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Does lake thermocline depth affect methyl mercury concentrations in fish?

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Abstract Climate change is projected to increase mean temperature of northern lakes by the end of the twenty-first century. To simulate this scenario, during 2004–2007 we imposed artificial mixing in Halsjärvi, a small, polyhumic, boreal lake in southern Finland, to increase the depth of the thermocline by ~ 1.5 m. The aims of the experiment were to evaluate potential effects of climate change on biogeochemical cycles, especially of mercury, and on food web structure, productivity and biodiversity in dystrophic lake ecosystems. Following the initial depression of the thermocline in the experimental lake, the methyl mercury (MeHg) concentration in small European perch (*Perca fluviatlis* L.) decreased and remained lower throughout the study. In contrast, perch in a nearby reference lake exhibited increased MeHg during the same period. The δ^{15}N values of the muscle tissue of perch were similar in both lakes over the study period, suggesting that the trophic position of perch remained unchanged. However, δ^{13}C values of perch became clearly more negative in Halsjärvi, probably due to the greater mixing of the water column that resulted in changes in the carbon sources for the food web. A marked decrease of epilimnetic MeHg concentrations recorded during the experiment was considered to be the main reason for the decreased MeHg concentrations in perch. The results suggest that oxygen-related changes induced by climate change will be more important than direct temperature changes for MeHg accumulation in fish in small humic lakes with persistent oxygen deficiency in the hypolimnion.

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Introduction

An effect of climate change on ecosystem sensitivity to mercury (Hg) has been proposed by many studies. Climate change can alter the methylation of mercury or the mobilization of methyl mercury (MeHg) by associated increase in temperature and rainfall intensity, runoff from catchments and water level fluctuations (e.g. Monson 2009). For instance, Bodaly et al. (1993) suggested an increase in net methylation rates in the surface sediments of boreal lakes due to climate change. Increased hydrological loading of organic matter, and inorganic and methyl mercury due to climate change has also been suggested (Grigal 2003; Martinez Cortizas et al. 2007). Indeed, climate change effect was proposed to be a possible explanation of the post 1995 increase of fish mercury concentrations in Minnesota (Monson 2009).

One of the major effects of climate change on freshwater lakes will be on the thermal regime, which influences ecosystem structure and function (Wrona et al. 2006). The stratification of lakes can be manipulated artificially by increasing the input of mixing energy. Lowering the thermocline depth will increase the total heat content of the lake, and thus experimental manipulation of the thermocline depth is one way of simulating the effects of a warming climate (Lydersen et al. 2008). The manipulation also simulates the effect of wind speeds which are expected to be increased according to many climate change scenarios (Saloranta et al. 2009).

The overall effect of mixing on Hg dynamics in lakes could be either an increase or a decrease of MeHg production and concentration in biota depending on which processes and environmental characteristics determine most of the MeHg production rate. Warm, shallow, organic rich lake sediments are often important zones of net methylation, and the extent of these sediments in a lake may affect the conversion of Hg to MeHg (Bodaly et al. 1993; Munthe et al. 2007). Further, the presence of an anoxic hypolimnion significantly enhances net MeHg bioaccumulation in stratified lakes (Watras et al. 2006). Additional mechanisms may be important for the bioaccumulation of MeHg, such as the overall response of the phytoplankton community and the food chain structure from producers to fish (Gorski et al. 2008).

A whole-lake experiment (THERMOS) was conducted during 2004–2007 in which the stratification pattern of a small humic lake was manipulated. The aim of the study was to evaluate the effects of change in thermocline depth on biogeochemical cycles, food web structure, productivity, and biodiversity in dystrophic lake systems. The effects of manipulation on the Hg and MeHg cycle in the lake were also studied and a decrease in methyl mercury produced in the lake during the summer months was found (Verta et al. 2010). In the present study, the impacts of these changes on MeHg concentrations in small European perch (*Perca fluviatlis* L.) were examined.

Materials and methods

Site description

As a reference lake for the study we used Valkea-Kotinen, which is located in a small headwater catchment in a remote, unmanaged forested area in southern Finland. The catchment of the lake is part of a protected conservation area and receives only background levels of air pollution. Typical of glaciated boreal regions, the catchment contains areas of forested mineral soil at higher elevations, and peatlands at lower elevations and adjacent to the lake. The mineral soils in the catchments are predominantly podzols developed in shallow glacial drift or till deposits (Starr and Ukonmaanaho 2001; Holmberg et al. 2006). The forest cover consists mainly of old-growth mixed stands of Norway spruce (*Picea abies*), birch (*Betula* spp.) and aspen (*Populus tremula*) with some large individuals of Scots pine (*Pinus silvestris*). The experimental lake, Halsjärvi, was selected from the same area to be as similar as possible to Valkea-Kotinen with respect to catchment area, lake area, bathymetry and water quality (Fig. 1, Tables 1, 2). The distance between the lakes is 4.5 km.

