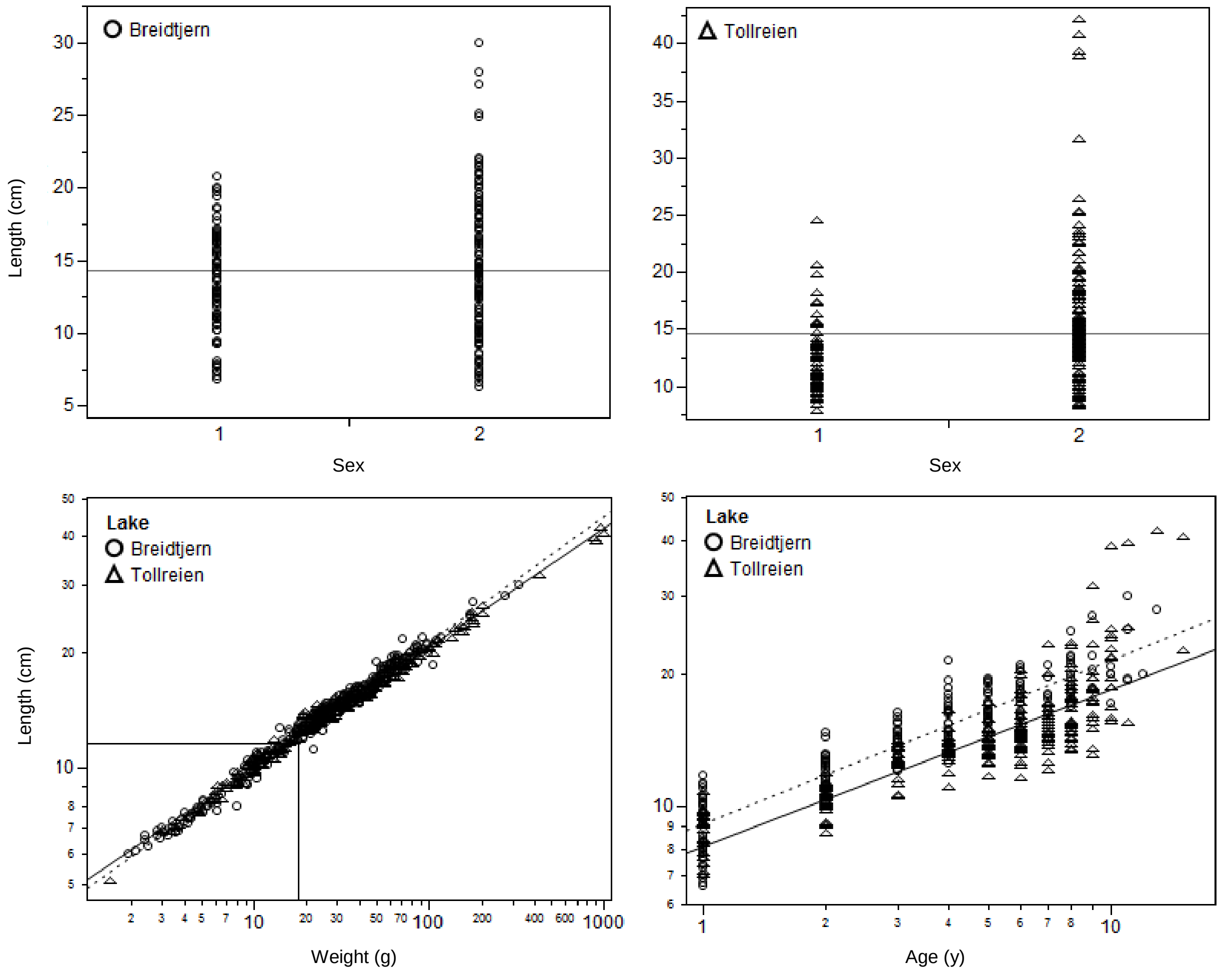

Figure 3 The relationship between length and sex (top) for the Breidtjern (left) and Tollreien (right) populations, length and weight (bottom left) and length and age (bottom right) for all data included in the study (n = 562). The linear length versus weight regression (Tollreien; r^2 = 0.98, and Breidtjern; r^2 = 0.99) is based on log transformed values of both weight and length. Slopes for the Breidtjern data (dotted line; y = -4.6 + 3.01x) are similar to the Tollreien data (solid line; y = -5.1 + 3.19x). The regression lines intersect at (x, y) = (12.5, 19.5), indicated by the solid lines. The linear length versus age regression is also based on log transformed values; Tollreien (solid line) r^2 = 0.72, y = 2.1 + 0.3x; Breidtjern (dotted line) r^2 = 0.85, y = 2.2 + 0.4x. The horizontal line in the length versus sex plots, show the mean length for the lake-specific data sets (14.0 and 14.4 cm for Breidtjern and Tollreien, respectively).

