

## Seasonal variation of mercury and $\delta^{15}\text{N}$ in fish from Lake Heddalsvatn, southern Norway

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### ABSTRACT

Seasonal variations of mercury (Hg) concentration in muscle tissue of northern pike (*Esox lucius* L), European whitefish (*Coregonus lavaretus* L) and European perch (*Perca fluviatilis* L) have been investigated in Lake Heddalsvatn, southern Norway. Our data confirmed seasonal differences in the Hg concentrations in fish, but the causes for that may vary depending on fish species. Length (pike and perch) and age (perch and whitefish) were the strongest explanatory factors for the Hg fluctuations. Fish caught in December (pike and whitefish) had the highest Hg concentrations, while the concentrations in May were higher than in September for all 3 species, statistically significant for perch ( $P < 0.0001$ ) and close to significant for pike ( $P = 0.07$ ). Higher  $\delta^{15}\text{N}$  signatures were found in December and May compared with September, but these differences might be related to fish body size variations. There was a large range of  $\delta^{13}\text{C}$  signatures in the investigated fish, but no significant differences between seasons neither within nor between species.

**Key words:** Mercury, fish, seasonal variations, stable isotopes, Lake Heddalsvatn.

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### INTRODUCTION

Mercury (Hg) is naturally present in waters at very low concentrations. The more toxic form of Hg, the monomethylmercury,  $\text{CH}_3\text{Hg}^+$  (MeHg), is a toxicant readily accumulated by aquatic biota. Although in the water phase MeHg only represents 3-5% (interquartile range) of the total Hg in boreal lakes in South-East Norway (Braaten *et al.*, 2014), more than 95% is in the methylated form in muscle tissue and directly obtained from the diet (Huckabee *et al.*, 1979; Bloom, 1992). Therefore, the methylation of inorganic Hg is a crucial process regulating Hg levels in fish (Korthals and Winfrey, 1987). Microbial Hg transformations play an important role in the Hg cycle, the production of MeHg occurs primarily through methylation of inorganic Hg by sulfate-reducing bacteria (Benoit *et al.*, 2001) and iron-reducing bacteria (Kerin *et al.*, 2006).

In addition to the methylation rates, there exist a number of other environmental, ecological and physiological factors influencing the bioaccumulation of Hg in aquatic ecosystems (Watras *et al.*, 1998; Ullrich *et al.*, 2001). These factors may mislead the interpretation of the results of many studies, leading to erroneous conclusions and hiding other possible relationships (MassDEP, 2006). Therefore, while apparent Hg concentrations in specific fish tissue may vary over time, the relative influence of factors such as environmental fluctuations (pH, temperature, dissolved organic carbon, nutrient availability) and changes in fish physiology should be

taken into account when results are interpreted (Mass-DEP, 2006). Concentrations of Hg can be influenced by fish size (Gilmour and Riedel, 2000), a factor that can easily vary among sampling periods. Hence, seasonal changes in the Hg concentrations in aquatic ecosystems have been investigated and documented in many studies aiming to describe seasonal patterns in freshwater food webs (Meili and Wills, 1985; Staveland *et al.*, 1993; Slotton *et al.*, 1995; Park and Curtis, 1997; Gorski *et al.*, 1999; Farkas *et al.*, 2003; Murphy *et al.*, 2007; Zhang *et al.*, 2012). On the other hand, some studies record no seasonal changes in Hg concentrations (Farkas *et al.*, 2000; Foster *et al.*, 2000; Burger *et al.*, 2009).

It is hypothesized that increased growth rates in the fish will lead to reduced mass-specific MeHg concentration by somatic growth dilution (Stafford *et al.*, 2004; Karimi *et al.*, 2007). Some studies have documented that different fish growth rates can contribute to modify Hg concentrations in fish muscle when comparing fish of similar lengths (Simoneau *et al.*, 2005). Furthermore, as fast growing fish during summer assimilate more biomass relative to Hg than slow growing fish, it is assumed that the ingested Hg becomes diluted by a larger body mass. In other words, fish with low activity costs are expected to contain lower levels of Hg in their muscle tissue (Trudel and Rasmussen, 2006). On the other hand, the opposite occurs during the winter period, with little fish growth and higher Hg concentrations (Lindqvist *et al.*, 1991). During this starvation period the fish catabolize their own muscle tissue for energy, reducing