Both lakes are highly humic headwaters with water colour around 200 mg Pt l^{-1} and have comparable levels of TOC and main nutrients; however, Valkea-Kotinen is more acidic than L. Halsjärvi (Table 2). The primary production in the lakes is

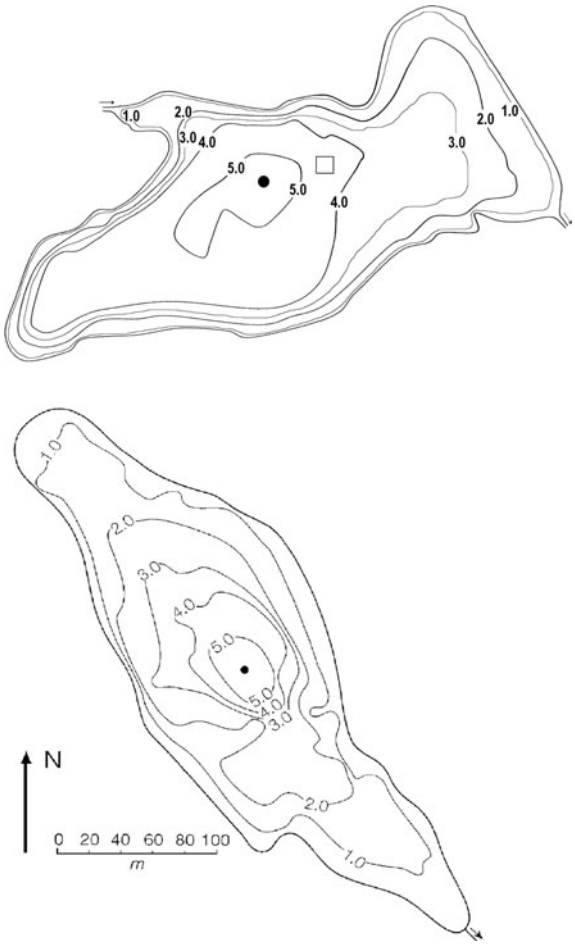

Fig. 1 Bathymetric maps of the lakes Halsjärvi (*top*) and Valkea-Kotinen (*bottom*). The sampling point for water quality is indicated for each lake with a *black dot* and the location of the mixing equipment in Halsjärvi with an *open square*

largely based on phytoplankton, as littoral macrophytic vegetation and periphyton are scarce due to the restricted photic zone of these humic lakes. European perch (*Perca fluviatilis*) and northern pike (*Esox lucius*) occur in both lakes, while Halsjärvi also contains the cyprinid species roach (*Rutilus rutilus*), bleak (*Alburnus alburnus*) and bream (*Abramis brama*).

Main experimental characteristics

The primary objective of the thermocline manipulation was to simulate projected future increase in lake mean temperature (i.e. heat content) and wind speed during the open water season by the end of the twenty-first century due to climate change. The heat content of the lake was calculated by multiplying the density of each water layer by the layer temperature, water-specific heat capacity and layer thickness (Korhonen 2002). A one-dimensional lake simulation model (MyLake; Saloranta and Andersen 2007) was used to simulate the daily vertical distribution of lake water temperature and thus density stratification. The MyLake model was already used in the planning phase of the manipulation experiment to select the target depth for lowering the thermocline. The MyLake model was set up and calibrated at the reference lake Valkea-Kotinen, at the manipulation lake Halsjärvi and at the nearby larger L. Pääjärvi, and both model sensitivity tests and model runs with climate change scenarios were carried out. Two different greenhouse gas emission scenarios, A2 and B2 (IPCC 2001; generally less greenhouse gas emissions in the B2 than in the A2 scenario) were used in model simulations which, when using ECHAM4 circulation model and A2 scenario, resulted in a prediction of 5°C increase in air temperature and 8% increase in wind speed. A detailed description of the model calibration and scenario results is given by Saloranta et al. (2009).

Table 1 Morphological and hydrographical characteristics of the lakes Halsjärvi and Valkea-Kotinen, and estimated areal proportion of total sediments in contact with the epilimnion in the lakes during June–August of each year of the experimental period

	Halsjärvi (experimental lake)	Valkea-Kotinen (reference lake)
Latitude	61°14″	61°15″
Longitude	25°08″	25°04″
Elevation (m)	131	156
Catchment area (km^2)	0.58	0.3
Lake area (ha)	4.7	4.1
Lake maximum depth (m)	5.9	6.4
Lake mean depth (m)	2.8	2.5
Lake volume (m^3)	130,000	103,000
% area of epilimnetic sediments		
2004	28	60
2005	77	55
2006	77	50
2007	60	60

Table 2 Water quality of the lakes Halsjärvi and Valkea-Kotinen during the mixing experiment with Halsjärvi

	Lake Halsjärvi		Lake Valkea-Kotinen	
	Epilimnion	Hypolimnion	Epilimnion	Hypolimnion
pH	6.5 (6.8)	6.5 (6.5)	5.2 (5.3)	5.4 (5.5)
Alkalinity, mmol l^{-1}	0.13 (0.17)	0.51 (0.42)	0.01 (0.01)	0.05 (0.07
Conductivity, mS m^{-1}	4.7 (4.8)	7.8 (6.9)	2.7 (2.6)	3.1 (3.1)
Colour, mg Pt l^{-1}	196 (136)	245 (271)	173 (177)	190 (251)
Ca, mg l^{-1}	5.0 (5.1)	7.8 (6.9)	2.1 (2.0)	2.7 (2.6)
Fe, mg l^{-1}	0.6 (1.0)	7.3 (6.9)	0.2 (0.3)	0.7 (0.8)
SO_4, Mg l^{-1}	9.6 (9.0)	8.6 (7.6)	3.0 (4.2)	3.1 (4.0)
TOC, mg l^{-1}	14.8 (8.4)	7.6 (6.2)	14.0 (13.3)	n.a. (16.1)
P_{tot}, μg l^{-1}	10.3 (10.7)	6.4 (7.8)	18.7 (14.1)	21.2 (21.7)
N_{tot}, μg l^{-1}	469 (334)	563 (522)	497 (461)	590 (679)
TotHg, ng l^{-1}	2.7 (1.4)	2.2 (3.7)	2.9 (2.4)	4.4 (4.2)
MeHg, ng l^{-1}	0.58 (0.06), 0.04	0.48 (1.0), 0.53	0.36 (0.36)	2.4 (2.2)