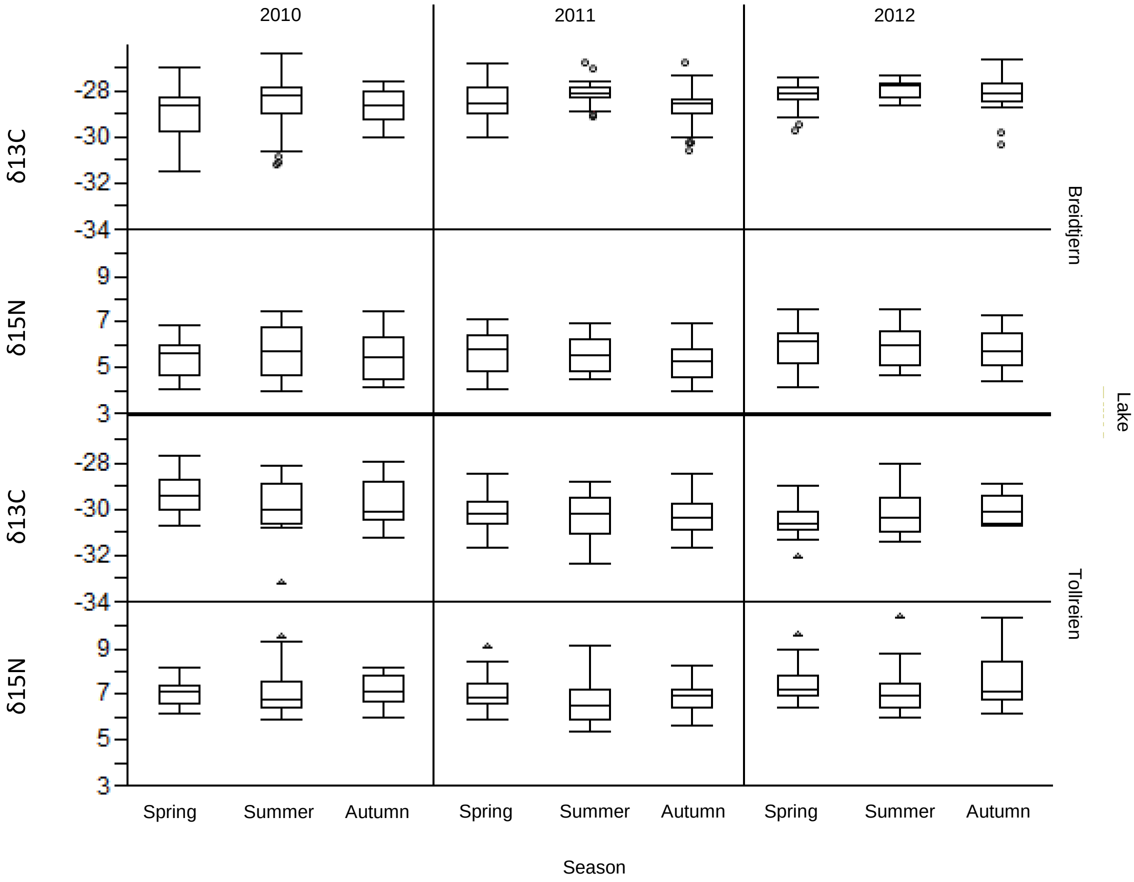

Figure 4 Box plots of seasonal $\delta^{13}C$ (top) and $\delta^{15}N$ (bottom, not adjusted) data in Breidtjern (above bold horizontal line) and Tollreien (below bold horizontal line). The horizontal line inside the box represent seasonal median value, the ends of the box represent 75th and 25th quantiles, and the end of the lines represent +/- 1.5*interquartile range. Values outside this range are shown as circles (Breidtjern) and triangles (Tollreien).

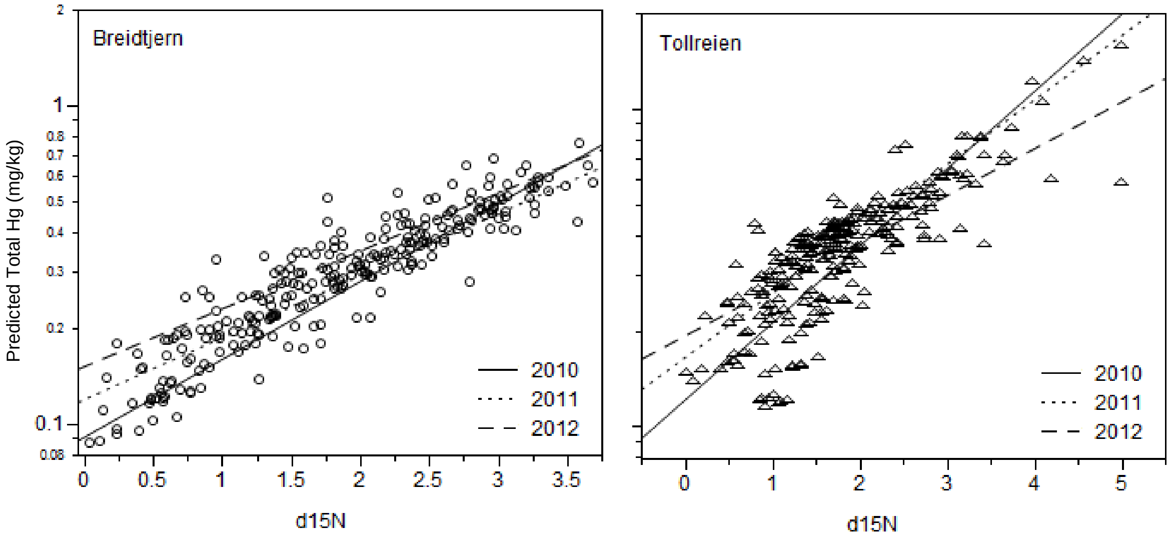

Figure 5 Log Hg concentrations (x-axes, ng/g) versus δ^{15}N values (y-axes, ‰, adjusted) for all fish included in the present study. Shown are Breidtjern (left panel, circles) and Langtjern (right panel, diamonds). Trophic magnification slopes for the years 2010 (unbroken lines), 2011 (dotted line) and 2012 (dashed line) is 0.56, 0.45 and 0.42 for Breidtjern. Similar numbers for Tollreien are 0.56, 0.47 and 0.34.