the body mass faster than the bound MeHg, with the subsequent increase of the overall Hg concentration in the fish muscle (Cizdziel *et al.*, 2002). Stable isotope analysis of nitrogen (N) and carbon (C) provide a powerful tool for characterizing food web structures in lakes and the pathways of energy flow through the food chain (Post, 2002). While the stable carbon isotope ratio ( $\delta^{13}\text{C}$ ) undergoes little fractionation ( $0.4\pm 1.3\%$  per trophic level) with assimilation of food, the stable nitrogen isotope ratio ( $\delta^{15}\text{N}$ ) is enriched by about  $3.4\pm 1\%$  relative to their diet (Post, 2002) and thus, useful for estimating trophic position of organisms (Minagawa and Wada, 1984; Peterson and Fry, 1987; Kling *et al.*, 1992). Consumers feed potentially on many sources, it has been shown that there is a duality of pelagic and benthic production in lakes, and fish are mobile consumers linking both habitats (Vander Zanden and Vadeboncoeur, 2002). Thus, cross-habitat feeding by fish will generate a mixture in their  $\delta^{13}\text{C}$  signatures; predators potentially feeding on different sources can lie in the same isotopic area (Soto *et al.*, 2013). The  $\delta^{15}\text{N}$  signatures of starving or natural fasting animals typically show elevated values (Hobson *et al.*, 1993). Starving (protein-limited) animals catabolize their own bodily proteins to use as energy and amino acid sources. The starvation and the subsequent deamination and transamination (internal rearrangement of N) are isotope fractionation processes, and might be the reason for the high  $\delta^{15}\text{N}$  values, generating an isotopic enrichment analogous to the process by which the tissues of animals at high trophic levels become  $^{15}\text{N}$  enriched (Gannes *et al.*, 1998). Since  $\delta^{15}\text{N}$  can be used to characterize trophic relationships in aquatic food webs, a simple linear relationship between Hg (with logarithmic transformation) and  $\delta^{15}\text{N}$  in organisms within a food web can be used to rate the biomagnification power of Hg. The slope of this regression (trophic magnification slope, TMS) has been used in many studies to quantify the biomagnification potential. The trophic magnification factor (TMF) also represents the average biomagnification of a pollutant per trophic level. When  $\text{TMF} > 1$  then Hg biomagnifies through the food web (Lavoie *et al.*, 2013).

In our study, we have investigated seasonal variations of Hg concentration ( $\text{mg kg}^{-1}$  ww) and  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures (‰) in muscle tissue from northern pike *Esox lucius* L, European whitefish *Coregonus lavaretus* L, and European perch *Perca fluviatilis* L, in Lake Heddalsvatn, southern Norway. As the captured fish represent various trophic levels from piscivorous to planktivorous or benthivorous fish, seasonal variations of Hg are studied both within and between fish species.

## METHODS

### Site description

Lake Heddalsvatn ( $59^{\circ} 33' \text{ N}$ ,  $9^{\circ} 13' \text{ E}$ ) is located in the county of Telemark, southern Norway. The lake is a

relatively big (surface area:  $13.39 \text{ km}^2$ ; lake volume:  $0.44 \text{ km}^3$ ), medium deep (maximum depth: 54 m; medium depth: 37 m) lake, located at 16 m asl, with a large catchment ( $5\,036 \text{ km}^2$ ). The lake is surrounded partly by mountain and woodland areas as well as some agricultural areas. The city Notodden (13,000 inhabitants) is situated in the northern end of the lake. Two main rivers flow into the lake from north, the River Heddøla and the River Tinnelva, the latter draining large parts of the Hardangervidda, a large high mountain plateau.

Historically, a potential local Hg source was a paper mill operating at the outlet of River Tinnelva until 1972. In addition, Hg has also been used as a fungicide in the surrounding agricultural areas, until prohibited in 1990. Today, the main known Hg source in remote areas, as in the Heddalsvatn area, is the long range atmospherically transported air-pollution Hg (Rannekleiv *et al.*, 2009).

### Sampling

Samples were collected over three seasons, December 2008, May 2010 and September 2010. The fish were caught by gillnets of different mesh sizes (from 10 mm to 52 mm) in order to cover a representative selection of various length and age classes of the 3 fish species investigated. Due to this fishing method the sampling replicates was not balanced between seasons and fish species. The fishing was conducted only in the northern part of Lake Heddalsvatn, a large shallow area, which is not necessarily representative of the entire lake. A total of 266 fish of the 3 species were sampled, 49 pike, 121 whitefish and 96 perch. Pike and whitefish were collected in all three seasons, while we were not able to catch perch in December, a period of the year where this species is very immobile (Borgstrøm and Hansen, 1987). Each fish was stored in individual plastic bags in a freezer ( $< -18^{\circ}\text{C}$ ) until analyzed for Hg and stable isotopes.

### Sample preparation and analysis

Age determinations were mainly done by examination of otoliths. Otoliths were first burned with a butane lighter and then sectioned on the concave side in the center of the otolith. The sectioned pieces were studied under a light microscope. In addition, the metapterygoid bone was dissected for age determination of pike, while the operculum was sampled as a supplementary support for age determination of perch.

Analyses of Hg were conducted on fish tissue taken from the mid dorsal muscle (two duplicates of 100 mg). Hg samples were analyzed by atomic absorption spectrometry, using a RA-915M Mercury Analyzer with a pyrolyzer PYRO-915+ (Lumex Instruments, St. Petersburg, FL, USA). A tuna fish standard (European Reference Material CE464) with a Hg concentration of 5240 ppb was

used for the calibration of the instrument. Prior to stable isotope analyses of N and C, a small dorsal muscle sample was dried in an oven for 24 h at 60–80°C before grounded to fine powder by mortar and pestle. Approximately 1 mg of dried material was then transferred into 9x15 mm tin capsules, and analyzed by the Norwegian Institute of Energy Technology (IFE). All isotope values are referring to primary standards. For C the reference standard is the marine carbonate, Pee Dee Belemnite (PDB) (Craig, 1953); for N the reference standard is the atmospheric nitrogen (Mariotti, 1983). The relationships between stable isotopes of C and N ( $\delta^{13}\text{C} = ^{13}\text{C}/^{12}\text{C}$  and  $\delta^{15}\text{N} = ^{15}\text{N}/^{14}\text{N}$ ) are reported in ‰ and expressed by the following equation:

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

where R represents the ratio between the heavy and light isotope, *i.e.*  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$ .