June–August mean values for epilimnion and hypolimnion are given for the reference years (2004 and 2007) with values for the years of mixing (2005 and 2006) shown in parentheses. Annual number of samples was 4–15. Note that for MeHg the years 2004 and 2007 are given separately because of great difference in epilimnetic concentrations between these years. Note also that the volume of the epilimnion was significantly higher in 2005 and 2006 than in 2004 and 2007 in Halsjärvi

A modified commercial aerator (MIXOX, Water-Eco Ltd.) was used for manipulating the thermocline depth. The aerator had a maximum pumping capacity of 15,000 m^3 day^{-1} (0.6 kW). After a testing and modification phase in 2004, the MIXOX aerator was installed in the middle of Halsjärvi after ice-off in early May 2005 and ran from May to September in 2005 and 2006. The experimental design is shown in Fig. 2 and the effects of the mixing on the temperature distribution and oxygen saturation are shown in Figs. 3 and 4. The effect of the mixing of Halsjärvi on the proportion of total lake sediments in contact with the epilimnion (estimated from the bathymetry and oxygen distribution) is shown in Table 1.

Water quality

The lakes were sampled weekly in 2004–2006 and biweekly in 2007 during the open water season. Water samples were taken over the deepest point of the lake. Temperature and oxygen profiles were measured at 0.5 m intervals, and other variables from integrated water samples representing three depth zones according to the stratification situation of the lake (i.e. epi-, meta- and hypolimnion). Water quality analyses were carried out in the laboratory of Lammi Biological Station, University of Helsinki, using SFS standard methods.

Total Hg and total MeHg were measured each year during May and at the end of summer stratification