We assumed that consumers become enriched on average by 3.4 ‰ per trophic level (Minagawa and Wada, 1984; Post, 2002). On the other hand,  $\delta^{13}\text{C}$  signatures are rather weakly enriched as trophic level increases, but they vary in the base of the food chain (Peterson and Fry, 1987). The biomagnification of Hg has typically been calculated with the following equation (eq. 2) (Lavoie *et al.*, 2013):

$$\text{Log}[\text{Hg}] = b \cdot \delta^{15}\text{N} + a \quad (\text{eq. 2})$$

where *b* is the trophic magnification slope (TMS) and *a* is the intercept, previously considered as an estimate of the concentration of Hg that is incorporated at the base of the food web.

The trophic magnification factor (TMF) of the ecosystem is calculated as the antilog of the TMS per trophic level (Lavoie *et al.*, 2013), *i.e.*:

$$\text{TMF} = 10^{(b \cdot 3.4\text{‰})} \quad (\text{eq. 3})$$

### Statistics

To explain the Hg fluctuations in different seasons, we tested the hypothesis of whether growth dilution or the opposite phenomenon caused by starvation/energy expenditures due to spawning/reproduction were the cause for these differences in Hg concentration. Statistical analyses included analysis of covariance (ANCOVA), using the Hg concentrations as the dependent variable, season as a nominal independent factor and length, age, body mass or  $\delta^{15}\text{N}$  as candidates for continuous covariates in the model. The minimum Akaike Information Criterion (AIC) was used to choose the best model. The model residuals were checked for normality, heteroscedasticity and serial correlations. The Mann Whitney test was used for the examination of specific differences of isotopic C burden,

both within species and between species. The data were also examined with linear regression analysis for correlations between Hg content and fish size (length or mass), age and stable isotopes for each fish species, with all seasonal data together. A logarithmic transformation of the Hg concentration, length, body mass and age values was used to improve normality of data. The regression residuals were tested for normality and a Durbin Watson test for serial correlations was used.

A significance level of 0.05 was used in the significance tests. All the statistical analyses were conducted by JMP Statistical Software (SAS, 2010) and/or Minitab 16 Statistical Software.

### RESULTS

Statistical data on morphology (total length, mass), age,  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  (‰) and Hg ( $\text{mg kg}^{-1}$  ww) from the 3 fish species investigated in Lake Heddalsvatn at three different seasons (December, May and September) are presented in Tab. 1. The Hg concentrations in the fish from Lake Heddalsvatn varied from 0.04 to 2.50  $\text{mg kg}^{-1}$  (ww), with the highest value found in the biggest and eldest individual of pike (112 cm, 9.8 kg and 16 years). The highest average Hg concentrations and most enriched  $\delta^{15}\text{N}$  signatures were found in pike, followed by perch, and with the lowest average values present in whitefish.

The  $\delta^{15}\text{N}$  signatures were significantly higher ( $P < 0.05$ ) in May and December compared with September for all three species, except for the fact that we have no fish data for perch in December. The  $\delta^{15}\text{N}$  signatures in December and May were relatively similar. The TMS in the northern part of Lake Heddalsvatn for all fish species and seasons was  $0.19 \pm 0.02$ . The Hg concentration was calculated to increase by a factor (TMF) of 4.29 per trophic level in the lake food web. The TMS including all fish species for the months of May, September and December was  $0.27 \pm 0.04$ ,  $0.14 \pm 0.03$  and  $0.15 \pm 0.04$ , respectively. There were not significant ( $P > 0.05$ ) differences in the  $\delta^{13}\text{C}$  signatures between species nor between seasons within a species. However,  $\delta^{13}\text{C}$  in whitefish had a significantly higher variance than pike and perch, which might reflect a broader ecological niche with regard to carbon sources (zooplankton, terrestrial surface insects and zoobenthos). During all seasons, significant positive correlations existed between Hg concentration in muscle tissue and length, body mass, age, and  $\delta^{15}\text{N}$  in perch and pike (Tab. 2), except for the correlation between Hg and  $\delta^{15}\text{N}$  in pike in September ( $P = 0.099$ ). For whitefish, also significant positive correlations existed between Hg concentration in muscle tissue and length, mass and age, while regarding  $\delta^{15}\text{N}$ , a significant positive correlation ( $P = 0.006$ ;  $R^2 = 18.0$ ) with Hg was only found in September (Tab. 2).

The covariance analysis for perch showed that both length and age contributed to the variation in Hg concen-

trations (all variables log-transformed) together with season (Fig. 1, Tab. 3). The model was highly significant ( $P < 0.0001$ ) and explained 81% of the variations in log Hg concentrations. Length was the most important explanatory variable ( $F_{1,92} = 35.8$ ,  $P < 0.0001$ ), followed by age ( $F_{1,92} = 10.3$ ,  $P = 0.002$ ) and season ( $F_{1,92} = 7.0$ ,  $P = 0.01$ ). No significant interactions between season and length or age could be proved, hence the effect of length and age did not differ between seasons. The  $\delta^{15}\text{N}$  values did not provide any further significant contribution to the model ( $P = 0.23$ ). The concentration of Hg in perch was significantly higher in May 2010 ( $0.35 \text{ mg kg}^{-1}$ ) than in September 2010 ( $0.26 \text{ mg kg}^{-1}$ ) after adjusting to geometric mean length (22.8 cm) and age (4.4 yr). The perch caught during September 2010 were significantly bigger (23.4 cm, 169 g) compared to those caught in May 2010 (21.1 cm, 125 g) after adjusting for differences in age distribution. There was no significant variation ( $P < 0.0001$ ) in the length-mass relationship between seasons, maintaining the same mass at a given length (May: 158 g and September: 154 g).

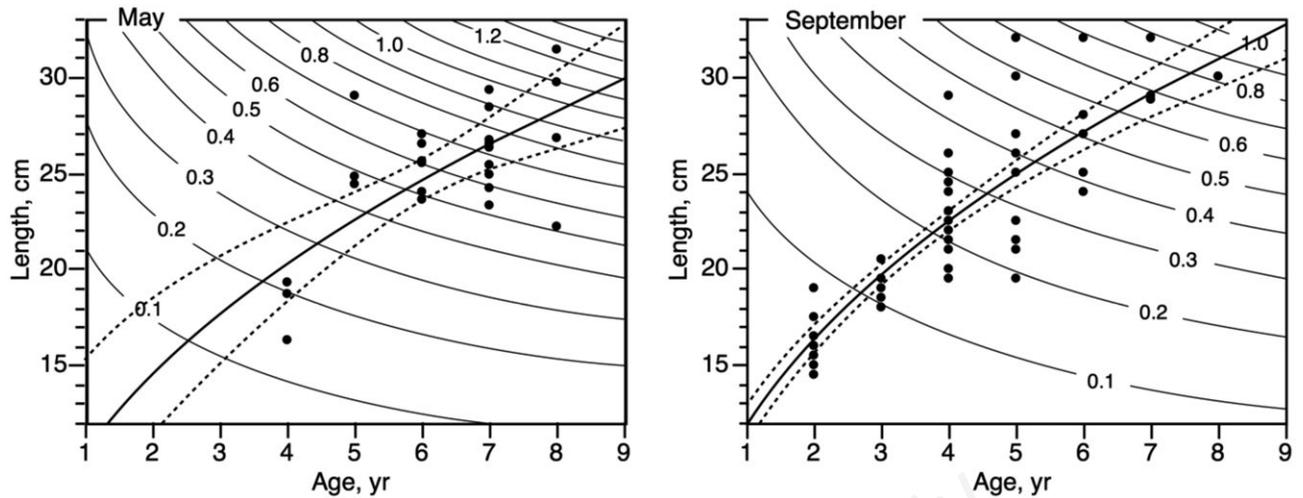
The covariance analysis between Hg concentrations in pike and length (both log-transformed) for different seasons showed that the interaction between length and season was insignificant ( $P = 0.43$ ), indicating parallel regressions of Hg concentration on length (both log-trans-

formed) for the different seasons (Fig. 2, Tab. 3). The model was highly significant, explaining 84% of the variation in log Hg concentrations ( $P < 0.0001$ ). Length was the most important variable ( $F_{1,44} = 131.8$ ,  $P < 0.0001$ ), whereas the effect of season was close to significance ( $F_{2,44} = 2.74$ ,  $P = 0.07$ ). Neither  $\delta^{15}\text{N}$  nor age (log-transformed) could provide any further significant contribution to the model ( $P = 0.99$  and  $0.26$ , respectively). The length-adjusted Hg mean for pike, adjusted to geometric mean length (48.9 cm) was higher in December 2008 ( $0.60 \text{ mg kg}^{-1}$ ) than in May and September 2010 ( $0.47$  and  $0.43 \text{ mg kg}^{-1}$ , respectively). A *post-hoc* test (least square means contrast) indicated that the length adjusted Hg mean of December 2008, could be significantly higher than that of May and September 2010 ( $P = 0.02$ ). For the two different seasons in 2010 (May-September) the pike length at a given age (average: 4.8 yr) was slightly shorter ( $P = 0.04$ ) in September (46.2 cm) compared with May (52.2 cm). However, there were not significant ( $P = 0.2$ ) variations in the length-mass relationships between May (637 g) and September (669 g), maintaining the same condition in both months.

For whitefish the covariance analysis showed that the most important factor for Hg variations is the age. The interaction between age and season was insignificant ( $P = 0.45$ ), indicating parallel regressions for the different

**Tab. 1.** Summary data for the length, mass, age, total mercury concentration, measured  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in muscle tissue for the three fish species in Lake Heddalsvatn, southern Norway.