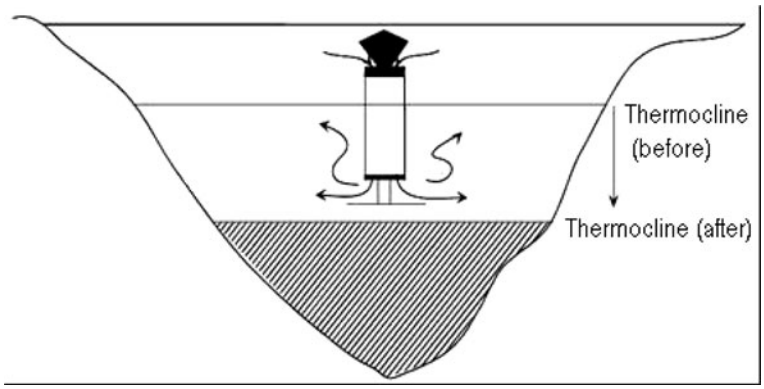

Fig. 2 Schematic diagram showing the mixing of the water column in Halsjärvi

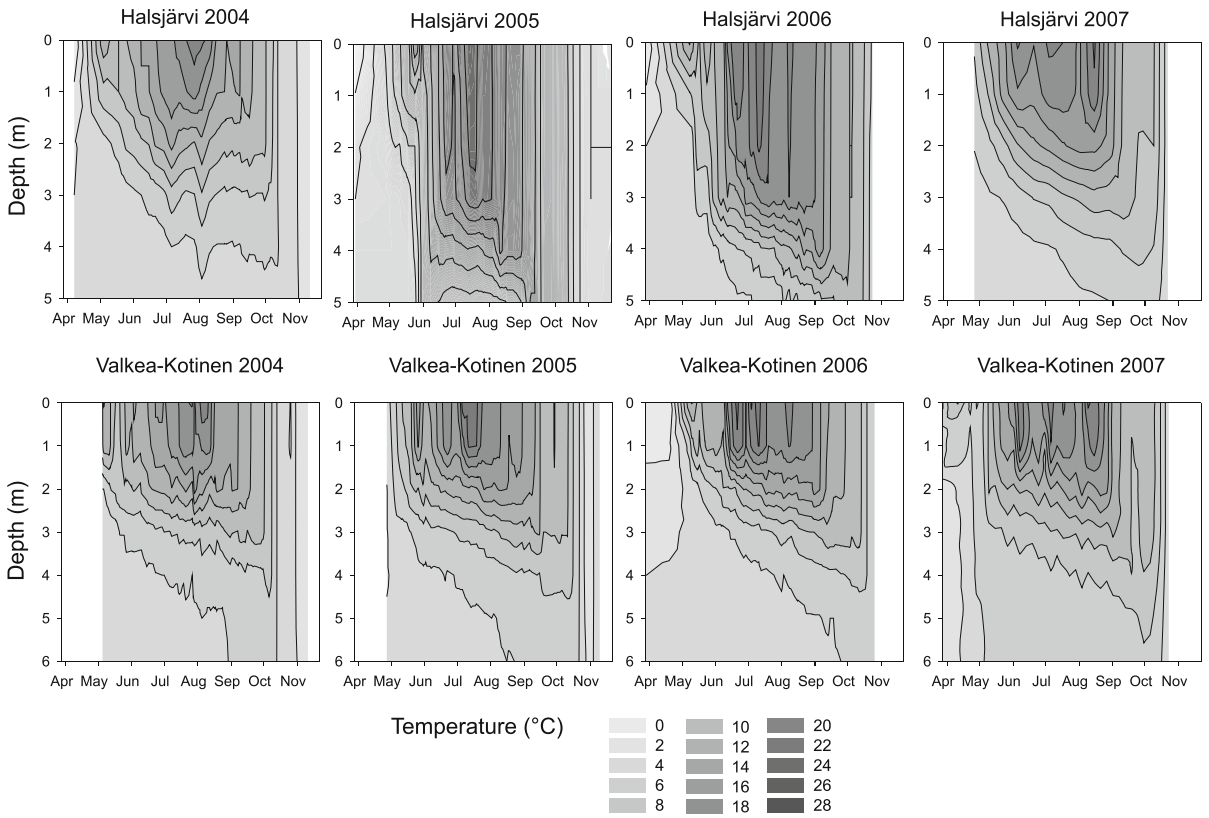

Fig. 3 Interpolated seasonal development of temperature in the experimental lake Halsjärvi (*top*) and in the reference lake Valkea-Kotinen (*bottom*) during the study period 2004–2007

(late August) at 0.5–1 m intervals and after autumn overturn (early December). Samples were collected in acid washed Teflon bottles (250 ml), kept cool during transportation to IVL laboratory, Sweden, for analyses, and preserved with 0.5 ml 30% HCL (Merck, Suprapur) within 1 day of collection. For details of Hg sampling and analyses, see Verta et al. (2010).

Sampling of fish

Small perch for Hg analyses were captured from the Halsjärvi ($n = 75$) and Valkea-Kotinen ($n = 54$) in September 2004–2007 with special gillnets targeted at small fish (1.5 × 20 m; 5 m panels of mesh sizes 5, 6.25, 8 and 10 mm). The nets were located randomly to the depth zone of 1.5–2.5 m in both lakes. The original intention was to use 0+ perch of 60–70 mm in total length. However, because 0+ perch were not available each year, larger (up to 110 mm total length) and older (up to age of 4 years) were included in the samples. To avoid the effects of age dependence on the MeHg concentrations of perch (Metsälä and Rask 1989; Rask et al. 2007), perch of total length 80–90 mm and age 2+ years were used (Halsjärvi $n = 45$, Valkea-Kotinen $n = 20$, 5–20 individuals per lake each year) in calculations. The fish were kept frozen at −25°C before mercury and stable isotope analyses. The samples of 2006 from L. Valkea-Kotinen were accidentally lost.

Mercury analyses

Analyses of total Hg were made on samples of dorsal white muscle from perch. All the mercury analyses were performed at one time to avoid any possible effects of temporal variation in analysis. Total Hg was analysed at the West Finland Regional Environment Centre using an automatic analyzer (Leco AMA