Species (n)	Season (n)	Statistics	Length (cm)	Mass (g)	Age (yr)	Hg ppm (ww)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
Pike (49)	May (23)	Mean±sd	57.1±16.7	1581±1227	6±2	0.64±0.33	-27.8±0.7	9.5±1.1
		Median	58.9	1320	5	0.58	-27.7	9.4
		Min-max	27.5-87.0	125-4250	3-10	0.15-1.30	-29.3- -27.0	7.8-11.7
	Sept. (18)	Mean±sd	39.9±14.5	619±808	4±2	0.34±0.20	-27.8±0.9	8.2±1.4
		Median	35.8	277	4	0.27	-28.0	8.5
		Min-max	25.0-77.0	52-3060	2-7	0.16-0.86	-29.4- -25.9	3.6-10.1
	Dec. (8)	Mean±sd	63.3±24.4	2661±3009	7±4	0.95±0.74	-28.4±1.4	9.5±1.7
		Median	64.8	2000	6	0.76	-28.0	9.7
		Min-max	26.3-112.0	111-9800	2-16	0.07-2.50	-31.6- -27.2	6.3-11.7
Whitefish (121)	May (24)	Mean±sd	34.6±5.4	471±316	7±3	0.15±0.07	-27.5±1.3	8.7±0.5
		Median	33.6	367	6	0.06-0.38	-27.9	8.7
		Min-max	28.3-50.0	208-1609	3-15	0.14	-29.8- -24.5	7.7-9.9
	Sept. (41)	Mean±sd	27.0±5.5	194±152	4±2	0.10±0.04	-27.8±1.3	8.0±0.7
		Median	25.5	123	4	0.09	-28.1	7.9
		Min-max	19.5-44.0	54-743	2-11	0.04-0.21	-30.7- -23.9	6.7-9.7
	Dec. (56)	Mean±sd	35.5±7.1	456±303	7±4	0.23±0.28	-28.2±1.9	8.7±0.9
		Median	34.7	310	6	0.15	-28.3	8.7
		Min-max	21.4-48.7	65-1245	2-22	0.07-2.07	-32.7- -24.0	5.6-11.0
Perch (96)	May (26)	Mean±sd	25.2±3.4	244±109	6±1	0.68±0.33	-27.6±0.7	9.2±0.7
		Median	25.6	228	7	0.66	-27.6	9.3
		Min-max	16.3-31.4	45-503	4-8	0.11-1.32	-28.9- -25.5	8.1-10.8
	Sept. (70)	Mean±sd	22.5±4.6	175±128	4±2	0.32±0.31	-27.9±0.9	8.3±1.1
		Median	21.8	120	4	0.18	-27.8	8.5
		Min-max	14.5-32.0	35-567	2-8	0.06-1.33	-31.0- -25.3	4.3-10.5



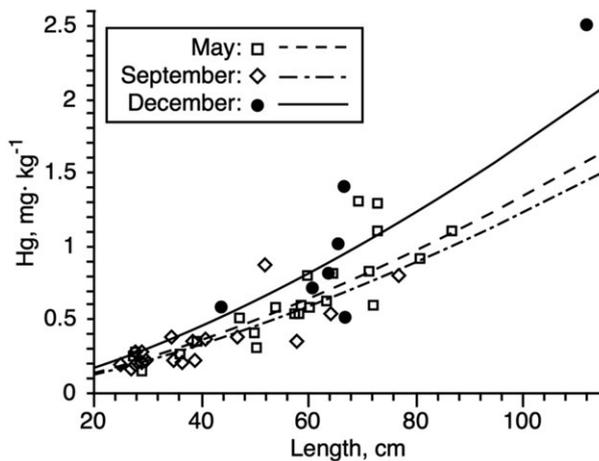
**Fig. 1.** Contour plots of mercury concentration ( $\text{mg}\cdot\text{kg}^{-1}$ ) in perch in May (left plot) and September (right plot) as a function of length and age (log-transformed variables). The plots are based on an ANCOVA model. Growth curves (with 95% confidence interval) are superimposed on the plots (linear regressions on log-transformed variables).

**Tab. 2.** Linear regression equations for the relationships between mercury concentration and the length (cm), mass (g), age and stable nitrogen isotope ( $\delta^{15}N$ ) for pike, whitefish and perch, collected during three different months in Lake Heddalsvatn, southern Norway.

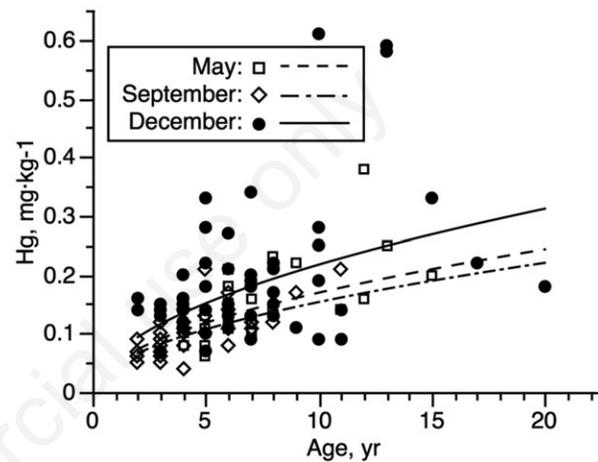
Species	Season (n)	Regression equation	R <sup>2</sup>	P
Pike	May (23)	$\text{LogHg} = -2.98 + 1.57 \cdot \text{LogLength}$	82.5	0.000
		$\text{LogHg} = -1.73 + 0.49 \cdot \text{LogMass}$	80.1	0.000
		$\text{LogHg} = -1.02 + 1.08 \cdot \text{LogAge}$	79.4	0.000
		$\text{LogHg} = -1.90 + 0.17 \cdot \delta^{15}N$	59.5	0.000
	Sept. (18)	$\text{LogHg} = -2.48 + 1.24 \cdot \text{LogLength}$	68.8	0.000
		$\text{LogHg} = -1.44 + 0.36 \cdot \text{LogMass}$	69.0	0.000
		$\text{LogHg} = -0.97 + 0.81 \cdot \text{LogAge}$	52.7	0.001
		$\text{LogHg} = -1.11 + 0.07 \cdot \delta^{15}N$	16.1	0.099
	Dec. (8)	$\text{LogHg} = -4.34 + 2.35 \cdot \text{LogLength}$	86.7	0.001
		$\text{LogHg} = -2.57 + 0.75 \cdot \text{LogMass}$	86.7	0.001
		$\text{LogHg} = -1.26 + 1.37 \cdot \text{LogAge}$	69.2	0.010
		$\text{LogHg} = -2.37 + 0.23 \cdot \delta^{15}N$	74.6	0.006
Whitefish	May (24)	$\text{LogHg} = -3.00 + 1.39 \cdot \text{LogLength}$	22.4	0.020
		$\text{LogHg} = -1.72 + 0.33 \cdot \text{LogMass}$	16.0	0.053
		$\text{LogHg} = -1.53 + 0.80 \cdot \text{LogAge}$	55.4	0.000
		$\text{LogHg} = -1.36 + 0.058 \cdot \delta^{15}N$	2.8	0.434
	Sept. (41)	$\text{LogHg} = -2.81 + 1.25 \cdot \text{LogLength}$	36.5	0.000
		$\text{LogHg} = -1.83 + 0.36 \cdot \text{LogMass}$	33.5	0.000
		$\text{LogHg} = -1.39 + 0.62 \cdot \text{LogAge}$	50.3	0.000
		$\text{LogHg} = -1.80 + 0.096 \cdot \delta^{15}N$	18.0	0.006
	Dec. (56)	$\text{LogHg} = -2.09 + 0.87 \cdot \text{LogLength}$	9.8	0.019
		$\text{LogHg} = -1.38 + 0.25 \cdot \text{LogMass}$	10.2	0.016
		$\text{LogHg} = -1.19 + 0.55 \cdot \text{LogAge}$	24.8	0.000
		$\text{LogHg} = -1.21 + 0.052 \cdot \delta^{15}N$	2.7	0.230
Perch	May (26)	$\text{LogHg} = -5.40 + 3.69 \cdot \text{LogLength}$	62.1	0.000
		$\text{LogHg} = -2.50 + 0.97 \cdot \text{LogMass}$	58.3	0.000
		$\text{LogHg} = -2.08 + 2.32 \cdot \text{LogAge}$	51.6	0.000
		$\text{LogHg} = -2.21 + 0.21 \cdot \delta^{15}N$	22.3	0.015
	Sept. (70)	$\text{LogHg} = -5.67 + 3.73 \cdot \text{LogLength}$	76.3	0.000
		$\text{LogHg} = -2.98 + 1.08 \cdot \text{LogMass}$	76.9	0.000
		$\text{LogHg} = -1.76 + 1.88 \cdot \text{LogAge}$	71.0	0.000
		$\text{LogHg} = -1.88 + 0.15 \cdot \delta^{15}N$	14.3	0.001

seasons (Fig. 3, Tab. 3). The model between Hg concentration and age (both log-transformed) for different seasons was significant ( $P=0.0001$ ) and explained 49% of the Hg variations. The effect of the age was the most important factor ( $F_{1,116}=50.38$ ,  $P<0.0001$ ) followed by the effect of season ( $F_{2,11}=9.77$ ,  $P=0.0001$ ). Neither  $\delta^{15}\text{N}$  nor length (log-transformed) could provide any further significant contribution to the model ( $P=0.86$  and  $0.24$ , respectively). The concentration of Hg in whitefish at a given age (av-

erage: 6.1 yr) was significantly higher in December 2008 ( $0.16 \text{ mg kg}^{-1}$ ) compared with May 2010 ( $0.12 \text{ mg kg}^{-1}$ ) and September 2010 ( $0.11 \text{ mg kg}^{-1}$ ), while the difference between May and September was not significant. However, significant seasonal mass-length relationship was revealed, as the whitefish in May 2010 (306 g) were significantly heavier at a given length compared with September 2010 (284 g) and December 2008 (254 g). There was a large variation in  $\delta^{13}\text{C}$  signatures in the investigated



**Fig. 2.** Mercury concentrations in pike as a function of length and season. The regression lines are based on an ANCOVA model (log-transformed variables).



**Fig. 3.** Mercury concentrations in whitefish as a function of age and season. The regression lines are based on an ANCOVA model (log-transformed variables).

**Tab. 3.** Mercury concentration (log) as a function of length (log) and season in pike as a function of age (log) and season in whitefish, and of length, age and season in perch.