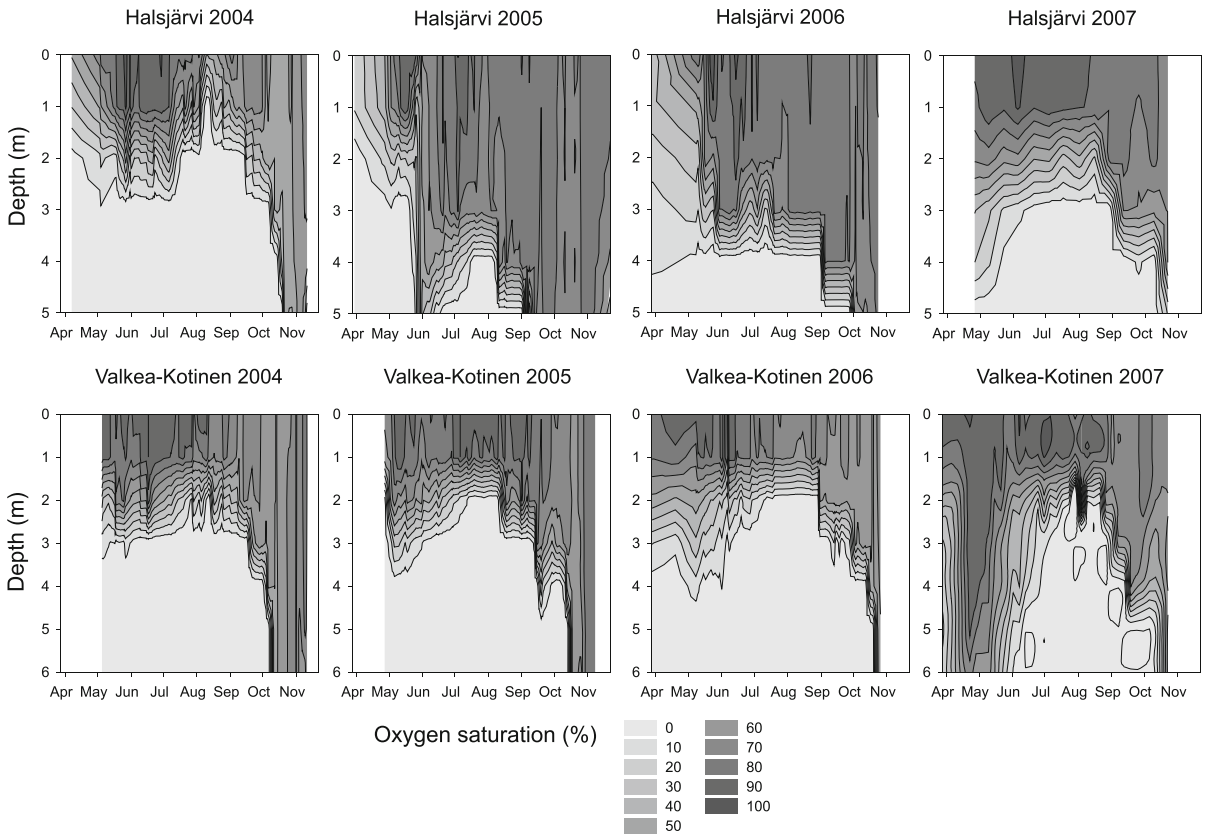

Fig. 4 Interpolated seasonal development of oxygen saturation in Halsjärvi (*top*) and in Valkea-Kotinen (*bottom*) during the study period 2004–2007

254). The samples of fish muscle were dried and then thermally burned. The decomposition products were pumped through a catalyst to an amalgamator for a selective gold trap of mercury. The Hg trapped in the amalgamator was released by short heating and measured by an atomic absorption spectrophotometer at 253.65 nm. Two sets of reference samples were used: Oyster Tissue (NIST) and Cod Muscle (BCR 422, lyophilised), with the certified concentrations of 0.0371 mg kg^{-1} ($\pm$10%) and 0.559 mg kg^{-1} ($\pm$10%). The measured mean concentrations were 0.0369 mg kg^{-1} (SD = 0.00114) and 0.563 mg kg^{-1} (SD = 0.0109) showing good performance of the Hg analysis. Annual between-lake differences in MeHg concentrations of perch muscle tissue were analysed using a two tailed independent *t* test with pooled variances. Year-to year variations within the lakes were analysed using ANOVA and Tukey multiple comparison (SYSTAT 12).

Stable isotope analyses

For stable isotope analyses (SIA), a sample of dorsal white muscle was freeze-dried to a constant weight and ground to fine powder using a mortar and pestle. Stable isotopes of carbon and nitrogen were analysed at the University of Jyväskylä, Finland, using a Carlo Erba Flash EA1112 elemental analyser connected to a Thermo Finnigan DELTAplusAdvantage continuous flow stable isotope-ratio mass spectrometer (CF-IRMS). Results are expressed using the standard δ notation as parts per thousand (‰). The reference materials used were secondary standards of known relation to the international standards of Vienna Pee Dee belemnite (for carbon) and atmospheric N_2 (for nitrogen). Dried pike white muscle was used as an internal working standard and two replicate standards were run repeatedly after every 10 samples in each sequence. Internal precision was always better than

0.2‰, based on the standard deviation of replicates of the standards. Between-lake differences in SIA values of δ^{15}N‰ and δ^{13}C‰ of perch muscle tissue were analysed using a two tailed independent t test with pooled variances. Year-to year variations within the lakes were analysed using ANOVA and Tukey multiple comparison (SYSTAT 12).

Results

The overall response of the lake to the experimental mixing

As it is typical of these small boreal lakes, the spring overturn was incomplete and both lakes exhibited anoxic conditions in the hypolimnion from late winter through summer until autumn overturn. The oxic epilimnion only extended to 1–2.5 m depth in Halsjärvi during summer 2004 before the manipulation experiment (Fig. 4). In the reference lake, Valkea-Kotinen, the temperature and oxygen stratification was similar to that in Halsjärvi in 2004 and remained unchanged throughout the study period with the exception of a complete spring turnover in spring 2007 (Figs. 3, 4).

The response of L. Halsjärvi to experimental mixing was clearly seen as a 1.5–2 m deepening of the thermocline in summer 2005 and 2006 (Fig. 3). Furthermore, the manipulation did not affect surface temperatures significantly but increased the metalimnetic and hypolimnetic temperatures by up to 8°C. The increase in the mean heat content in the manipulation experiment was 11.1 MJ m^{-3}, equivalent to a 2.6°C increase in the water mass mean temperature in May–October (Forsius et al. 2010), indicating that the lake manipulation experiment well represented the average simulated future increase in summer/autumn heat content, predicted to be 9.5 MJ m^{-3} (Saloranta et al. 2009). With the deepening of the oxygenated epilimnion in Halsjärvi due to mixing (Fig. 4), the area of sediments in contact with the epilimnion increased considerably (Table 1), resulting in an increase in oxic–anoxic water–sediment interfaces.

The MeHg concentration in the epilimnion of Halsjärvi was 0.58 ng l^{-1} in August 2004 prior to mixing, and decreased to 0.06 ng l^{-1} in the years of mixing. The concentration remained at this low level in 2007, 1 year after mixing (Table 2). In contrast, MeHg concentrations in the anoxic hypolimnion were higher (0.36–2.4 ng l^{-1}) throughout the study period in both lakes. A similar pattern was recorded for total mercury concentration, although the decrease in epilimnetic concentration due to mixing was not as marked as for MeHg concentrations (Table 2).