Species	Term	B	B lower 95% CL	B upper 95% CL	$\beta$	P	Hg <sub>adj</sub> (ppm ww)
Pike	Intercept	-6.333	-7.338	-5.327	0.000	<0.0001	
	Dec. 2008	0.188	0.024	0.351	0.198	0.026	0.60
	May 2010	-0.050	-0.163	0.063	-0.055	0.373	0.47
	Sept. 2010	-0.137	-0.278	0.004	-0.143	0.056	0.43
	Log length	1.448	1.194	1.702	0.830	<0.0001	
Whitefish	Intercept	-2.928	-3.184	-2.672	0.000	<0.0001	
	Dec. 2008	0.199	0.106	0.291	0.333	<0.0001	0.16
	May 2010	-0.050	-0.166	0.066	-0.075	0.397	0.12
	Sept. 2010	-0.149	-0.258	-0.039	-0.258	0.0084	0.11
	Log age	0.522	0.376	0.668	0.533	<0.0001	
Perch	Intercept	-10.254	-12.338	-8.171	0.000	<0.0001	
	May 2010	0.155	0.039	0.271	0.148	0.001	0.35
	Sept. 2010	-0.155	-0.039	-0.271	-0.148	0.001	0.26
	Log length	2.548	1.702	3.393	0.541	<0.0001	
	Log age	0.740	0.283	1.197	0.326	0.002	

*B*, Parameter estimates with confidence level (CL). *b*, standardized parameter estimates; Hg<sub>adj</sub>, length or age adjusted means. Pike, mercury (Hg) adjusted to geometric mean length: 48.9 cm; analysis of covariance:  $R^2=0.84$ ;  $P<0.0001$ . Whitefish, Hg adjusted to geometric mean age: 6.1 yr; analysis of covariance:  $R^2=0.49$ ;  $P<0.0001$ . Perch, Hg adjusted to geometric mean length and age: 22.8 cm, 4.4 yr; analysis of covariance:  $R^2=0.81$ ;  $P<0.0001$ .

whitefish (-23.9‰ to -32.7‰), but not significant variations between the seasons.

## DISCUSSION

In boreal areas, like in Scandinavia, little fish data exist from the cold and normally snow rich winter periods, when most lakes are covered by ice. The cold and dark environment present at that time of the year implies minor biological production at all ecosystem levels. Accordingly, the activities of many fish species, including feeding activity, are at minimum (Greenfield *et al.*, 2005) and various degrees of starvation likely appear in the fish populations. Conversely, during the growth season (May-October), feeding activity, growth rate and growth efficiency are high (Lindqvist *et al.*, 1991), and the body mass of the fish increases rapidly (Talbot, 1993). Consequently, seasonal variations in Hg concentrations and  $\delta^{15}\text{N}$  in fish should be expected as these parameters are expected to respond to variations in growth (decrease in Hg and  $\delta^{15}\text{N}$ ) and starvation (increase in Hg and  $\delta^{15}\text{N}$ ). The evaluation of seasonal variations of Hg concentrations in fish from our study was implemented in different years, 2008 and 2010. We assumed that there was no influence on the interpretation of the data regarding temporal variation from the year 2008 to the year 2010, and that the seasonal variations have a stronger influence on the fish Hg concentrations. Braaten *et al.* (2014) tested temporal variations on THg and MeHg concentrations in three boreal lakes and showed a minimal but not significant variation within the two years of their study (2010-2012). Major watershed disturbances (such as forest fires or clearcutting) or local point sources of pollution would imply a possible variation in inter-annual concentrations of Hg, but such events have not occurred in lake-near areas during the investigated period.

There exist differences in fish Hg concentrations between sexes and these differences have primarily been attributed to energy expenditures during the spawning season and growth rates (Gewurtz *et al.*, 2011; Stacy and Lepak, 2012). Sex determination was not conducted in our study; however, as the Hg concentrations were normalized to body mass and/or length when comparing between seasons, we assumed that it is indirectly accounting for possible sex-specific differences. The TMS obtained in our study (0.19) is comparable to a recent report throughout different regions and ecosystems worldwide, which shows mean values of  $0.16 \pm 0.11$  for total Hg and  $0.24 \pm 0.08$  for MeHg (Lavoie *et al.*, 2013). The comparison of the TMS values during different months could lead to misinterpretations due to the unbalanced replication of samples in the different seasons, which may influence the slope and the TMF. The regression may as well be substantially influenced by the number of trophic levels included in the samples (Borgå *et al.*, 2012), which varies greatly between each season sampled in the study. There

was no significant species specificity in the  $\delta^{13}\text{C}$  signatures. The average  $\delta^{13}\text{C}$  signatures for all fish species in our study lake (Tab. 1) have typical values for energy sources derived from pelagic areas (-28.4‰; Vander Zanden and Rasmussen, 1999). However, it is well-known that fish are a mobile species and integrate both pelagic and benthic food webs in lakes and they derive energy opportunistically from both habitats (Post *et al.*, 2000; Vander Zanden and Vadeboncoeur, 2002). Unfortunately, prey organisms were not sampled to estimate the percent contribution from littoral or pelagic sources to each individual. Stable isotope signatures of fish that feed on different food sources may lie in the same isotopic position, while truly belonging to different niches. Hence, it is a tentative assumption to only consider measured  $\delta^{13}\text{C}$  signatures of the fish to assess their energy sources. Therefore,  $\delta^{13}\text{C}$  did not add explanatory power to the description of Hg concentrations within the food web of the lake.