Mercury concentrations and stable isotope ratios in perch

Methyl mercury concentrations in the muscle of small perch were equivalent in the experimental lake, Halsjärvi, and in the reference lake, Valkea-Kotinen (0.1–0.15 mg/kg (ww) in both lakes) at the start of the study in 2004 (Fig. 5). After mixing of the water column in Halsjärvi started in 2005, the mean perch Hg concentrations decreased significantly and remained <0.1 mg/kg (ww) (ANOVA, $F = 20.97$, df = 3,41, $P < 0.001$; Tukey test $P < 0.001$ for 2004 vs. other years). In the reference lake, Valkea-Kotinen, an increasing trend in the Hg concentrations of perch was recorded at the same time (Fig. 5), up to 0.2 mg/kg (ww) in 2007 (ANOVA, $F = 11.54$, df = 2,17, $P = 0.001$; Tukey test $P < 0.001$ for 2004 vs. 2007 and 0.033 for 2005 vs. 2007). Consequently, the differences between the lakes became significant during the study period (2005: $t = -5.934$, df = 10, $P < 0.001$; 2007: $t = -11.594$, df = 24, $P < 0.001$).

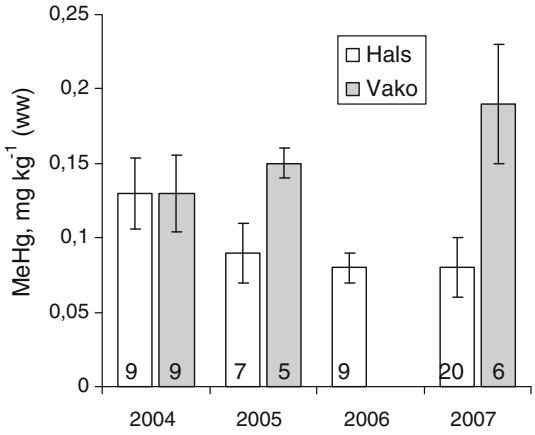

Fig. 5 The methyl mercury concentrations (mean ± SD) in small perch (total length 70–80 mm, age 2+ years) in the lakes Halsjärvi ($n = 45$) and Valkea-Kotinen ($n = 20$) during 2004–2007. The artificial mixing in Halsjärvi took place in the summers 2005 and 2006

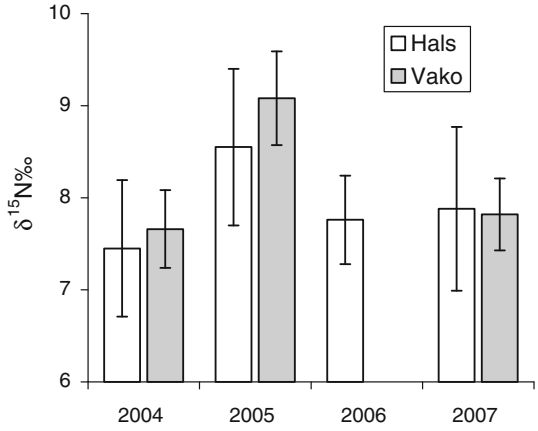

Fig. 6 Nitrogen stable isotope values (δ^{15}N‰ mean ± SD) of small perch from Halsjärvi ($n = 45$) and L. Valkea-Kotinen ($n = 20$) during 2004–2007. The artificial mixing in Halsjärvi took place in the summers 2005 and 2006

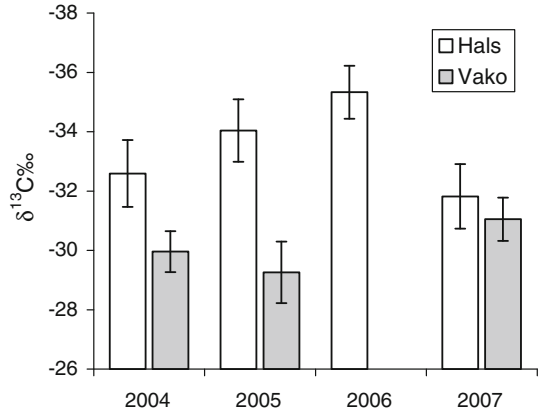

Fig. 7 Carbon stable isotope values (δ^{13}C‰ mean ± SD) of small perch from Halsjärvi ($n = 45$) and L. Valkea-Kotinen ($n = 20$) during 2004–2007. The artificial mixing in Halsjärvi took place in the summers 2005 and 2006

The SIA analyses showed that δ^{15}N values for perch (Fig. 6) ranged between 6 and 10‰ in both lakes over the study period from 2004 to 2007 with no significant differences between the lakes ($t = -0.913$, df = 62, $P = 0.362$). Among the years, the mean δ^{15}N values were between 7.5 and 7.9‰ in both lakes with the exception of 2005 when values were close to 9‰, differing significantly from the other years in both Halsjärvi (ANOVA, F = 2.609, df = 3,41, $P = 0.064$; Tukey $P = 0.043$) and Valkea-Kotinen (ANOVA, F = 17.869, df = 2,16, $P < 0.001$; Tukey $P < 0.001$).

The δ^{13}C of perch (Fig. 7) from Halsjärvi was consistently more negative than that of perch from Valkea-Kotinen over the study period ($t = -6.955$, df = 62, $P < 0.001$). Consequently, the annual δ^{13}C values were significantly different between the lakes in 2004 ($t = -7.797$, df = 16, $P < 0.001$) and 2005 ($t = 7.797$, df = 10, $P < 0.001$) but not in 2007 ($t = -1.477$, df = 23, $P = 0.153$).

A clear response in the δ^{13}C values was recorded in Halsjärvi following experimental mixing of the water column (Fig. 7). The mean δ^{13}C values were around −32‰ in the reference years 2004 and 2007, whereas during the years of mixing, 2005 and 2006, the values were significantly more negative, at around −35‰ (ANOVA, $F = 25.62$, df = 3,41, $P < 0.001$, Tukey $P = 0.046$, 0.001). In the reference lake,. Valkea-Kotinen, there was less year to year variation in the δ^{13}C values but still some significant difference (ANOVA, $F = 6.44$, df = 2,16, $P = 0.009$; Tukey $P = 0.064$, 0.09).

There was no relation between MeHg concentrations of perch and their and δ^{15}N values (Fig. 8), but there was a significant positive correlation ($r = 0.486$, $P < 0.001$ for the whole data) between the MeHg and δ^{13}C values of perch (Fig. 9).

Discussion

Mercury is known to bioaccumulate along aquatic food chains resulting in highest concentrations in the uppermost levels of the ecosystems, particularly in predatory fish (e.g. Häsänen and Sjöblom 1968; Fagerström and Åsell 1973; Wren and MacCrimmon 1986; Rask et al. 1994; Hinck et al. 2009). The nitrogen isotope ratios of perch were analysed in the present study because δ^{15}N values reflect the trophic position of organisms in food webs (e.g. Jardine et al. 2006). The total range of δ^{15}N values across all the perch samples was from 5.9 to 10.3‰ in both lakes and probably mainly reflects individual variation in diet, for example relative contributions of zooplankton and macro invertebrates which are the typical food items perch in these lakes (Rask 1986). In addition, some perch may have been partly cannibalistic, especially on perch fry, which would tend to raise their δ^{15}N values. An average increase in δ^{15}N of about 3‰ between diet and consumer has been widely reported (e.g. Post 2002) so the observed δ^{15}N

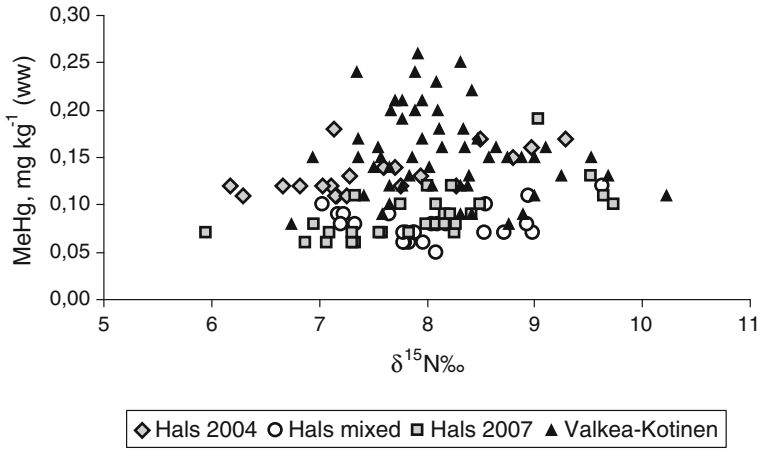

Fig. 8 The methyl mercury concentrations in small perch related to their δ^{15}N values in the lakes Halsjärvi ($n = 75$) and Valkea-Kotinen ($n = 54$) during 2004–2007. For Halsjärvi, the values for the reference years (2004 and 2007, $n = 20 + 30$) and for the years of experimental mixing (2005 and 2006, $n = 25$) are indicated separately

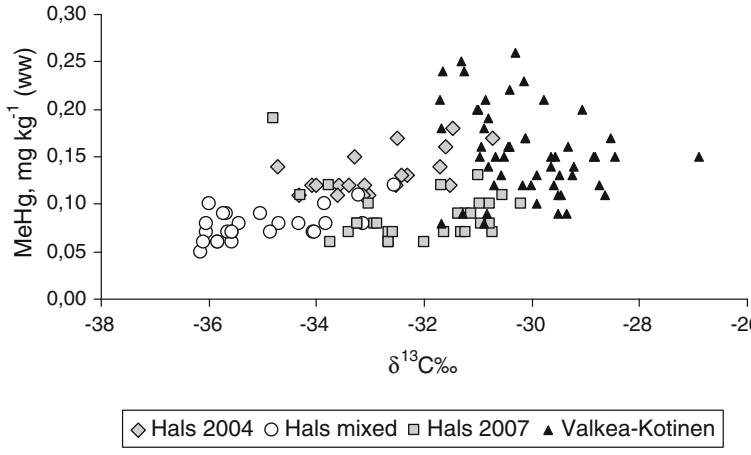

Fig. 9 The methyl mercury concentrations in small perch related to their δ^{13}C values in the lakes Halsjärvi ($n = 75$) and Valkea-Kotinen ($n = 54$) during 2004–2007. For Halsjärvi, the values for the reference years (2004 and 2007, $n = 20 + 30$) and for the years of experimental mixing (2005 and 2006, $n = 25$) are indicated separately

variation indicates a difference of about one trophic level or more between some perch individuals in the lakes of the present study. However, the δ^{15}N values of perch in Halsjärvi clearly overlapped between the years, and the mean perch δ^{15}N values did not differ significantly between the two lakes or between years. This suggests that perch occupied similar trophic positions in both lakes and that their trophic position essentially did not change over time. Consequently, changes in trophic position of perch can not explain the observed changes in perch Hg concentrations in the lakes.