Our data confirm seasonal variations in the Hg concentrations in fish from Lake Heddalsvatn, but the causes may vary depending on fish species. Generally, the highest concentrations of Hg and  $\delta^{15}\text{N}$  in muscle tissue of fish (only pike and whitefish caught) were observed during winter time (December). Perch showed clear seasonal variations in the Hg concentrations. After the spawning in late April-May, the fish population increased in mass rapidly throughout the summer with subsequent decrease in the Hg concentration in muscle tissue. The most likely explanation is the somatic growth dilution of Hg, as also shown by Stafford *et al.* (2004), where Hg concentrations decreased with fish growth. Our results also concurred with the seasonal Hg patterns described by Meili (1991) in Swedish lakes, where the Hg concentrations were higher during May compared to September for all fish species investigated (pike, perch and roach). Both length and age were important explanatory factors for the Hg concentrations in the perch population, indicating the tight relationship between bioenergetics and Hg accumulation in fish (Trudel and Rasmussen, 2006). It is hypothesized that Hg concentration increases over time when the uptake rate of Hg exceeds the sum of excretion rate and growth dilution rate. That is, older fish will have higher Hg concentrations than younger fish at a given length. An old fish has had a longer exposure time to accumulate Hg than a young fish, even though both have achieved the same length. This phenomenon is more pronounced in populations with individuals with a stagnated growth pattern or in older individuals that have reduced growth efficiency, due to the fact that metabolic costs are relatively higher in older individuals (Trudel and Rasmussen, 2006). The seasonal changes of Hg in muscle tissue of pike could not be explained by growth dilution, since there was no measurable growth from May to September in the fish caught. An explanation might be the single capture site within a restricted area of the lake, which may have

biased the results. In large and deep lakes, whitefish might represent various sub-populations of different phenotypes occupying specific niches (Harrod *et al.*, 2010), but all sub-populations basically feed on zooplankton and benthos. This likely explains the very low concentration of Hg in all three investigated seasons. Despite so, seasonal variations of Hg in whitefish were documented. However, this cannot be explained by growth dilution since the fish caught in September at the end of the growth season, were smaller than those caught in May. Based on our data, no adequate reasons for this fact can be found. The wide range in  $\delta^{13}\text{C}$  in whitefish (-23.9‰ to -32.7‰), which indicates large variation in habitats and/or feeding strategy, may have influenced on the results. Additionally, similar  $\delta^{13}\text{C}$  signature variations in whitefish have been found in Lake Norsjø, the first downstream lake from Lake Heddalsvatn (Jensen, 1954).

The higher  $\delta^{15}\text{N}$  signatures found in fish in Lake Heddalsvatn in December and May compared with September, concur with a previous study by Gannes *et al.* (1998) where they affirm that the processes by which the tissues of animals at high trophic levels become enriched in  $\delta^{15}\text{N}$ , probably apply to starving animals. As May and December belong to cold and dark winter-period in lakes at high latitudes, minor biological activity and production occur. Accordingly, the feeding rates of fishes are at minimum, and starvation with subsequent  $\delta^{15}\text{N}$  enrichment might occur. However, the significant seasonal variations in  $\delta^{15}\text{N}$  in all three species in our study were mainly related to fish body size variations. For pike and whitefish, the lower  $\delta^{15}\text{N}$  signatures in September were associated with a decrease in mean fish size. For perch, the decrease from May to September also coincided with a decrease in mean fish size. There were no significant differences in the length adjusted seasonal means in  $\delta^{15}\text{N}$  for pike and whitefish ( $P > 0.05$ ), whereas a small statistical significant reduction from May to September ( $\Delta^{15}\text{N} = -0.7$  ‰) could be detected for perch ( $P = 0.02$ ).

In Norway, the human consumption limit for fish is 0.5 mg Hg kg<sup>-1</sup>(ww) in muscle tissue of perch and whitefish and 1.0 mg Hg kg<sup>-1</sup> regarding pike (Codex Alimentarius Commission, 1991). Predicted results by linear regression analyses, showed that bigger individuals of pike (100 cm, 9.0 kg) and perch (27 cm, 0.302 kg) are needed to exceed this limit in September, compared with fish caught in May (pike: 79 cm, 3.6 kg; perch: 24 cm, 0.187 kg). As only a few individuals of pike and no perch were caught in December, these data are not incorporated in this comparison. For the planktivorous and benthivorous whitefish, the concentrations of Hg are generally well below these limits during all seasons.

## CONCLUSIONS

The investigation of pike (piscivorous), whitefish (planktivorous, benthivorous) and perch (generalist) in

Lake Heddalsvatn has documented seasonal variations in the muscle tissue Hg concentrations, disregarding confounding factors such as fish length, age or  $\delta^{15}\text{N}$  signatures. However, length was the most important explanatory variable for Hg concentrations in fish tissue from pike, while age was the most important factor for whitefish. Both length and age combined were the best explanatory factors for perch.

The highest Hg concentrations were revealed in the fish species caught in December (pike and whitefish). The Hg concentrations in May were higher than in September for all 3 species, it was of statistical significance for perch and close to significant for pike. The interplay between low feeding rates during the winter (starvation) and somatic growth dilution during summer is likely the main factor for seasonal Hg fluctuations in fish in high latitude lakes as Lake Heddalsvatn. For perch, this was the main explanation for the Hg seasonal variations. Regarding whitefish, it is well known that several subpopulations occur in the lake, varying in size, condition factor, and use of littoral habitat for feeding and/or spawning. Despite so, a significant positive correlation was found between Hg in muscle tissue of whitefish and age, and that the Hg concentrations differed between seasons.

Due to seasonal variations of Hg in fish from Lake Heddalsvatn, bigger perch and pike can be eaten during autumn before the consumption limits of 0.5 mg kg<sup>-1</sup> (perch) and 1.0 mg kg<sup>-1</sup> (pike) are reached, while Hg in all whitefish was below these limits.

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