The mean δ^{13}C values of perch from Valkea-Kotinen showed irregular variation across years but in Halsjärvi showed a clear response to the thermocline manipulation. During the mixing in 2005 and 2006, the mean δ^{13}C values of perch from Halsjärvi were significantly lower, at around −35‰, than those in the reference years 2004 and 2007 (around −32‰). The lower δ^{13}C values occurred concurrently with lower mercury concentrations in perch muscle tissue, and also with the decrease of MeHg concentration in the epilimnetic water reported by Verta et al. (2010). Although there was a significant correlation between the Hg and δ^{13}C values of perch, this may have been the result of two independent processes. The experimental mixing of Halsjärvi decreased the volume of the anoxic zone and increased the area of sediment–water oxic–anoxic interface, which may have led to more access of methane-derived carbon, via methane-oxidising bacteria (MOB), for the food web up to perch. At the same time the mixing-induced decrease in the volume of anoxic water layers probably reduced the synthesis of MeHg by sulphate reduction and resulted in lower MeHg concentrations in the water as suggested by Verta et al. (2010), leading to lower Hg values in perch.

Although it is not possible to identify with certainty the cause of the drop in perch $\delta^{13}C$, a plausible explanation is that it reflects increased incorporation of methane-derived carbon into perch tissue in Halsjärvi. There is considerable evidence that much of the methane produced in anoxic lake sediments becomes incorporated into bacterial biomass when it is utilized by abundant MOB at oxic–anoxic interfaces. This MOB production can then be exploited by primary consumers, particularly by chironomid larvae in lake sediments (e.g. Jones et al. 2008). Since the biogenic methane produced in lake sediments has very low $\delta^{13}C$ values (typically −60‰ or lower), incorporation of this methane carbon into primary consumers can impart to them a characteristically low $\delta^{13}C$ signature. Chironomid larvae can have $\delta^{13}C$ values as low as −60‰, and zooplankton values as low as −55‰ have also been reported. Therefore, higher trophic level consumers, such as fish, which include an appreciable proportion of these isotopically light primary consumers in their diet can also be expected to exhibit reduced $\delta^{13}C$ values.

Unfortunately samples of perch prey items from the lakes were not available for SIA. However, there have been previous reports of small forest lakes in Finland containing primary consumers with low $\delta^{13}C$ values, both chironomid larvae down to −55‰ (e.g. Jones and Grey 2004; Jones et al. 2008) and zooplankton down to −40‰ (e.g. Jones et al. 1999; Taipale et al. 2008). The experimental mixing of Halsjärvi and the consequent lowering of the thermocline and expansion of the oxic part of the total lake volume, is likely to have increased the spatial extent of sediment surface oxic–anoxic interfaces, increased the production of MOB and hence the availability of their production to chironomid and zooplankton consumers, and also increased the oxic ecosystem space available to perch for foraging. All these factors would be expected to increase the potential transfer of isotopically light methane carbon to perch in Halsjärvi, leading to the observed significant reduction in perch mean $\delta^{13}C$ values in 2005 and 2006. Following cessation of artificial mixing, these factors evidently rapidly reverted to their pre-mixing state, and the relatively rapid turnover times (weeks to months) of isotope ratios in fish muscle mean that by autumn 2007 the perch in Halsjärvi were again showing $\delta^{13}C$ values equivalent to those in the reference year 2004. The overall range of perch $\delta^{13}C$ values from −35.4 to −26.2‰ was high, and probably again reflects individual diet variation, and particularly differences between individuals on the extent to which their diet had included isotopically light prey items with a high proportion of methane-derived carbon.

A cause and effect relationship between the mixing-induced decrease in epilimnetic MeHg concentrations and the decrease in perch MeHg concentration is probable. This is because the decrease was clear in both and the concentrations in epilimnetic water and in perch also remained low after the mixing in 2007. The major cause for the decreased MeHg concentration in epilimnetic water remains uncertain but may be driven by enhanced demethylation or increased MeHg sedimentation or a combination of both these processes.

Bodaly et al. (1993) hypothesized that the higher Hg concentrations in smaller and warmer lakes in Ontario, Canada, would be a result of temperature-related differences in gross and net methylation rates in lakes, mainly in epilimnetic sediments. Later, Harris and Bodaly (1988) showed through modelling that temperature/metabolic and growth effects on fish were of only secondary importance and that dietary exposure was probably the primary factor. They argued that this finding supported the methylation hypothesis, but did not exclude other possible factors. In our experiment the area of warm epilimnetic sediments increased simultaneously with a decrease in water MeHg concentrations and in fish Hg concentrations. This would indicate that, although the dominance of dietary exposure is valid (lower water MeHg concentration is followed by lower food item MeHg), the methyl mercury production in epilimnetic sediments can hardly be the main source of MeHg in fish. In that case we would have expected to find higher MeHg concentration in water and fish after mixing.

Concluding remarks

The results of the study indicate that the decrease in methyl mercury production and concentration in the water due to the mixing experiment was followed within a few months by a decrease in Hg concentrations in small perch. Furthermore, as water MeHg

concentrations remained low during the summer after the experiment, so did the Hg concentration in perch. Concurrent with the changes in mercury processes, significant changes in carbon sources in the food chains of Halsjärvi took place. The relation of the changes in carbon dynamics with the MeHg formation and uptake in perch remains an open question, but it appears that these are two independent consequences of the oxycline change. These results support the hypothesis that possible oxygen-related changes caused by climate change will be far more important than possible direct temperature changes for MeHg in fish in small boreal lakes with regular and persistent oxygen deficiency in the hypolimnion.

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